

Clinical and Radiographic Evaluation of Platelet Rich Plasma in Combination with Demineralised Freeze-Dried Bone Allograft in the Treatment of Periodontal Intrabony Defects: A Comparative Study

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ABSTRACT

Aim: Demineralized Freeze-Dried Bone Allograft (DFDBA) has repeatedly demonstrated significant improvements in soft and hard clinical tissue parameters for the treatment of intraosseous periodontal defects. Platelet-derived growth factor (PDGF) has the primary effect of a mitogen, initiating cell division. It was shown that osteoblasts proliferate in response to PDGF alone or with the addition of a progression factor to induce mitosis. However there is no evidence to evaluate whether a combination of PRP and DFDBA-Allograft enhances the clinical outcome compared to treatment with DBM mixed with saline solution. Therefore, the purposes of this study were to compare the clinical and radiographic outcomes obtained with the combination of PRP and DFDBA to those obtained with DFDBA mixed with saline solution in the treatment of periodontal intrabony defects.

Materials and Method: This study was carried out for a period of 12 months. 20 intrabony defects in 10 patients were divided into experimental and control sites. The experimental sites were debrided and grafted with a combination of Platelet Rich Plasma and DFDBA-Allograft. The control sites were debrided and grafted with DFDBA-Allograft with saline. Probing depth, clinical attachment level and gingival margin position were recorded at baseline, 3, 6, 9 and 12 months. Standardized radiographs were also documented at these recalls.

Results: On completion of 12 months, the DFDBA + PRP sites had significantly lower mean PD (7.0 mm versus 2.10 mm; $P < 0.003$) and CAL (7.0mm versus 1.90 mm; $P < 0.003$) compared to the DFDBA+saline sites. At 12months, there was no significant difference between the study groups for mean gingival margin position ($P > 0.05$).

Conclusion: Overall, both therapies led to significant improvements of the investigated parameters. The combination of PRP and DFDBA-Allograft was more effective in terms of improving clinical parameters than DFDBA-Allograft alone. There is a need for further long term controlled studies evaluating the adjunctive benefits of a combination of PRP



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and DFDBA-Allograft as compared to DFDBA-Allograft alone in the treatment of periodontal intrabony defects.

Keywords: Allografts, Guided Periodontal Tissue Regeneration, Platelet Rich Plasma.

INTRODUCTION

Periodontal disease is one of the most prevalent afflictions worldwide and is the major cause of tooth morbidity and mortality. The goals of periodontal therapy include the arrest of periodontal disease progression and regeneration of structures lost to disease where appropriate¹. Moderate to severe periodontal defects are often not amenable to osseous resection without further compromise in the supporting structures of the involved and adjacent teeth. When applicable, regeneration of the lost bone and periodontal attachment improves the support of the tooth and hopefully its long term prognosis. Although many attempts have been made to regenerate alveolar bone support and the attachment apparatus, predictable success has proved elusive². This can be accomplished by using regenerative surgical procedures like root biomodification, use of bone replacement grafts, Guided Tissue Regeneration (GTR) and growth factors^{2,3}.

Recently, the attention of periodontal researchers and clinicians has focused on the use of polypeptide growth factors for periodontal regeneration⁴⁻⁷. Growth factors that seem to play an important role in periodontal and bone wound healing are platelet derived growth factor (PDGF) and transforming growth factor- β (TGF- β), Insulin like growth factor (IGF), Vascular endothelial growth factor (VEGF) and Epithelial growth factor (EGF)⁸. PDGF has been shown to exert a favourable effect on periodontal regeneration as measured by increase in clinical attachment levels and osseous defect fill in humans⁹. Platelet-derived growth factor (PDGF) has the primary effect of a mitogen, initiating cell division¹⁰. It was shown that osteoblasts proliferate in response to PDGF alone or with the addition of a progression factor to induce mitosis^{11,12}. Transforming growth factor-beta (TGF- β), a multifunctional growth factor that is chemotactic for bone cells, increases the differentiated function of osteoblasts, osteoblast precursors, and extracellular matrix formation, such as type I collagen^{13,14} and stimulates the proliferation of gingival fibroblastic cells, the formation of blood vessels, the remodelling of

extracellular matrix, and the formation of granulation tissue during the healing of periodontal tissue¹⁵. PDGF and TGF- β are abundant in the alpha granules of platelets¹⁶. Platelet-rich plasma (PRP) is an autologous source of PDGF and TGF- β . TGF- β when coated onto β -Tricalcium phosphate pellets substantially stimulates cell proliferation and differentiation of osteoblast lineage cells and induces bone formation¹⁷.

A convenient and economical approach to obtain autologous PDGF and TGF- β is the use of platelet rich plasma (PRP)¹⁸. Delivery of autologous platelets to periodontal wounds can increase the local concentration of growth factors, which may enhance the healing outcomes. Demineralized freeze dried bone allograft (DFDBA) is a frequently used bone graft replacement material that forms bone by osteoinduction¹⁹. The freeze drying process destroys cells while maintaining cellular morphology and chemical integrity. Substances present in the demineralised bone graft material, bone morphogenetic proteins, stimulate local cell cycles to produce new bone²⁰. Autologous platelet gel combined with bone grafts has been used successfully for regeneration of bone defects²¹. PRP has been used successfully to enhance the clinical outcome obtained with guided tissue regeneration (GTR) and bone grafts in the treatment of intrabony defects^{22,23}. Use of combination of PRP and bone replacement grafts in periodontal regenerative therapy offers interesting perspective to the clinician. However, only a few studies have tested the efficacy of a combination of PRP and various types of bone grafts in the treatment of intrabony defects and there is no evidence to evaluate whether a combination of PRP and DFDBA enhances the clinical outcome compared to treatment with DFDBA mixed with saline solution. Therefore, the purposes of this study were 1) to compare the clinical and radiographic outcomes obtained with the combination of PRP and DFDBA to those obtained with DFDBA mixed with saline solution in the treatment of periodontal intrabony defects 12 months after flap surgery and 2) to investigate the possible additional effects of PRP on the treatment of intrabony defects by comparing the use of PRP plus DFDBA to DFDBA with saline solution.

Study Sample: 10 patients who reported to the outpatient Department of Periodontics, Faculty of Dental Sciences, Ramaiah University, Bangalore, Karnataka, fulfilling the inclusion and exclusion criteria were included in this study. All subjects were explained the purpose of the study and an informed consent approved by the ethical committee was signed by them. All patients were enrolled from 2009 to 2010. Inclusion criteria were, patients in the age group between 20-40 years, having periodontal pocket with probing depth ≥ 6 mm, radiographic evidence of periodontal osseous defects and patients who have not undergone any type of regenerative periodontal therapy 6 months prior to the initial examination. Exclusion criteria were patients who were medically compromised or under therapeutic regimen that may decrease the probability of soft tissue and bone healing, patients who were pregnant or lactating, smokers and allergic to materials and drugs used or prescribed in this study.

Study Design: 10 patients (3 males and 7 females) with a total of 20 sites were selected after the completion of initial phases in all the patients. These selected sites in each individual were divided into control and experimental sites randomly. They were treated according to split mouth technique. Experimental sites were treated with open flap debridement followed by placement of a combination of PRP with DFDBA and control sites were treated with open flap debridement followed by placement of DFDBA.

Clinical parameters: The following clinical parameters were recorded at baseline, 3, 6, 9 and 12 months post operatively.

A. Vertical measurements for determination of probing depths, clinical attachment levels and gingival margin position:

1. Fixed reference point (FRP) to the base of the pocket (BOP)
2. Fixed reference point (FRP) to cemento-enamel junction (CEJ)
3. Fixed reference point (FRP) to gingival margin (GM)

All the measurements were standardized using customized acrylic stents with grooves, which were

prepared on the study model of the patients. The recordings were made using a UNC 15 probe (Hufriedy's). The calculations were made from the clinical measurements recorded: Probing depth: (FRP to BOP) – (FRP to GM), Clinical attachment level: (FRP to BOP) – (FRP to CEJ), Gingival margin position: (FRP to CEJ) – (FRP to GM).

B. Radiographic Measurement:

Intra oral periapical radiograph and digital radiograph (RVG) of each defect site were taken at baseline, 3, 6, 9 and 12 months post-surgery using long cone/paralleling technique.

Bone graft used in the study: Demineralized bone matrix graft material is made up of insoluble collagen and proteins that are non-collagenous in nature. This promotes growth of the bone cells because of its osteoinductive potential.

Preparation of Platelet Rich Plasma (PRP):

PRP was prepared according to the procedure described by Kazuhiro Okuda et al. One hour prior to the periodontal surgery 8-10 ml of whole blood was drawn from the patient's antecubital vein. Blood was collected in a vacutainer containing 10% citrate anticoagulant solution. The tubes were inverted several times to ensure the mixing of blood and anticoagulant. The sample tube was then spun in a standard centrifuge for 10 minutes at 2400 rpm to separate PRP and platelet poor plasma (PPP) from the red blood cell fraction. The PPP was discarded, leaving just about 1ml of PPP present above the buffy coat. The 1ml of PPP, the whole of buffy coat and 1ml of red blood cell fraction rich in newly synthesized platelets was pipetted out and transferred to another test tube without an anticoagulant. The test tubes were then centrifuged at 3600 rpm for 15 minutes, to separate PRP and PPP. The PRP was then drawn into a syringe and expressed into a sterile container.

Presurgical Procedure: All the selected patients, following an initial examination, treatment planning and discussion were given detailed instructions in self performed plaque control measures and were subjected to phase I periodontal therapy. Four to six weeks after phase I therapy the patients were subjected to surgical procedure.

Surgical Procedure: The patient was made to rinse with 0.2% Chlorhexidine digluconate mouth rinse for 30 seconds prior to the surgery. The standardized surgical procedures for the experimental and control sites were performed as follows:

- Surgical area was anesthetized using local anaesthetic (2% lignocaine with adrenaline 1:80000).
- Intracrevicular incisions were made and full thickness flap were elevated, to retain as much soft tissue as possible in order to obtain primary closure.
- Meticulous defect debridement and root planing were carried out to remove subgingival plaque, calculus, diseased granulation tissue and pocket epithelium. The surgical sites were irrigated with sterile saline and care was taken to keep the area free of saliva and flaps were engaged using 3-0 silk suture material utilizing an interdental direct suturing technique.
- Immediately before application the PRP was activated by clot initiator 10% calcium chloride and whole blood from the defect, within a few seconds the PRP preparation assumed a sticky gel consistency that would be relatively easy to apply to the surgical defects. The coagulated PRP and bone graft was placed up to the vertical height of the corresponding adjacent bone level.
- Flaps were repositioned to the pre-surgical level and the previously placed loose sutures at the sites were approximated and stabilised to achieve a primary closure.
- A periodontal dressing (Coe - pak) was placed on the surgical area. Post operative care included systemic administration of doxycycline HCL 200 mg for the first day followed by 100 mg/day for another 6 days along with a combination of Ibuprofen 400mg and Paracetamol 325mg given twice daily for three days. 0.2% chlorhexidine gluconate rinse was prescribed twice daily for a period of 2 weeks. Periodontal dressing and sutures were removed one week post surgery.

Post-Surgical Care: Patients were given routine post surgical instructions

- Apply the ice pack in intervals for 1hour after surgery.
- If any unusual pain or bleeding from the operated site is felt, report to the doctor immediately.
- Not to brush the teeth in the surgical area.
- Not to eat anything hard or hot for 24 hours post surgically
- Not to feel for the surgical area with tongue or with finger.
- Smoking should be avoided.

Sutures were removed after 1 week.

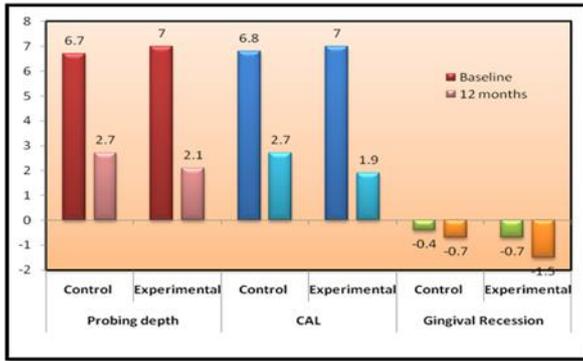
Post Surgical Evaluation:

Clinical Evaluation: The patients were evaluated clinically at 3, 6, 9 and 12 months post surgically. The customized acrylic stent was placed on each defect site. Measurements were made using UNC-15 graduated periodontal probe for attachment gain, pocket depth and gingival recession.

Radiographic Evaluation: All the sites in both experimental and control groups were subjected to radiographic assessment. IOPA radiographs and digital radiographs (RVG) were taken for each site at 3, 6, 9 and 12 months post surgically.

Statistical Analysis: The following methods of statistical analysis have been used in this study. Data was entered in Microsoft excel and analysed using SPSS (Statistical Package for Social Science, Ver.10.0.5) package. Analysis-of-variance procedures require the following assumptions: Each of the groups is an independent random sample from a normal population and in the population; the variances of the groups are equal.

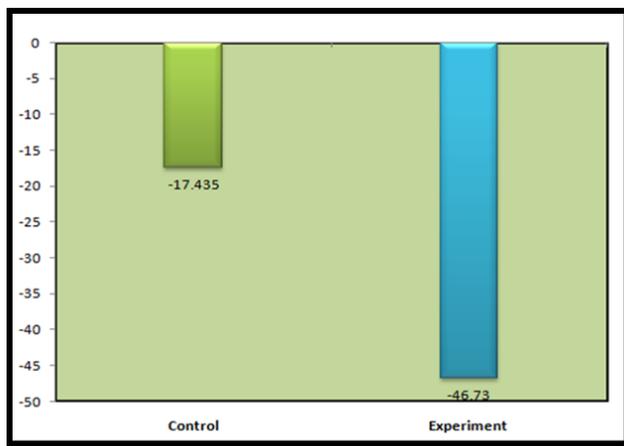
If F value is significant there is a significant, difference between group means. Then LSD comparison test was used to detect significant difference between group means. The student't' test was used to determine whether there was a statistical difference between groups in the parameters measured. In all the above test "p" value of less than 0.05 was accepted as indicating statistical significance.



Graph 1: Mean values of probing depth, clinical attachment level & gingival recession of experimental & control groups at baseline & 12 months.



Fig 1b: Experimental site IOPA after 12 months.



Graph 2: Comparison of percentage of bone fill in experimental and control group.



Fig 2a: Control site IOPA at baseline.



Fig 1a: Experimental site IOPA at baseline.



Fig 2b: Control site IOPA after 12 months.

RESULTS

In the present study, 10 patients (3 males and 7 females) in the age group of 20-45 years, fulfilling the inclusion and exclusion criteria, contributing to a total of 20 intrabony defects were recruited. These 20 periodontal intrabony defects were then randomly assigned into experimental and control sites. All enrolled subjects completed the study. All sites healed uneventfully. At 12 months, the DFDBA + PRP sites had significantly lower mean PD (7.0 mm versus 2.10 mm; $P < 0.003$) and CAL (7.0mm versus 1.90 mm; $P < 0.003$) compared to the DFDBA+ saline sites. At 12months, there was no significant difference between the study groups for mean gingival margin position ($P > 0.05$). Within-group comparisons showed that the treatment of the intrabony defects with DFDBA + PRP or DFDBA + saline led to an overall clinical improvement in PD and CAL at 12 months compared to baseline. At 12 months, the mean probing depth (Mean \pm standard deviation) in mm for DFDBA + PRP sites was 7.00 ± 1.054 and 6.70 ± 1.059 for DFDBA+ saline sites. The mean probing depth measurements were less in the DFDBA + PRP when compared with the DFDBA+ saline sites at 9th months ($p=0.019$) and 12th months ($p=0.003$) postoperatively. At 12 months, the mean clinical attachment level measurements did not differ significantly between DFDBA + PRP and DFDBA+ saline sites at baseline, but there was statistically significant difference between the group at 9th and 12th mth with the p value being .019, .003 respectively. Mean values of probing depth, clinical attachment level & gingival margin position of both the groups at baseline & 12 months are shown in Graph 1. The mean IOPAR bone level (\pm standard deviation) in mm was 6.50 ± 2.173 at baseline and 3.40 ± 0.843 at 12thmth in the DFDBA + PRP sites and 6.10 ± 2.025 at baseline and 4.50 ± 1.179 at the 12thmth in the DFDBA+ saline sites. There was a statistical significant difference in the radiographic bone level within the groups when the baseline and 12thmth measurements were compared with the p value being $p=0.027$ (Table.1). The mean RVG bone level (\pm standard deviation) in mm was 6.93 ± 2.35 at baseline and 3.510 ± 0.834 at 12thmth in the experimental group and 6.45 ± 2.38 at baseline and 5.36 ± 2.068 at the 12thmth in the control group. There was a statistical significant difference in the radiographic bone level within the groups when the baseline and 12thmth

measurements were compared with the p value being $p=0.017$ (Graph 2 and Figures 1 and 2).

DISCUSSION

The present study was designed to evaluate PRP as an adjunct to DFDBA in treating intrabony osseous defects. Subject age, gender, and teeth with osseous defects treated were similar in both groups at baseline. Each subject demonstrated excellent oral hygiene and a generally healthy gingival condition throughout the study. This investigation showed that both treatment modalities significantly improved clinical and radiographic parameters between baseline and 12 months. However, the DFDBA + PRP treatment group showed statistically significantly greater PD reduction (2.10 ± 0.738 mm) and CAL gain (1.90 ± 0.56 mm) compared to the DFDBA + saline treatment group (PD = 2.70 ± 0.483 mm; CAL = 2.70 ± 0.48 mm). Richardson et al 1999²⁴ and Bender et al 2005²⁵ evaluated the treatment of human vertical intrabony defects with DFDBA at 6months post surgery and found a PD reduction of 2.0 and 2.8 mm and a CAL gain of 2.6 and 2.4 mm, respectively. The present results with DFDBA were more favourable for PD reduction and similar for CAL gain than reported in the earlier investigations and when PRP was added as an adjunct to DFDBA in the present study, the clinical results were more impressive. Similar results have been reported by Matteo Piemontese et al 2008²⁶ using a combination of demineralised freeze-dried bone allograft in combination with platelet- rich plasma. The mean IOPA radiographic bone level (\pm standard deviation) in mm was 6.50 ± 2.173 at baseline and 3.40 ± 0.843 at 12thmth in the experimental group and 6.10 ± 2.025 at baseline and 4.50 ± 1.179 at the 12thmth in the control group. There was a statistical significant difference in the radiographic bone level within the groups when the baseline and 12thmth measurements were compared with the p value being $p=0.027$. The mean of percentage of bone fill in the control group obtained was 24.63% whereas the percentage of bone fill in experiment group was 45.54%. The mean RVG bone level (\pm standard deviation) in mm was 6.93 ± 2.35 at baseline and 3.510 ± 0.834 at 12thmth in the experimental group and 6.45 ± 2.38 at baseline and 5.36 ± 2.068 at the 12thmth in the control group. There was a statistical significant difference in the radiographic bone level within the groups when the baseline and 12thmth measurements were compared with the p value

being $p=0.017$. The mean percentage of bone fill in the control group obtained was 17.43% whereas the percentage of bone fill in experiment group was 46.73%. The hard tissue assessment for IOPA radiographs were assessed using the grids and for digital images was done using AUTOCAD software where the region of interest of the pre and post operative radiographs of both the experimental and the control was demarcated digitally and the difference between the pre and post operative radiographs was calibrated. Demineralised bone matrix (DBM) being radiolucent material, was apparent on the radiographs at all the times so that the bone filling in the defect was easy to notice at different time intervals. In the present study gingival margin position was similar in both the groups which was in accordance with study done by Matteo Piemontese et al 2008²⁶ using a combination of demineralised freeze-dried bone allograft in combination with platelet- rich plasma.

However, the greater CAL gain and PD reduction observed in the DFDBA + PRP group in the present study may be explained by the additional biologic effects of PRP. The mechanism of action of PRP is attributed to several of its cellular effects. Marx et al²⁷ demonstrated the platelet stored growth factors and its specific cell receptors. PRP potently stimulated PDL cells and osteoblastic proliferation while inhibiting epithelial cell proliferation²⁸ and formed a gel-like material (fibrin clots) in several cell cultures of PDL or osteoblastic cells capable of up regulating collagen synthesis in the extracellular matrix²⁹. Another feature of the PRP enhanced graft is its high fibrinogen (fibrin glue) content that enhances wound stability and promotes a favourable scaffold for cellular migration³⁰. Because of its high fibrin content, the PRP preparation has a “sticky” characteristic that works as a haemostatic and stabilizing agent and may aid in immobilizing the blood clot and bone graft in the defect area, an important event in the early phases of wound healing in periodontal regenerative therapy³¹. Controversial results exist in the literature regarding the osteoinductive role of BMP in bone replacement grafting materials³²⁻³⁴. It can be speculated that because BMPs are members of the TGF super family, and they add to the effects of the growth factors within the platelets, ensuring a synergetic impact on the cell population of the wound^{35,36}. Although different preparations of

allograft material, from one distributor and among various distributors, may have different biologic activity, DFDBA remains a viable treatment modality to regenerate the periodontal attachment apparatus³⁷. Stricter standards from bone banks in evaluating the potency of their preparations, including the possibility of using bones from individuals under a specific age and/or free of bone diseases and/or using fresh bone and developing assays that can test the inductive capacity of the material prior to sales, may lead to more consistent and reliable clinical results. As reported by Hanna et al 2004³⁸ another combination periodontal therapy using PRP with bone-derived xenograft (BDX) for treating intrabony defects demonstrated an enhanced clinical result (PD reduction of 3.5 mm and CAL gain of 3.1 mm) compared to the use of BDX alone (PD = 2.53 mm and CAL = 2.31 mm) at 6 months. A study by Lekovic et al 2002²³ found PD of 3.9 mm and CAL of 3.3 mm with a combination of PRP and BDX and showed that GTR added no clinical benefit to PRP/BDX. Camargo et al 2002²² demonstrated CAL gain of 4.28 to 4.37 mm and HTF of 4.66 to 4.78 mm with triple combination therapy composed of PRP, bovine porous bone mineral, and GTR. Okuda et al 2005³⁹ evaluated PRP as an adjunct to hydroxyapatite (HA) in treating intrabony osseous defects. They found a more favourable clinical attachment compared to HA with saline. Growth factors present in high concentrations at inappropriate times or for an extended duration can adversely affect cell behaviour^{40,41}. The viability and proliferation of alveolar bone cells were suppressed by high PRP concentrations but were stimulated by low PRP concentrations (1% to 5%). Therefore, the large initial release of growth factors from PRP may result in suboptimal bone healing, or it may impede periodontal bone regeneration. This clinical study demonstrated a good soft and hard tissue response after treatment of intrabony defects with either demineralised freeze-dried bone allograft alone or in combination with platelet-rich plasma. Both treatment groups showed a significant PD reduction, CAL gain, hard tissue fill, and bone depth reduction at 12 months after surgery compared to baseline. At 12 months, there was a significantly greater PD reduction and CAL gain when PRP was added to DFDBA. This is in accord with other findings confirming the adjunctive clinical benefit of PRP when added to bone substitutes.

However, further long term controlled studies are justified towards evaluating the adjunctive benefits of a combination of (Platelet Rich Plasma + DBM) while using DBM bone graft alone, in the treatment of human periodontal osseous defects.

Clinical Relevance: The increased prevalence and incidence of periodontitis has been documented with increased osseous defects. These defects are often not amenable to osseous resection without further compromise in the supporting structures of the involved and adjacent teeth. When applicable, regeneration of the lost bone and periodontal attachment improves the support of the tooth and hopefully its long term prognosis. This study evaluated the efficacy of platelet rich plasma (PRP) and demineralised freeze-dried bone- allograft (DFDBA) in combination and DFDBA alone as a bone graft material in the treatment of periodontal intrabony defects.

Principal findings: Study showed that both the therapeutic approaches led to significantly greater reductions in probing depths and gains in clinical attachment level compared to baseline. The combination of Platelet Rich Plasma with demineralised freeze-dried bone- Allograft (DFDBA) was more effective than DFDBA alone in improving clinical attachment levels and reducing probing depths.

Clinical implications: Within the limits of the current study, can be concluded that both a combination of PRP and DFDBA or DFDBA alone bone graft treatment led to significant improvements of the investigated clinical parameters. The added benefits of PRP combined with DFDBA are not only statistical but also of clinical significance.

CONFLICT OF INTEREST

No potential conflict of interest relevant to this article was reported.

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