

Evaluation of Effect of T-PRF Over L-PRF as an Adjunct to the Bone Graft (DFDBA) in the Treatment of Intra Bony Defects - A Randomized Controlled Clinical and Radiographic Trial

Palaparthi Raja Babu¹, Raghaveena Ingle², Suryakanth Malgikar^{3*}, Vidya Sagar S¹, Juliet Josephin J³ and Abhinav Atchuta³

¹Professor, Department of Periodontology, Kaloji Narayana Rao University of Health Sciences, Telangana, India

²Consultant Periodontist, Bengaluru, India

³Reader, Department of Periodontology, Kaloji Narayana Rao University of Health Sciences, Telangana, India

***Corresponding Author:** Suryakanth Malgikar, Reader, Department of Periodontology, Kaloji Narayana Rao University of Health Sciences, Telangana, India.

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Abstract

Background: Periodontal regenerative therapy is rapidly evolving, with a major shift from bio compatible material to human derived biomaterials. Demineralized Freeze Dried Bone Allograft (DFDBA), has both osteoinductive and osteoconductive property, hence is utilized in periodontal bone defects. To enhance the regenerative outcomes, various additives viz commercial growth factors, PRP, PRF etc. are adjunctively used along with bone grafts. Titanium-prepared, Platelet Rich Fibrin (T-PRF) is one such new concentrate used in regenerative surgery.

Aim of the Study: The aim of the present trial was to evaluate clinically and radiographically the efficacy of autologous L-PRF (Leukocyte Platelet Rich Fibrin) and T-PRF as an adjunct to DFDBA in the treatment of intrabony defects in chronic periodontitis patients.

Materials and Methods: 30 intrabony sites were randomly assigned into two treatment groups as group I (Control): DFDBA + L-PRF and group II (Test): DFDBA + T-PRF. Venous blood samples were collected and PRF obtained after centrifugation was used in combination with bone graft (DFDBA) in intra bony defects. Clinical parameters PPD and RAL and radiographic defect depth of randomly selected sites in group I and group II were recorded at baseline, 3rd, 6th and 9th months after therapy.

Results: Within each group, significant improvements ($P < 0.05$) were found for all variables in 9-month follow-up compared with baseline. Greatest reduction in the PPD of 60.66% and RAL gain of 1.6 mm was found in group II and radiographic bone gain of 3.07 mm (21.64%) at the end of 9th month in group II. Percentage changes of radiographic defect depth in both the groups were found to be significant from baseline to 9th month, greatest reduction of 3.07 (21.64%) was found in group II at 9th month interval whereas in group I it was 2.14 (14.53%).

Conclusion: Significant improvements in clinical and radiographic parameters indicate success of regenerative therapy using T- PRF over L-PRF with DFDBA.

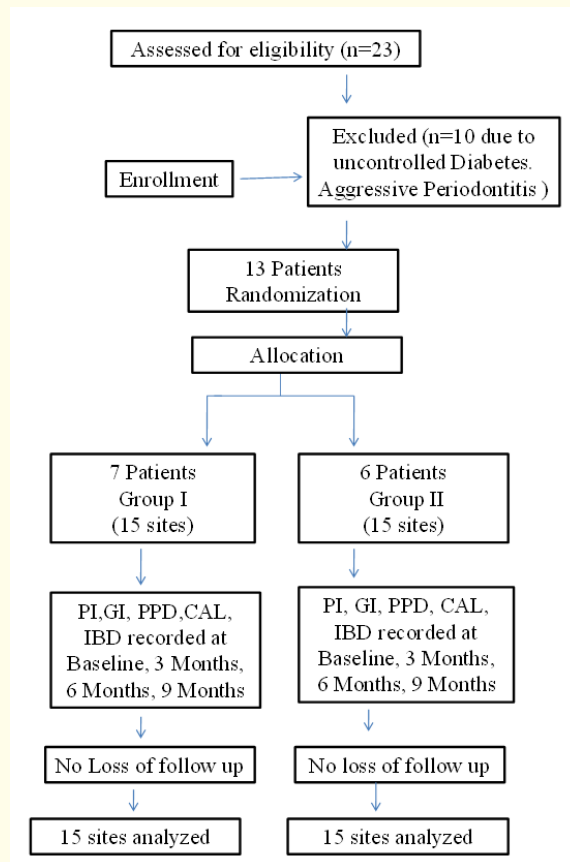
Keywords: Chronic Periodontitis; Periodontal Regeneration; Intrabony Defects; Platelet Rich Fibrin; Titanium - Platelet Rich Fibrin; Allograft; DFDBA

Introduction

The regeneration of periodontium requires biological events [1]. Regeneration of lost periodontal structures is the goal of periodontal therapy caused due to periodontal disease [2]. Several trials have used different grafting procedures in intrabony defects and have reported that the reconstruction of periodontal tissues is difficult to obtain [3] because of the limited capacity of the periodontium for regeneration [4]. Combined use of bone grafts, blood products like (PRF& PRP) and guided tissue regeneration have shown good results in periodontal regeneration simulating the progenitor cells to differentiate into periodontal ligament forming cells, cementoblasts or bone forming osteoblasts [5,6]. Platelet rich fibrin (PRF) is a second-generation platelet concentrate [7]. PRF described by Choukroun, *et al.* [8] can be obtained without any artificial biomodification. The PRF clot consist of strong natural fibrin matrix which contains all platelets and growth factors. Platelet rich fibrin clot produced in titanium test tubes was clinically identical to that produced in glass tubes but, activation of platelets with titanium provides a distinct characteristic including its increased biocompatibility [9].

Materials and Methods

The present single blinded randomized controlled clinical and radiographical trial was carried out at a single-centre to evaluate and compare the efficacy of Leukocyte- Platelet Rich Fibrin (L-PRF) as an adjunct to Demineralized Freeze Dried Bone Allograft (DFDBA) and Titanium- prepared Platelet Rich Fibrin (T-PRF) as an adjunct to Demineralised Freeze Dried Bone Allograft (DFDBA) in intrabony defects in chronic periodontitis patients. The institutional ethical committee approved the clinical study (Reg. No: IEC/2014/29). Helsinki's guidelines were followed.



Figure

Patient selection criteria

Untreated chronic periodontitis individuals were enrolled in the study. Chronic periodontitis was diagnosed if probing pocket depth \geq 5 mm and bone loss on radiography. Informed consent was obtained from the patients after explaining the procedure.

Inclusion criteria

- Individuals between the age 23 - 54 years.
- 20 permanent teeth should be present in the dentition.
- Atleast 1 site with periodontal pocket depth \geq 5 mm.
- Signed informed consent.
- Radiographic angular bone loss.

Exclusion criteria

- Individuals who are compromised with systemic disease.
- tooth mobility grade II and III.
- Furcation involvement grade III and IV.
- Pregnant women.
- lactating women.
- Subjects with previous history of periodontal treatment within 1 year.
- Aggressive periodontitis patients.

Sample size

Sample size was calculated using G power 3.1 software. 30 sites would be sufficient to achieve the required significance under 95% power with alpha error value set at 0.05.

Screening and examinations

Periodontal examination was done using UNC-15 probe for 13 patients with chronic periodontitis. At baseline, 3rd, 6th and 9th month clinical parameters Probing Pocket Depth (PPD) and Relative Attachment level (RAL) and radiographic defect depth of selected sites in group I and group II were recorded. Experienced periodontal examiner did all periodontal measurements. The probing angulation was standardized using an acrylic stent, on which a groove was marked representing the site chosen for the treatment based on the chart measurements earlier made on the patient. Measurements were done at selected sites and the reading was recorded to the nearest millimeter (Figure 1).



Figure 1: Pocket depth and clinical attachment recording using acrylic stent.

Randomization

Total 30 sites were selected according to inclusion and exclusion criteria and the selected sites were randomly divided by flipping the coin into two groups of 15 each:

1. Group I (Control): DFDBA + L-PRF.
2. Group II (Test): DFDBA + T-PRF.

All patients received thorough scaling and root planing. After baseline examination and recording of clinical parameters, all patients received routine oral hygiene instructions and full-mouth scaling and root debridement employing both hand instruments (Hu-Friedy, USA) and a piezoelectric ultrasonic hand piece (EMS) under local anaesthesia of 2% lidocaine with 1:80000 adrenaline until the operator achieved a hard, smooth and calculus free root surface.

Following materials were used in the study:

1. Surgical kit
2. Demineralised Freeze Dried Bone Allograft (DFDBA) from Tissue bank, TATA Memorial hospital, Mumbai.
3. Platelet Rich Fibrin (PRF) prepared from patient's own blood.

After 4 weeks evaluation was done and patients were scheduled for surgery.

PRF preparation

After taking informed consent of the patient, around 9 ml of whole venous blood was withdrawn from antecubital vein by Venipuncture and was collected in one 15 ml sterile test tube (either titanium test tubes (Figure 2a or glass test tubes) or 10 ml of whole venous blood was withdrawn and collected equally (5 ml each) in two sterile test tubes (either titanium test tubes for T-PRF or glass test tubes

for L-PRF) without anticoagulant and was immediately centrifuged in a centrifugal machine [Remi 8c (Figure 2b)] at 3500 revolutions per minute (RPM) for 12 minutes. A pipette was used to remove upper straw-colored layer and middle fraction i.e. the fibrin clot was removed from the tubes using sterile tweezers and was placed on sterile woven gauze, which is the PRF (Figure 2c). The T-PRF collection protocol in human subjects is similar to the conventional PRF protocol.



Figure 2: 2a: Titanium test tubes. 2b: Centrifuge (REMI 8C). 2c: PRF plug.

Surgical procedure

After anesthetizing the area, a sulcular incision was given and full-thickness mucoperiosteal flap was elevated. Thorough debridement was performed using Gracey curettes (Hu-Friedy, USA) and Demineralized Freeze-Dried Bone Allograft and Leukocyte- platelet rich fibrin were filled in the defects [Group I] or with Demineralized Freeze-Dried Bone Allograft and Titanium prepared platelet rich fibrin [Group II] according to randomly assigned groups. The flaps were approximated using a 3-0 (non-resorbable) black-braided silk suture. The operated area was covered with periodontal pack for 1 week postoperatively.

Maintenance program

All patients received (Amox 500 mg thrice daily) for 7 days and (Ketorolac DT twice daily) for 3 days. Periodontal dressing and Sutures were removed after one week.

Radiographic evaluation

Radiographic evaluation at the operated sites was done at baseline, 3rd, 6th and 9th month. A fixed reference point like cusp tip or the incisal edge to base of the defect was used to record radiographically. Long cone paralleling technique using film holders (RINN XCP™, DENTSPLY) was used. A special software (University of Texas Health Science Center at San Antonio UTHSCSA Image Tool™ to evaluate bone fill. A grid incorporated into the IOPA was used for standardization of the radiographs [10].

Statistical analysis

SPSS - software 20.00 program (SPSS Inc., Chicago, IL, USA) was used to analyze the data. Clinical parameters like probing pocket depth, relative attachment level and radiographic defect depth were compared between group I and group II at various study intervals

using dependent t test in intra group comparison and intergroup comparison was done by using independent t test. Statistically significant if $p < 0.05^*$.

Results and Discussion

Improvement in clinical and radiographic parameters (Table 1 and 2) like PPD when compared between the two groups at baseline to 3 months, 3 months to 6 months and 6 months to 9 months is 0.33 mm, 0.80 mm and 1.60 mm respectively, which was found to be statistically significant at the end of 9 months in the test group (Figure 3 and 4). Gain in RAL when compared between the two groups at baseline to 3 months, 3 months to 6 months and 6 months to 9 months is 0.34 mm, 0.47 mm, 0.80 mm respectively (Figure 5 and 6), which was statistically significant at the end of 9 months. Similarly, IBD (bone fill in mm) when compared between the two groups at baseline to 3 months, 3 months to 6 months and 6 months to 9 months is 0.06 mm, 0.59 mm and 0.27 mm respectively, which is statistically significant at the end of 9 months in favor for the test group (Figure 7-10).

Parameters	Baseline (Mean ± SD)			3 Months (Mean ± SD)			6 Months (Mean ± SD)			9 Months (Mean ± SD)		
	Group I	Group II	P- Value	I	II	P- Value	I	II	P	I	II	P- Value
PPD (mm)	7.40 ± 0.99	8.13 ± 1.06	0.0598	5.60 ± 0.99	6.00 ± 1.31	0.3526	4.47 ± 0.92	4.40 ± 0.74	0.8277	4.07 ± 0.92	3.20 ± 1.01	1.0188*
RAL (mm)	8.07 ± 1.22	8.67 ± 1.45	0.2303	6.33 ± 1.29	6.60 ± 1.64	0.6244	5.13 ± 1.25	4.93 ± 1.16	0.6530	4.73 ± 1.22	3.73 ± 1.10	0.0258*
IBD (mm)	14.72 ± 1.95	14.20 ± 2.07	0.4853	13.79 ± 1.98	13.20 ± 2.20	0.4524	12.98 ± 1.87	11.81 ± 2.03	0.1112	12.58 ± 1.85	11.12 ± 2.01	0.0487*

Table 1: Intergroup comparison of probing pocket depth, relative attachment level and intrabony defect depth (in mm).

*: $P < 0.005$ - Significant.



Figure 3: Probing depth baseline group I (DFDBA + L-PRF).

Parameters and groups	Baseline to 3 months			3months to 6 Months			6 Months to 9 Months		
	I	II	P- Value	I	II	P- Value	I	II	P- Value
PPD (mm)	1.80	2.13	0.3864	2.93	3.73	0.0562	3.33	4.93	0.0002*
RAL (mm)	1.73	2.07	0.3994	1.20	1.67	0.0787	0.40	1.20	0.0108*
IBD (mm)	0.93	0.99	0.7610	0.80	1.39	0.0599	0.41	0.68	0.0481*

Table 2: Clinical changes of probing pocket depth, relative attachment level and intrabony defect depth at various time intervals (in mm).
 *: P < 0.005 - Significant.

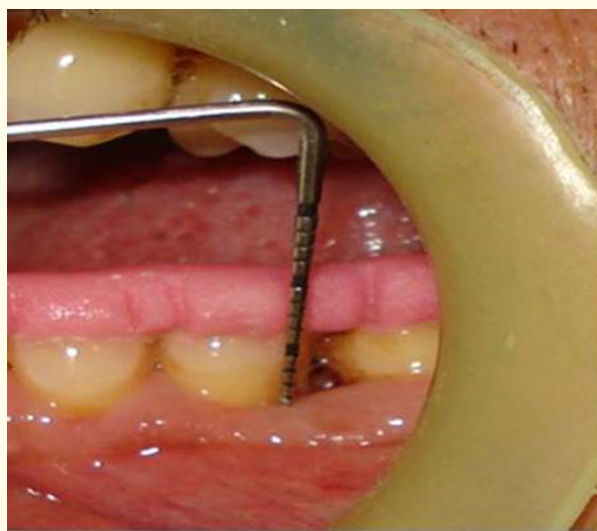


Figure 4: Probing depth 9 month group I (DFDBA + L-PRF).



Figure 5: Probing depth baseline group II (DFDBA + T-PRF).



Figure 6: Probing depth 9 month group II (DFDBA + T-PRF).

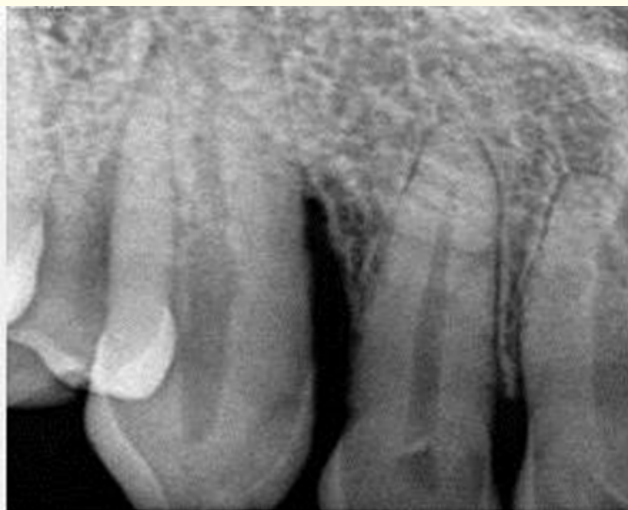


Figure 7: Radiographic defect depth (in mm) baseline group I (DFDBA + -PRF).



Figure 8: Radiographic defect depth (in mm) 9 month group I (DFDBA + L-PRF).



Figure 9: Radiographic defect depth (in mm) baseline group II (DFDBA + T-PRF).



Figure 10: Radiographic defect depth (in mm) baseline group II (DFDBA + T-PRF).

Discussion

Several studies have reported good results with L-PRF [11] but the silica activators in the glass tubes may be health hazard. To eliminate potential negative effects of silica from dry glass or glass-coated plastic tubes, modification was done of the initial L-PRF method by changing the structure of the tubes and by using a more biocompatible and hemocompatible material, titanium [11], which has highest strength-to-weight ratios and corrosion resistance among metals. The present study evaluated the clinical efficacy of T-PRF matrix in the treatment of a hard tissue defect. In our study, a significant reduction PPD and gain in RAL was found. Significant gain in radiographic bone fill in the periodontal intrabony defect was also observed.

A study by Ozdemir, *et al.* [12] evaluated testing the newly described autologous titanium platelet rich fibrin (T-PRF) platelet product in an animal model and investigated the effectiveness of T-PRF *in vivo* on wound healing in connective tissue. The study concluded that T-PRF could induce the formation of new bone connective tissue. Rummelhart, *et al.* [13] CAL gain was 1.7mm in our study RAL gain was 1.67 mm in group II at the end of 6 months which was not significant. The results of the present study between both group I and group II showed statistically significant results at the end of the 9th months study in reduction of probing pocket depth, gain in relative attachment level and radiographic bone fill. However greater improvement in soft and hard tissue parameters was seen in group II than group I.

PRF helps in osteoclastogenesis by promoting the secretion of osteoprotegerin in osteoblast cultures [14] it can also stimulate osteogenic differentiation of human dental pulp cells by upregulating osteoprotegerin and alkaline phosphatase expression [15]. PRF membrane stimulates Growth factors such as Transforming Growth Factor and Platelet-Derived Growth Factor for sustained release [16] up to 28 days [17] during remodeling. PRF not only increases cell attachment and proliferation [18] but also enhances OPG and ALP expression [19]. Hence, DFDBA and PRF combination is expected to have a synergistic action on the parameter of radiographic defect fill in the present study. This is the first study where T-PRF was used with DFDBA for periodontal intrabony defects.

Takemoto, *et al.* [20] correlated between titanium oxide layers on oxidized titanium (Ti) substrates and platelet adhesion in their study. Histological structure of T-PRF in the literature has shown that fibrin of T-PRF is more tightly woven and thicker than that of the classic L-PRF. According to the properties of the titanium by Tunali, *et al.* [9] titanium prepared PRF is 3rd platelet concentrate, and titanium tubes may be more effective at activating platelets than glass tubes used in preparation of platelet rich fibrin by Choukroun. Titanium-prepared platelet rich fibrin (T-PRF) is more effective at activating platelets, when compared with L-PRF. The adjunctive use of DFDBA + T-PRF could improve clinical and radiographical parameters as T-PRF could induce the formation of new bone with new connective tissue attachment.

Conclusion

Within the limitations of the study it can be concluded that using T-PRF over L-PRF, the additional benefit in PPD reduction, RAL gain and radiographic defect depth reduction or bone fill at the end of 9th month.

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Conflict of Interest

No conflict of interest.

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