Original Article

Comparative evaluation of regenerative potential of injectable platelet-rich fibrin and platelet-rich fibrin with demineralized freeze-dried bone allograft in the treatment of intrabony defects: A randomized controlled clinical study

ABSTRACT

Background: Injectable platelet-rich fibrin (i-PRF) being in liquid form keeps graft particles clumped together forming agglutinated steak of bone graft. It has been shown to contain more platelets and long-term deliverance of growth factors in comparison with platelet-rich fibrin (PRF).

Aim: The aim of the present study was to assess regenerative potential of i-PRF and comparing it with PRF, along with demineralized freeze-dried bone allograft (DFDBA) in the treatment of intrabony alveolar defects.

Materials and Method: Thirty defect sites in 15 patients with bilateral intrabony defects were assigned randomly into two groups (Group I (Control group)- DFDBA + PRF and Group II (Test group)-DFDBA + i-PRF). Gingival index (GI), plaque index (PI), pocket probing depth (PPD), and relative attachment level (RAL) were recorded at baseline, 3 months, and 6 months. Linear bone growth (LBG) was recorded radiographically at baseline and 6 months.

Statistical Analysis: ANOVA test and post hoc Tukey test were used to assess intragroup comparison of clinical parameters. Paired t-test was used to assess intragroup comparison of the radiographic parameter. Unpaired t-test was used to assess intergroup variations in all the clinical as well as radiographic parameters.

Results: Statistically significant PPD reduction ($P = 0.005$) and RAL gain ($P = 0.003$) were found in Group II than in Group I, and no significant difference was found in other parameters. Percentage LBG was higher in Group II than Group I but the difference was not statistically significant.

Conclusion: i-PRF with DFDBA showed more favorable results as compared to PRF with DFDBA in the management of intrabony periodontal defects.

Keywords: Growth factors, intrabony defects, platelet concentrate, platelet-rich fibrin

INTRODUCTION

Periodontitis is a multifactorial inflammatory disease of the supporting tissues of teeth and is signified by attachment loss and osseous defects. Bone destructive patterns in periodontitis acquire characteristic forms, more commonly as horizontal and vertical bone defects. Vertical intrabony defects acquire angular pattern and the defect base lies apical to the adjoining bone.[1] These defects are compliant to reduction of pocket with the help of regenerative periodontal treatment.

Bone grafts are utilized like a filler and a frame to aid in the formation of alveolar bone. These comprise of autografts,

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allografts, alloplasts, and xenografts. Allografts are derived from different individuals of genetically similar species. Demineralized freeze-dried bone allograft (DFDBA) has got osteoinductive property due to the presence of bone morphogenetic proteins (BMP 2, 4, and 7) that permit rapid revascularization and hard tissue growth in the osseous defects. Its osteoconductive property is due to its ability to create as well as maintain the space.[2]

Although many attempts have been made to regenerate alveolar bone by using biomaterials such as bone grafts and guided tissue regeneration (GTR), predictable success has proved elusive. There is a need of additional stimuli to enhance the regenerative process by promoting cell migration, attachment, proliferation, differentiation, and matrix synthesis. These functions can be achieved by using various growth factors like platelet-derived growth factors (PDGF), basic-fibroblast growth factor (b-FGF), transforming growth factor-β (TGF-β), insulin-like growth factor (IGF), etc.[3] Convenient and cost-effective approach to obtain such autologous growth factors is the use of platelet concentrates.

Platelet concentrates being autogenous, reduce the chances of immune-mediated adverse reactions and release various growth factors. Platelet-rich plasma (PRP), the first-generation platelet concentrates, being in liquid state had shown promising results with bone grafts.[4]

However, several limitations such as two-step centrifugation procedure and use of anticoagulants that suppress wound healing led to the emergence of second-generation platelet concentrate as platelet-rich fibrin (PRF).

PRF is obtained by centrifugation of blood in glass tubes in only one-step, without any anticoagulants, thus simplifying its production. It has dense framework of fibrin that protects the growth factors from proteolysis.[5] Its fibrin matrix has three-dimensional structure, which causes entrapment of cytokines, leukocytes, and various growth factors. It contains more platelets, leukocytes, and growth factors than PRP.[6]

An advancement was made to develop PRF in liquid state. Injectable platelet-rich fibrin (i- PRF) is next step PRF and has the properties of advanced PRF in liquid form. It was developed by Miron *et al*.[7] in 2015, by low-speed centrifugation concept in plastic tubes.[7] Slow and short centrifugation speed results in retaining higher number of growth factors, platelets, leukocytes as compared to PRF. Leukocytes help in tissue regeneration by recruiting various cell types (fibroblasts, osteoblasts) and favoring more sustained growth factor release during wound healing. i-PRF induces increased fibroblastic proliferation, migration, periodontal ligament cell growth, osteoblasts differentiation, and release of various growth factors.

iPRF coagulates after few minutes and thus can be used with other biomaterials. When mixed with particulate bone graft, it clots in few minutes, encapsulate the bone particles in a very nice way, thus allowing carving of the bone graft and giving it a compact form.[8]

Many studies have shown synergistic regenerative effect of PRF when combined with DFDBA in intrabony defects; this study was an attempt to evaluate and compare the regenerative potential of i-PRF along with DFDBA in intrabony periodontal defects.

MATERIALS AND METHOD

Materials

Commercially available DFDBA of particle size 500–1020 μm was used as the material for the study.

Patient selection

A randomized controlled clinical trial was carried out in the Department of Periodontology. It was conducted as per Helsinki Declaration and approved by MMU with Ref. no. 1443, dated 15.03.2019.

Inclusion criteria included systemically healthy patients, aged between 25 and 55 years, with almost two identical (one on either side of arch) interproximal intrabony defects, based on radiographic evidence and probing pocket depth (PPD) of 6 mm or more. Exclusion criteria included patients with systemic diseases or therapeutic regimen that could affect healing of the soft tissue or bone, allergic to chlorhexidine, one-walled defects, parafunctional habits (bruxism), history of periodontal surgery in last 6 months, pregnant or lactating women, smokers, and alcoholics.

Each patient was given detailed verbal and written descriptions of risks and benefits of treatment with the consent to the treatment agreement. After analysis of the pre-treatment records and satisfactory response to phase-I therapy, the patients were re-evaluated after 4 weeks. Patients who satisfied the inclusion criteria were finally selected for the study.

Study method

Thirty intrabony defects in 15 patients were selected for the study. Two interdental intrabony defect sites in each patient on contralateral side of same arch were assigned

randomly into two groups- Group I (Control group)- Open Flap Debridement (OFD) with placement of DFDBA and PRF and Group II (Test group) - Open flap debridement with placement of DFDBA and i-PRF.

Clinical parameters

The clinical parameters include PI-site specific,[9] GI-site specific,[10] PPD, and RAL; radiographic parameter includes LBG. Single investigator assessed all the parameters.

PPD was measured from gingival margin to the base of the pocket using University of North Carolina (UNC-15) probe. RAL was measured as distance from the apical end of the customized occlusal stent to the base of the pocket using UNC-15 periodontal probe. Vertical grooves were made in the stent for proper alignment of the probe and to ensure reliability and reproducibility for the future comparisons.

LBG was calculated as distance from cementoenamel junction (CEJ) to the base of the defect. At 6 months postoperatively, LBG was calculated by subtracting bone defect at 6 months (A1) from bone defect at baseline (A0) (A0- A1)

Percentage Linear bone growth was calculated from formula as

$$\frac{\text{Bone defect at baseline - Bone defect at 6 months}}{\text{Bone defect at baseline}} \times 100$$

To facilitate comparisons between serial radiographs, standardization was done by attaching X-ray grid with intraoral periapical radiographs (IOPA) and using paralleling technique. The grid had calibrations in millimeters that could be counted to assess the bony defect fill on successive radiographs [Figure 1a, b and Figure 2a, b].

Surgical procedure

Surgical procedure was carried out in the selected patients under aseptic conditions. The area was → anaesthetized → with → 2% → lignocaine → with → adrenaline → (1:80,000). → Full → thickness mucoperiosteal Kirkland flap was raised on both buccal and lingual sides using periosteal elevator. Granulation tissue was removed and root planning was done using Gracey and universal curettes (HU-Friedy). The anatomy of intrabony defect was assessed for its number of walls, depth, and width.

Preparation of PRF[11]

10 ml of blood was drawn from a peripheral vein in selected patients with a sterilized disposable syringe and was collected in a pre-sterilized borosilicate test tube without any addition of the anticoagulant. Blood was then immediately centrifuged at 2700 rpm for 12 minutes in a centrifuge unit. PRF was then separated from the red blood corpuscles base with the help of sterilized tweezers and scissors and was then transferred onto a sterile Dappen Dish.

Preparation of i-PRF[7]

Five ml of blood was drawn from a peripheral vein in selected patients with a sterilized disposable syringe and was collected in a pre-sterilized plastic red top vacutainer without any addition of the anticoagulant. Blood was then immediately centrifuged at 700 rpm for 3 minutes in a centrifuge unit [Figure 3a]. Following centrifugation, the upper liquid layer of i-PRF was separated from the red blood corpuscles base with the help of sterilized syringe. i-PRF collected in the syringe was added to DFDBA particles, which within 10–15 minutes

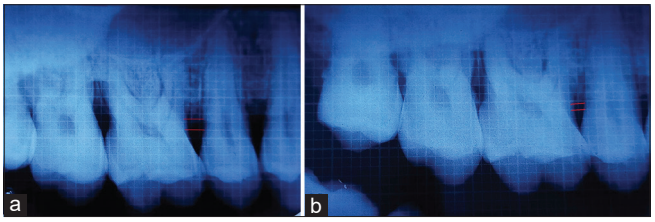

Figure 1: (a) Periapical radiograph of Group I at baseline. (b) Periapical radiograph of Group I at 6 months

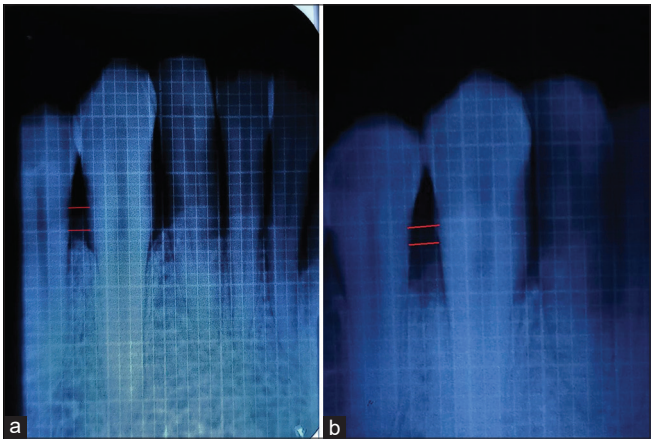

Figure 2: (a) Periapical radiograph of Group II at baseline. (b) Periapical radiograph of Group II at 6 months

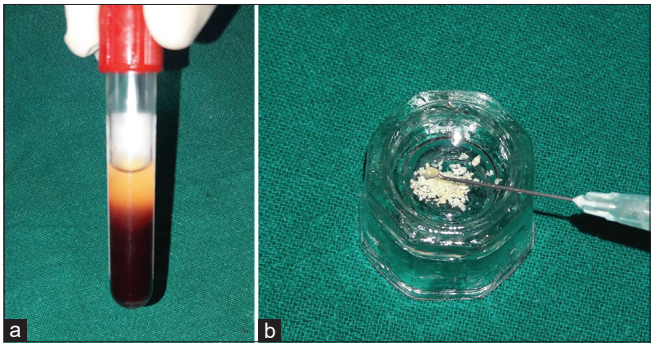

Figure 3: (a) iPRF obtained after centrifugation. (b) Mixing of iPRF with DFDBA and formation of solid mass

coagulated to form a homogenous agglutinated solid mass [Figure 3b].

Graft and PRF/i-PRF placement: After thorough debridement, defect site was presutured with 3–0 silk suture [Figure 4a and Figure 5a].

Group I site: DFDBA and PRF were mixed together and placed within the intrabony defect [Figure 4b].

Group II site: The solid graft mass (iPRF + DFDBA) was placed within the intrabony defect [Figure 5b]. At both the sites, the mucoperiosteal flaps were sutured and covered with periodontal pack. All patients were prescribed systemic antibiotic Novamox - LB (Amoxycillin) 500 mg thrice daily for 5 days and anti-inflammatory Combiflam thrice daily for 3 days to reduce postoperative pain and edema. 0.2% chlorhexidine mouthwash was prescribed twice daily for 4 weeks to maintain local plaque control.

Patients were recalled 24 hours postoperatively to evaluate any signs of complications such as pain, discomfort, swelling, hematoma, and hemorrhage. After one week, the periodontal pack and sutures were removed. Assessment of oral hygiene was done at 1, 3, and 6 months post-surgically, and oral hygiene instructions were explained.

Statistical analysis

All the clinical parameter, values obtained at different intervals (baseline, 3 months, and 6 months) and radiographic parameter values obtained (baseline and 6 months) were subjected to statistical analysis. For intragroup comparison of the clinical parameters: **"ANOVA test"** and **"Post Hoc Tukey"** test was performed. For intragroup comparisons of the radiographic parameter, **"Paired t-test"** was performed. For intergroup variations in all the clinical as well as radiographic parameters, **"Unpaired t-test"** was performed.

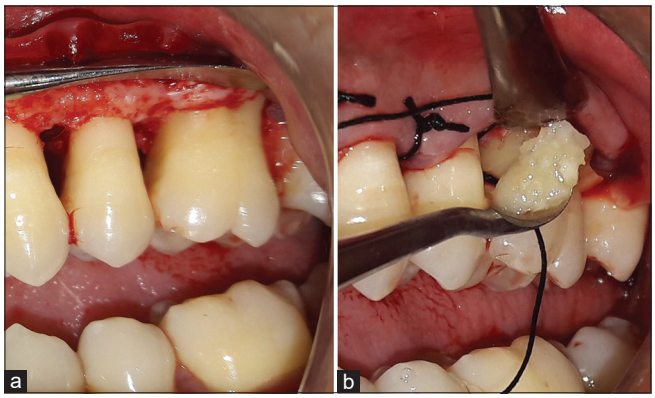

Figure 4: (a) Defect site in Group I. (b) Placement of DFDBA with PRF at defect site

Statistical Package for Social Science (SPSS) software 19.00 program (SPSS Inc., Chicago, IL, USA) was used for the analysis of data. $P < 0.05$ was considered as statistically significant.

RESULTS

All the 15 participants completed 6 months follow-up period with no postoperative complications. There was statistically highly significant PI reduction at 3 months and statistically significant reduction at 6 months in both the groups. There was statistically highly significant GI reduction at 3 months and statistically significant reduction at 6 months in Group I and statistically significant reduction in Group II at both 3 and 6 months [Table 1].

On comparing the two groups, the difference in mean PI and GI reduction scores was statistically non-significant at all time intervals [Table 2].

There was statistically highly significant PPD reduction and difference in RAL at 3 months and 6 months in both the groups [Table 3]. On comparison, Group II showed more statistically significant PPD reduction and difference in RAL than Group I at 6 months [Table 4].

Bone defect depth reduction in both the groups at 6 months from baseline was statistically significant [Table 5]. On comparing the two groups, the difference was statistically non-significant [Table 6].

Percentage gain in LBG of Group II was found to be more than Group I, though the difference was statistically non-significant [Table 7].

DISCUSSION

PRF is considered as a healing biomaterial with inbuilt regenerative capacity. It acts as source of growth factors like PDGF, FGF-basic, VEGF, and angiopoietin, thus facilitating endothelial cell migration resulting in angiogenesis.[12] It

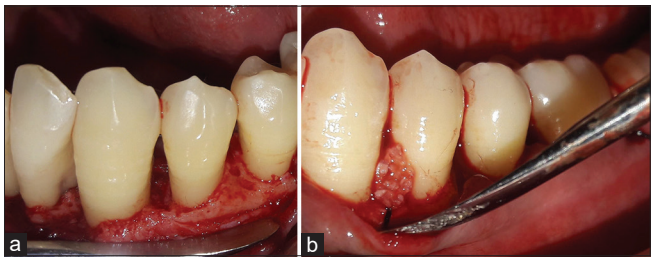

Figure 5: (a) Defect site in Group II. (b) Placement of DFDBA with iPRF at defect site

Table 1: Intragroup comparison showing mean and mean differences in plaque index and gingival index of Group I and Group II at different intervals

		Plaque index				Gingival index			
	Assessment Interval	Mean±SD	Mean difference from baseline	*F*	*P*	Mean±SD	Mean difference from baseline	*F*	*P*
Group I	Baseline	1.08±0.0.15	-	28.674	-	1.17±0.15	-	31.91	
	3 Months	0.82±0.17	0.26		0.000**	0.93±0.14	0.24		0.000**
	6 Months	0.63±0.15	0.45		0.001*	0.72±0.15	0.45		0.001*
Group II	Baseline	1.03±0.18	-	18.61	-	1.13±0.12	-	35.13	-
	3 Months	0.80±0.19	0.23		0.000**	0.92±0.12	0.21		0.001*
	6 Months	0.63±0.15	0.40		0.002*	0.70±0.16	0.43		0.002*

**$P<0.001$ is highly significant *$P<0.05$ is significant

Table 2: Intergroup comparison showing mean differences in plaque index and gingival index of Group I vs Group II at different intervals

	Assessment Interval	Plaque index		Gingival index	
		Mean difference	*P*	Mean difference	*P*
Group I vs Group II	Baseline	0.05	0.429	0.04	0.526
	3 Months	0.02	0.807	0.01	0.734
	6 Months	0.00	1.000	0.02	0.784

Table 3: Intragroup comparison showing mean and mean differences in probing pocket depth and relative attachment level of Group I and Group II at different intervals

		Probing pocket depth				Relative attachment level			
	Assessment interval	Mean±SD	Mean Difference from baseline	*F*	*P*	Mean±SD	Mean Difference from baseline	*F*	*P*
Group I	Baseline	6.07±1.22	-	25.028	-	11.26±2.01	-	119.2	-
	3 Months	3.4±0.50	2.67		0.000**	8.66±1.29	2.60		0.000**
	6 Months	2.4±0.50	3.67		0.000**	7.66±1.29	3.60		0.000**
Group II	Baseline	6±0.92	-	86.492	-	9.93±1.57	-	195	-
	3 Months	3.13±0.35	2.87		0.000**	7.13±1.50	2.80		0.000**
	6 Months	2±0	4.00		0.000**	6±1.51	3.93		0.000**

**$P<0.001$ is highly significant *$P<0.05$ is significant

Table 4: Intergroup comparison showing mean differences in probing pocket depth and relative attachment level of Group I vs Group II at different intervals

Group I vs Group II	Probing pocket depth			Relative attachment level		
	Assessment interval	Mean Difference	*P*	Assessment interval	Mean Difference	*P*
	Baseline	0.07	0.868	Baseline	1.33	0.053
	3 Months	0.27	0.105	3 Months	1.53	0.006*
	6 Months	0.40	0.005*	6 Months	1.66	0.003*

**$P<0.001$ is highly significant *$P<0.05$ is significant

Table 5: Intragroup comparison showing mean and mean differences in bone defect depth of Group I and Group II at different intervals

Group	Assessment interval	Bone defect depth			
		Mean±SD	Mean Difference	*t*	*P*
Group I	Baseline	2.43±0.72	-	17.48	-
	6 Months	1.4±0.60	1.03		0.000**
Group II	Baseline	2.2±0.64	-	17.48	-
	6 Months	1.16±0.58	1.04		0.000**

**$P<0.001$ is highly significant *$P<0.05$ is significant

aids in healing by releasing fibronectin, PDGF, TGF-β, fibrin that control integrin expression, fibroblast proliferation, and migration, thus expecting rapid soft tissue healing and less postsurgical discomfort.

Table 6: Intergroup comparison showing mean differences in linear bone growth of Group I vs Group II at different intervals

	Assessment interval	Mean Difference	*P*	Mean Difference (Baseline – 6 Months) [A0 – A1]	*P*
Group I vs Group II	Baseline (A0)	0.23	0.362	-0.01	1.000
	6 Months (A1)	0.24	0.293		

Table 7: Intergroup comparison showing mean difference in percentage linear bone growth of Group I vs Group II at different time intervals

	Assessment interval	Mean±SD	Mean Difference	*t*	*P*
Group I	At 6 Months [A0 – A1]	44.58%±11.89%	-	-	0.000**
Group II		48.78%±11.13%	-		
Group I vs Group II			-4.20%	-0.998	0.327

***P*<0.001 is highly significant **P*<0.05 is significant

i-PRF is third-generation platelet concentrate. It consists of more platelets, leucocytes, endothelial cells, and stem cells, hence called as "blood concentrate" and not just a platelet concentrate.[13] Comparing PRF with iPRF, PRF has dense fibrin structure, whereas i-PRF being in liquid or gel form has loose structure, more interfibrous space, more uniform cell distribution, more platelets, and more growth factors in comparison with PRF.[14] iPRF releases significantly higher levels of long-term release of growth factors PDGF-AA, PDGF-AB, EGF, IGF-1 even after 10 days, playing an important and integral role in the formation of bone. Collagen I synthesis has been seen to be the highest in iPRF compared to other platelet concentrates.[15] Thus, better regenerative capacity is expected from iPRF than PRF.

According to the available data, this is the first reported study of using iPRF and DFDBA in intrabony defects, so direct comparisons with other studies are not possible. In the present study, statistically significant reduction in the PI and GI scores at 6 months in both the groups can be attributed to disruption of local factors in maintenance phase and positive patient motivation. On comparing the two groups, results were found to be statistically non-significant.

Significant reduction in PPD and the difference in RAL at 6 months in both the groups are similar to studies conducted in the literature when PRF and DFDBA were used in the treatment of intrabony defects.[2,16] However, Group II showed more statistically significant PPD reduction and RAL than Group I at 6 months.

Gain in linear bone height (LBG) in Group I and Group II at 6 months from baseline was statistically significant. Chandradas ND *et al*.[17] observed significantly greater gain in LBG at 9 months when PRF and DFDBA were used in the treatment of intrabony defects than PRF alone. David A. Mott *et al*.[18] had shown that during demineralization process of DFDBA, some of the growth factors are lost, thus rendering the graft incapable of inducing spontaneous osteogenesis. They showed that in-vitro osteoblasts proliferation rate increased when DFDBA was supplemented with growth factors like TGF, IGF, PDGF, bFGF, VEGF as compared to DFDBA alone. Thus, growth factors in platelet concentrates augmented the osteogenic capacity of DFDBA.

On intergroup comparison of gain in LBG, results were found to be statistically non-significant.

Percentage gain in LBG was found to be more in Group II than Group I, though the difference was statistically non-significant. Melek LN and El Said MM[19] observed significant mean gain in ridge width and height after 3 months when i-PRF and autogenous bioengineered tooth graft were used for the reconstruction of alveolar ridge after extraction.

Amaral Valladão C.A *et al*.[20] Lydia N. Melek and Maha R. Taalab[21] showed successful guided bone regeneration with bone grafts agglutinated with i-PRF at future implant placement sites. Increased osteogenic property of iPRF is also supported by an in-vitro study by Xuzhu Wang *et al*.[22] that showed threefold increase in migration and higher proliferation rate of cultured osteoblasts with i-PRF as compared to PRP.

i-PRF is a liquid platelet concentrate which forms a clot after sometime as a result of fibrin component and act as a dynamic hydrogel with uniform white blood cell and platelet aggregate distribution within this hydrogel.[23] When mixed with the particulate graft, it favors graft's integration, facilitates the graft's manipulation, stability, and improves its mechanical properties at the grafted area. i-PRF in association with a bone graft seems to be promising; however, more long-term randomized controlled clinical studies with a longer study period and a larger sample size are needed to substantiate its real clinical benefits.

CONCLUSION

The results revealed statistically significant improvements in (PPD) reduction and changes in (RAL) with DFDBA and

i-PRF (Group II) as compared to DFDBA and PRF (Group I). As percentage gain in LBG was found to be more in Group II as compared to Group I though the difference was statistically non-significant, it is concluded that i-PRF in combination with DFDBA gave better results. Considering the autologous nature, minimal cost, and time, iPRF can be incorporated as a regenerative material in intrabony defects.

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Conflicts of interest

There are no conflicts of interest.

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