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ORIGINAL ARTICLE

Platelet rich fibrin combined with decalcified freeze-dried bone allograft for the treatment of human intrabony periodontal defects: a randomized split mouth clinical trail

ASHISH AGARWAL[1], NARINDER DEV GUPTA[2] & AVIKAL JAIN[1]

[1]*Deparment of Periodontics, Institute of Dental Sciences, Bareilly, India, and* [2]*Department of Periodontics, Dr. Z. A. Dental College, Aligarh, India*

Abstract

Objective. Polypeptide growth factors of platelet rich fibrin (PRF) have the potential to regenerate periodontal tissues. Osteoinductive property of demineralized freeze-dried bone allograft (DFDBA) has been successfully utilized in periodontal regeneration. The aim of the present randomized, split mouth, clinical trial was to determine the additive effects of PRF with a DFDBA in the treatment of human intrabony periodontal defects. ***Materials and methods.*** Sixty interproximal infrabony defects in 30 healthy, non-smoker patients diagnosed with chronic periodontitis were randomly assigned to PRF/DFDBA group or the DFDBA/saline. Clinical [pocket depth (PD), clinical attachment level (CAL) and gingival recession (REC)] and radiographic (bone fill, defect resolution and alveolar crest resorption) measurements were made at baseline and at a 12-month evaluation. ***Results.*** Compared with baseline, 12-month results indicated that both treatment modalities resulted in significant changes in all clinical and radiographic parameters. However, the PRP/DFDBA group exhibited statistically significantly greater changes compared with the DFDBA/saline group in PD (4.15 ± 0.84 vs 3.60 ± 0.51 mm), CAL (3.73 ± 0.74 vs 2.61 ± 0.68 mm), REC (0.47 ± 0.56 vs 1.00 ± 0.61 mm), bone fill (3.50 ± 0.67 vs 2.49 ± 0.64 mm) and defect resolution (3.73 ± 0.63 vs 2.75 ± 0.57 mm). ***Conclusion.*** Observations indicate that a combination of PRF and DFDBA is more effective than DFDBA with saline for the treatment of infrabony periodontal defects.

Key Words: *Chronic periodontitis, intrabony defect, periodontal regeneration, platelet rich fibrin*

Introduction

Various regenerative modalities have been investigated for the management of intrabony periodontal defects, e.g. Bone grafts (BG) and substitutes, guided tissue regeneration, growth factors, enamel matrix derivatives and combined approaches [1]. Decalcified freeze-dried bone allograft (DFDBA) contains bone morphogenetic proteins (BMPs) that aid in mesenchymal cell migration, attachment and osteogenesis; have both osteoinductive as well as osteoconductive activity and the ability to create and maintain the space. DFDBA has been proposed as an effective regenerative material for osseous defects [2–4].

Polypeptide growth factors (PGFs) revealed a potential application in wound healing by promoting periodontal regeneration via cell proliferation, angiogenesis, chemotaxis and differentiation. Autologous blood concentrates constitute a safe and convenient approach to deliver high concentrations of PGFs to periodontal surgical wounds [5,6].

Recent studies examining the effectiveness of PGFs, used alone or in combination with other materials and techniques, have been conducted with autologous platelet-rich plasma (PRP) [7–9], recombinant platelet-derived growth factor [10,11] and platelet rich fibrin (PRF) [12,13]. Among platelet concentrates, PRF belongs to a group of second-generation blood autologous preparations that was originally described by Choukroun et al. [14]. The PRF preparation process creates a gel like matrix that contains high concentrations of non-activated, functional, intact platelets contained within a fibrin matrix that release a relatively constant concentration of growth factors over a period of 7 days [15,16].

Dohan Ehrenfest et al. [17,18] demonstrated that PRF induced a significant and continuous stimulation and proliferation of human primary cultures of

Correspondence: Dr Ashish Agarwal, Senior Lecturer, Department of Periodontics, Institute of Dental Sciences, Pilibhit Bypass Road, Bareilly 243006, India. Tel: +91 94 5344 2418. E-mail: drashish.aag@gmail.com

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gingival fibroblasts, dermal pre-keratinocytes, pre-adipocytes and maxillofacial osteoblasts.

Keeping the above facts in mind, the addition of PRF to DFDBA may enhance periodontal regeneration as compared with those sites treated with BG alone. At present, to the authors' knowledge, there are very few published clinical controlled trials on PRF that compare the results of PRF with DFDBA to the outcomes of DFDBA alone in the treatment of infrabony periodontal defects. Therefore, the present study was conducted for testing the hypothesis that PRF would augment the regenerative effects of DFDBA in human intrabony defects.

Materials and methods

Thirty-two pairs of infrabony periodontal defects in 32 patients (18 men and 14 women, mean age = 52 ± 7 years), suffering from moderate-to-severe chronic periodontitis, were selected for the study at the outpatient Department of Periodontics, Dr Z. A. Dental College, Aligarh, India. The study was conducted from April 2010 to January 2013. The research was designed as a randomized, double-blinded, parallel, controlled clinical trial that employed a split-mouth design for the comparison of periodontal outcomes using DFDBA/saline and DFDBA/PRF preparation in the treatment of intrabony periodontal defects.

The inclusion criteria were the presence of a matched pair of interproximal, intrabony defects with probing depth (PD) ≥ 6 mm when evaluated 8 weeks after phase I therapy with defect depth ≥ 4 mm, in asymptomatic posterior teeth. Osseous defects needed to have two and/or three walls. The plaque and gingival indices, associated with interested tooth, achieved following re-evaluation of initial therapy had to be ≤ 1. Radiographic evidence of intrabony defects had to exist as revealed by periapical films taken with the long-cone parallel technique. The exclusion criteria for the study were the presence of any systemic disease, patients taking any medication, pregnancy or lactation, smokers, previously treated for periodontal reasons, one-wall defects and furcation involvement.

The study protocol, risks, benefits and procedures were explained and written informed consent was obtained from every patient. The study was approved by the ethical committee of Dr Z. A. Dental College, Aligarh and all the examinations, treatment and procedures of this study followed the Declaration of Helsinki of 1975, as revised in 2000.

Initial therapy

Initial therapy consisted of detailed instructions regarding proper oral hygiene measures followed by full mouth scaling and root planing using hand and ultrasonic instruments under local anesthesia. Eight weeks following phase I therapy, a periodontal re-evaluation was performed to confirm the suitability of the sites for this periodontal surgical study. The study used a split-mouth design, in which two interproximal sites were randomly (toss of a coin, performed by the study therapists) assigned to the DFDBA with saline or DFDBA with the PRF group. One operator (AA) performed all the surgeries, whereas another operator (NDG) performed all the clinical and radiographic measurements without knowledge of the groups.

Pre-surgical clinical measurements

Clinical parameters recorded before the surgical procedures and at 12 months post-operatively included PD [measured from the gingival margin to the base of the pocket (tip of the probe in the pocket)], clinical attachment level (CAL), measured from the cementoenamel junction (CEJ) to the base of the pocket (tip of the probe in the pocket) and gingival recession (REC, measured from the CEJ to the level of the gingival margin), using customized acrylic stents with grooves to ensure a reproducible placement of the University of North Carolina no. 15 (UNC-15, Hu Friedy, Chicago, IL) periodontal probe. Plaque index (PI) [19] and modified sulcus bleeding index (mSBI) [20] were also measured.

Radiological assessments

Pre-operatively and 12 months post-operatively, intra-oral standardized radiographs were taken for the evaluation of radiographic bone level (RBL) using the paralleling technique and an individual film holder consisting of a bite block rigidly connected to an acrylic dental splint to achieve identical film placement at each evaluation. The differences between pre- and post-operative RBL measurements were considered as the radiographic bone loss/gain. Radiographic measurements were done as (1) distance from the CEJ to the deepest point of the vertical bone defect (BD), (2) distance from the CEJ to the alveolar crest (AC) and (3) distance from the AC to BD (Figures 1 and 2). Measurements were obtained utilizing an adhesive millimeter grid (Meyer-Haake, Oberursel, Germany).

The most coronal area where the periodontal ligament (PDL) maintained an even width was identified to measure the most apical extension of the defect. The crossing of the silhouette of the alveolar crest with the root surface was defined as the alveolar crest. The differences between 12-month and baseline values of CEJ-BD indicated the amount of bone fill. The differences between CEJ-AC and AC-BD were identified as the amount of crestal bone resorption and as the resolution of intrabony defect, respectively.

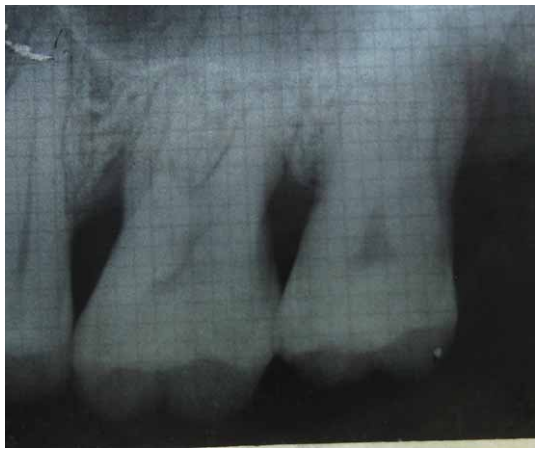


Figure 1. Pre-operative radiograph.

PRF preparation

The PRF was produced according to the protocol developed by Choukroun et al. [14]. On the day of surgery, 10 ml of blood was drawn from each patient by venipuncture of the antecubital vein. Blood was collected in a sterile glass test tube without any anti-coagulant. The test tube was immediately centrifuged using a refrigerated centrifugal machine at 400 g for 12 min. Because of differential densities, it resulted in the separation of three basic fractions: a base of red blood cells at the bottom, acellular plasma on the surface and, finally, a PRF clot between the two. The top layer was pipetted out with the sterile dropper; the middle layer (PRF) was removed and placed in a sterile dappen dish. This clot was either minced into small pieces and mixed with graft material or pressed between two sterile compresses to obtain a membrane.

Surgical procedure

Following administration of local anesthesia, buccal and lingual sulcular incisions were made and the mucoperiosteal flaps were elevated. Care was taken to preserve as much inter-proximal soft tissue as possible. Complete debridement of the defects, as well as scaling and root planing to ensure root smoothness, were achieved with the use of an ultrasonic device and hand instruments.

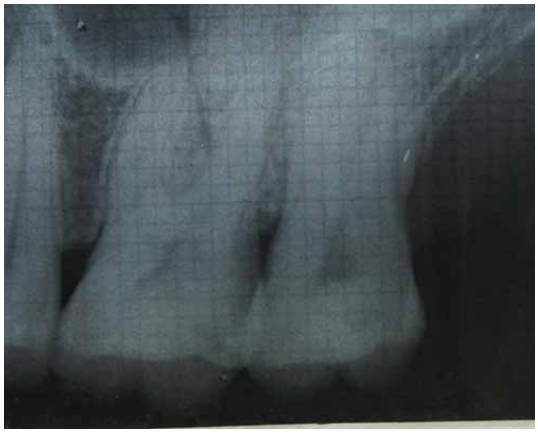


Figure 2. 12 months post-operative radiograph.

DFDBA (LifeNet Health, Virginia Beach, VA) was mixed with PRF or saline at a proportion of 1:1 (v/v) and filled into the defect to the same level as the most coronal existing bony defect wall during the surgical procedures according to treated group. Care was taken not to overfill defects. A membrane of compressed PRF was trimmed and adapted over the grafted defects in both of the groups. Membranes were extended over the periphery of the defect in the buccal and lingual directions and secured in place using 5–0 gut sutures anchored to the adjacent teeth. Flaps in both groups were repositioned and sutured with 3–0 non-absorbable black silk surgical suture (Ethicon, Johnson & Johnson, Somerville, NJ) using an interrupted technique (Figures 3, 4, 5 and 6). Periodontal dressing was placed over the surgical area; 500 mg amoxicillin, three times daily for 7 days; 800 mg ibuprofen, three times daily were prescribed, along with chlorhexidine digluconate (CHX) rinses (0.12%) twice daily for 2 weeks.

Post-operative follow-up care

Periodontal dressing and sutures were removed 2 weeks post-operatively. Surgical wounds were gently cleansed with 0.2% CHX on a cotton swab. Thereafter, gentle brushing with a soft toothbrush was recommended. At 8 weeks post-operatively, each patient was reinstructed about proper oral hygiene measures. Patients were examined weekly for 1 month after surgery and then at 3, 6 and 9 months. Post-operative care included reinforcement of oral hygiene and mechanical plaque control whenever necessary.

Post-surgical measurements

PI, SBI, PD, CAL and REC were recorded 12 months after the initial surgery. Soft tissue measurements were repeated with previously used acrylic stents. A second IOPA (after 12 months) of the same treated sites was taken and radiographical measurements were performed from the baseline and 12-month radiographs.

Primary and secondary outcome measures

The primary outcome measure of the study was CAL gain and secondary outcomes included PD, defect resolution, bone fill, PI and mSBI.

Statistical analysis

The results were averaged (mean ± SD) for each clinical and radiographic parameter at baseline and

Table I. Demographical data of the study.

Characteristics	DFDBA with PRF (30 sites in 15 patients)	DFDBA with saline (30 sites in 15 patients)
Male	10	7
Female	5	8
Osseous wall		
3 wall defects	7	9
2 wall defects	6	3
Combined 2 and 3 wall defects	17	18
Teeth treated		
Mandibular premolars	3	4
Maxillary premolars	8	8
Maxillary molars	10	8
Mandibular molars	9	10

12 months. The difference between each pair of measurements was calculated (baseline–12 months). The paired t-test was applied to assess the statistical significance between time points within each group for the clinical and radiographic parameters. Inter-group comparison was made by unpaired t-test. The Chi-square test was used for the assessment of frequency distribution between groups. Statistical significance was set at $p < 0.05$. Power calculations were performed before the study was initiated. To achieve a 90% power and detect mean differences of 1 mm for clinical parameters (PD, CAL, bone fill) between groups, 25 sites per group were required. The mean intra-examiner standard deviation of differences in repeated PD measurements and CAL measurements was obtained using single passes of measurements with a periodontal probe (correlation coefficients between duplicate measurements; $r = 0.95$).

Results

A total of 30 of 32 patients completed the study, while two patients (four sites) did not return for follow-up examinations. The type and number of teeth and bone defects evaluated are shown in Table I. Over the course of the study, there were no infectious episodes and no other adverse complications in any treatment site. During the study period the oral hygiene level and the number of bleeding sites remained stable or improved with respect to the values detected at baseline.

At the 12-month examination (Table II) statistically significant treatment effects were observed in both groups in terms of clinical and radiographical parameters ($p < 0.001$). On inter-group comparison, there was a statistically significant greater PPD reduction, CAL gain, REC, defect resolution and bone fill; while there was a non-significant reduction in alveolar crest resorption, PI and BOP were determined in DFDBA + PRP (Table III).

Table II. Clinical and radiographic measurements (in mm; mean ± SD) at baseline and 12 months (n = 30 for DFDBA/saline and PRF/DFDBA group).

	Baseline	12 months	Change	p-value
DFDBA/saline group				
PPD	7.12 ± 0.78	3.52 ± 0.79	3.60 ± 0.51	< 0.001*
CAL	8.18 ± 0.99	5.57 ± 1.17	2.61 ± 0.68	< 0.001*
REC	1.07 ± 0.41	2.07 ± 0.86	–1.00 ± 0.61	< 0.001*
CEJ-AC	4.03 ± 1.21	4.30 ± 1.30	–0.26 ± 0.25	< 0.001*
AC-BD	5.20 ± 0.71	2.45 ± 0.63	2.75 ± 0.57	< 0.001*
CEJ-BD	9.23 ± 1.30	6.75 ± 1.28	2.49 ± 0.64	< 0.001*
PI	0.62 ± 0.22	0.58 ± 0.23	0.03 ± 0.10	< 0.001*
BOP	0.95 ± 0.27	0.89 ± 0.30	0.07 ± 0.15	0.02
PRF/DFDBA group				
PPD	7.13 ± 0.88	2.98 ± 0.46	4.15 ± 0.84	< 0.001*
CAL	8.18 ± 1.04	4.45 ± 0.80	3.73 ± 0.74	< 0.001*
REC	1.05 ± 0.46	1.52 ± 0.60	–0.47 ± 0.56	< 0.001*
CEJ-AC	4.17 ± 1.29	4.40 ± 1.34	–0.23 ± 0.25	< 0.001*
AC-BD	5.32 ± 0.69	1.58 ± 0.49	3.73 ± 0.63	< 0.001*
CEJ-BD	9.50 ± 1.29	6.00 ± 1.15	3.50 ± 0.67	< 0.001*
PI	0.63 ± 0.20	0.60 ± 0.24	0.03 ± 0.11	< 0.001*
BOP	1.01 ± 0.27	0.98 ± 0.26	0.03 ± 0.08	0.03

*$p < 0.001$; highly significant.

The frequency distribution table (Table IV) shows more CAL gain and bone fill for the PRF associated group than the DFDBA with saline. For PRF/

Table III. Inter-group comparison of clinical and radiographical parameters (mean ± SD) from baseline to 12 months after treatment.

	DFDBA/saline (changes in 12 months)	PRF/DFDBA (changes in 12 months)	p-value
	Mean	Mean	Mean
PPD	3.60 ± 0.51	4.15 ± 0.84	< 0.05**
CAL	2.61 ± 0.68	3.73 ± 0.74	< 0.001*
REC	–1.00 ± 0.61	–0.47 ± 0.56	0.001*
CEJ-AC	–0.26 ± 0.25 (–6.8 ± 7.6)%	–0.23 ± 0.25 (–6.4 ± 9.9)%	0.613NS
AC-BD	2.75 ± 0.57 (53.0 ± 8.5)%	3.73 ± 0.63 (70.3 ± 8.3)%	< 0.001*
CEJ-BD	2.49 ± 0.64 (27.2 ± 7.3)%	3.50 ± 0.67 (37.2 ± 7.1)%	< 0.001*
PI	0.03 ± 0.10	0.03 ± 0.11	0.885NS
BOP	0.07 ± 0.15	0.03 ± 0.08	0.320NS

**$p < 0.05$; significant; *$p < 0.001$; highly significant.
NS, non-significant.

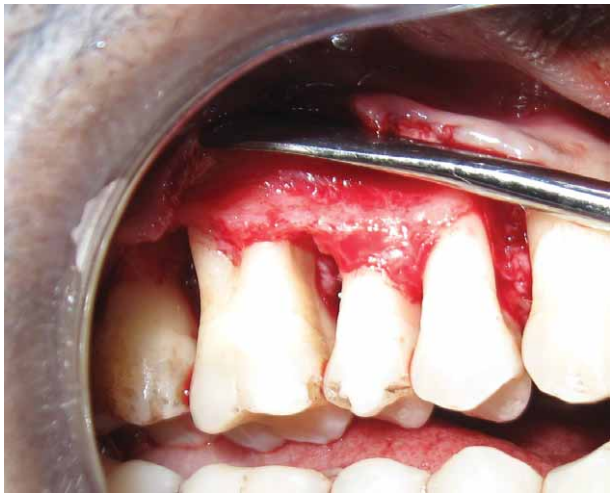
Table IV. Frequency distribution of clinical attachment gain and bone fill.

CAL change	Control group n (%)	Test group n (%)
≤ 2 mm	10 (33.3%)	—
> 2–3 mm	14 (46.7%)	9 (30%)
> 3–4 mm	6 (20%)	14 (46.7%)
> 4 mm	—	7 (23.3%)
CEJ-BD change (bone fill)		
≤ 2 mm	11 (36.7%)	—
> 2–3 mm	16 (53.3%)	12 (40%)
> 3–4 mm	3 (10%)	14 (46.7%)
> 4 mm	—	4 (13.3%)

DFDBA, most of the sites [14 (46.7%), seven (23.3%)] showed > 3–4 mm and > 4 mm of CAL gain. For DFDBA/saline, 14 (46.7%) and seven (23.3%) sites observed > 2–3 mm and > 3–4, respectively, while no site involved > 4 mm of CAL gain.

Discussion

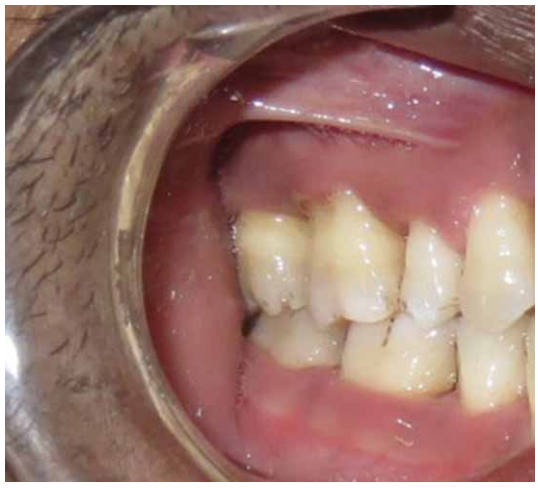
The present study was designed to evaluate PRF as an adjunct to DFDBA for the management of human periodontal infrabony defects. All baseline parameters were recorded with non-significant differences in both groups. The uneventful healing in all the sites was in agreement with previous studies [9,13,21], thus supporting the excellent properties of involved biomaterials for periodontal wound healing. Plaque accumulation and smoking are important factors that were shown to significantly influence the outcomes of regenerative periodontal surgery [22,23]. Because the present study excludes smokers and the patients maintained an acceptable oral hygiene throughout the study, therefore, it may be assumed that the careful patient selection was also responsible for the positive outcomes obtained in both groups.



Outcomes of this clinical trial have suggested that both treatment groups presented with significant improvement in clinical and radiographical parameters between baseline and 12 months. However, on inter-group comparison, DFDBA + PRP treatment showed significant advantages in terms of PPD, CAL, REC, alveolar crest resorption, defect resolution and bone fill.

In a previous study [9], DFDBA/saline demonstrated similar improvement in PD, CAL and defect fill, but more defect resolution than in the present study. Bender et al. [24] evaluated lesser PD reduction, CAL gain and bone fill, while greater mean percentage bone fill (37.0 ± 18.7%) and percent defect resolution (47.2 ± 25.3%). Improvement in clinical parameters (PD and CAL) of a recent trial [25] were less in magnitude as compared to the present study; possible reasons could be involvement of smokers and one wall defects. The data of our previous study [26] concluded similar changes in parameters as in the present study, despite involving non-contained one wall defects.

For PRF + BG, recent histological studies showed more benefits during bone healing in comparison to BG alone during augmentation in maxillary sinus, as revealed in this trial [27,28]. Bolukbasi et al. [29]



observed a histomorphometric increase in bone formation with the addition of PRF to BG for bone regeneration in surgically created bone defects in sheep tibia. Simon et al. [30] demonstrated that sites treated with platelet-rich fibrin matrix and DFDBA healed faster than those grafted with DFDBA and a membrane during clinical and histological comparison for 12 weeks of extraction socket healing. The present observations are not corroborated by a recent trial that proposed no additive benefits of PRF with BG for maxillary sinus augmentation [31]. During correlation of the results of above studies it should keep in mind that bone healing in infrabony periodontal defect is different as compared to mentioned bony cavities due to the chance of oral fluid contamination, lack of exact standardization, presence of less osteoprogenitor cells, and adjacent avascular tooth surface.

Scanty data is available regarding the use of autologous PRF in combination with BG in the treatment of infrabony defects. Thus, a direct comparison with other studies was not much. As similar to PRF, the first generation autologous platelet preparation (PRP) also supplies an elevated concentration of polypeptide growth factors at the surgical site. While monitoring changes in clinical and radiographic measurements in PRP with BG associated randomized trials [32], diverse inferences have been reported with respect to the present findings.

The recent findings with surgical re-entry support our results in terms of significant improvement with PRF/BG associated group in intrabony defects after 6 months [13]. In our previous study DFDBA + PRP showed lesser improvement after 12 months in PD (3.65 ± 0.63 mm), CAL (3.15 ± 0.50 mm), REC (–0.54 ± 0.59 mm), crestal resorption (–0.27 ± 0.25 mm), defect fill (3.02 ± 0.50 mm) and defect resolution (3.29 ± 0.53 mm) than DFDBA + PRF in the present study; the difference is more likely due to involvement of non-contained osseous defects in that study [26]. Piemontese et al. [9] determined more improvement for DFDBA + PRP in PD (4.6 ± 1.3 mm), but less in CAL (3.6 ± 1.8 mm), REC (–1.0 ± 1.3 mm), crestal resorption (–0.3 ± 1.3 mm), defect resolution (3.6 ± 1.7 mm) and bone fill (3.3 ± 1.5 mm).

Trials with no additive advantages of PGFs with bone graft in bony defects are also present in the literature. Choi et al. [33] histologically suggested that addition of PRP to autogenous bone graft retards new bone formation in osseous defects after 6 weeks of post-operative period. Variations in the PRP concentrations might influence the bone formation within the PRP-treated bone grafts. Growth factors present in high concentrations at inappropriate times or for an extended duration can adversely affect cell behavior. Viability and proliferation of alveolar bone cells are suppressed by high PRP concentrations, but are stimulated by low PRP concentrations [34].

Combining PRF with DFDBA resulted in significantly greater improvement in PD, CAL, REC, defect resolution and defect fill than DFDBA used with saline. However, the findings observed between the two treatment groups could not be attributed to significantly different resorption of alveolar crest, PI and BOP. Therefore, the differences in parameters observed between the two treatment groups in this trial can be explained due to the use of PRF with a high degree of certainty. The clinical superiority of the PRF involvement can also be confirmed by the frequency distribution data of CAL gain and bone fill supporting an additional significant benefit in terms of periodontal regeneration.

Magnitude of regenerative outcomes of other regenerative technologies like guided tissue regeneration, enamel matrix derivatives, combined approaches and PRP are comparable to the results of our modalities [35]. PRF is a biocompatible, bioresorbable, three-dimensional polymerized fibrin meshwork in which the platelet, leukocytes, cytokines, growth factors (such as transforming growth factor-β1, platelet-derived growth factor, vascular endothelial growth factor) and matrix glycoproteins (such as thrombospondin-1) are trapped and may be delivered for a certain time to play an essential role in wound repair, and provides a matrix for migration of tissue-forming cells like fibroblasts and endothelial cells, which are involved in angiogenesis and are responsible for re-modeling of the new tissue [36]. In vitro, PRF significantly improved proliferation of human osteoblasts in a dose-dependent manner and the expression of alkaline phosphatase activity was enhanced in a time-dependent manner with PRF [37]. The enhancement of phosphorylated extracellular signal-regulated protein kinase, osteoprotegerin and ALP may provide benefits for periodontal regeneration [38]. PRF entraps circulating stem cells due to which it leads to superior healing of large osseous defects where there is

migration of stem cells differentiating into the osteoblast phenotype [27].

The formability into the membrane, no requirement of bovine thrombin or other exogenous activators in the preparation process, slow release of growth factors during ≥ 7 days, antibacterial effects due to the presence of leukocytes, easy and fast processing as well as inexpensive chair side preparation make PRF the material of choice over other platelet concentrates [39]. On the other hand, the short shelf-life can be considered as a drawback of PRF. PRF membrane should be used immediately after preparation as it will shrink, resulting in dehydration and altering the structural integrity of PRF. Dehydration also results in the decreased growth factor content in PRF and leukocyte viability will be adversely affected, altering its biologic properties [37].

In clinical practice, pure two or three wall angular defects are uncommon, whereas the majority of the defects are present in combination manner [40]. Defects treated in this study were two wall, three wall or a combination of two and three wall defects. Regenerative potential of vertical defects depends on the defect topography. Therefore, due to the inherently low bone fill character, one wall defects have been excluded from the present study.

Heterogeneous regenerative outcomes are present in DFDBA-related previous studies due to variation in osteoinductive potential. Possible reasons for this could be the insufficient amount of bone morphogenic proteins and the inactivity of BMPs. Researchers have demonstrated that different preparations of allograft material, both from inter and intra distributors, may have different biological activity [41]. Assuming that the processing methods are standardized at individual bone banks, the fact that there was considerable variation in bone-induction ability among the samples suggests that there is an underlying difference in the inherent capacity of the bone to promote osteogenesis. These differences may be a function of donor age, previous exposure of pathology and/or drug therapy or genetic variation [41].

In conclusion, for human periodontal infrabony defects, addition of PRF to DFDBA significantly enhanced the regenerative output obtained compared with bone graft alone.

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Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

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