Clinical and radiographic evaluation of advanced platelet rich fibrin block compared to injectable platelet rich fibrin with nanohydroxyapatite in the management of periodontal intrabony defects: A randomized controlled trial

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Abstract. Periodontitis is a biofilm-induced inflammatory disease that leads to the destruction of periodontal tissues and eventual tooth loss. Mechanical therapy alone is often insufficient in managing deep intrabony defects, necessitating regenerative approaches. The present randomized controlled clinical trial aimed to compare the clinical and radiographic outcomes of advanced platelet-rich fibrin (A-PRF) block combined with injectable platelet-rich fibrin (i-PRF) and nanohydroxyapatite (nHA) in the treatment of periodontal intrabony defects. A total of 26 patients with stage III periodontitis presenting with three-walled intrabony defects were randomly allocated by coin toss into either the A-PRF block (test) group or the i-PRF + nHA (control) group. Clinical parameters, including probing pocket depth (PPD), relative clinical attachment level (RCAL), plaque index (PI) and gingival index (GI), as well as radiographic parameters, including intrabony defect fill and radiographic linear defect depth (RLDD), were assessed at baseline and at 6 months. At 6 months, the A-PRF block group demonstrated greater reductions in PPD (P<0.001), greater RCAL gain (P<0.001), greater defect fill (P<0.001) and a greater RLDD reduction (P=0.004) compared with the control group. Radiographically, the test group exhibited a significantly greater defect fill and reduction in defect depth. No significant differences were observed in PI and GI between the groups. Within the limitations of the study, the A-PRF block demonstrated superior clinical and radiographic outcomes compared with i-PRF combined with nHA, suggesting its potential as an effective regenerative modality for periodontal intrabony defects.

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Abbreviations: A-PRF, advanced platelet-rich fibrin; BMP, bone morphogenic protein; CBCT, cone beam computed tomography; GI, gingival index; i-PRF, injectable platelet rich fibrin; IDF, intrabony defect fill; IQR, interquartile range; L-PRF, leucocyte platelet rich fibrin; LSCC, low-speed centrifugation concept; nHA, nanohydroxyapatite; NcHA, nanocrystalline hydroxyapatite; OFD, open flap debridement; PRF, platelet-rich fibrin; PRP, platelet-rich plasma; PPD, probing pocket depth; PI, plaque index; RCAL, relative clinical attachment level; RLDD, radiographic linear defect depth; UNC-15, University of North Carolina-15 probe

Key words: periodontal regeneration, bone regeneration, intrabony defects, platelet-rich fibrin, nanohydroxyapatite graft, CBCT

Introduction

Periodontitis is an inflammatory disease that is caused by bacterial microorganisms, resulting in the destruction of the periodontium, which eventually leads to tooth loss. It is a complex infectious disease resulting from the response of the host to bacterial challenge (1). The biofilm and microflora present in dental plaque play a major role in the progression of periodontitis. Therefore, to reduce the microbial load, periodontal therapy is implemented, which mechanically removes the supra- and subgingival microbial deposits from the tooth and root surfaces (2). However, non-surgical periodontal therapy alone fails to remove pathogens from deep intrabony defects, leading to persistence of deep periodontal pockets and progression of bony destruction (3,4). Deep periodontal pockets are usually accompanied by angular intrabony defects where the base of the defect is apical to the surrounding bone. Intrabony defects can be classified as one-, two- or three-walled defects or combination defects depending on the number of osseous walls remaining around the pocket (5). Of these, two or three wall defects are most favorable for regenerative procedures (6).

Periodontal therapy aims to restore the lost structures of the periodontium through regeneration (7). Several therapeutic approaches have been developed to promote periodontal tissue regeneration, including the use of autogenous bone grafts and their substitutes, guided tissue regeneration employing barrier membranes alone, and combination therapies incorporating growth factors such as platelet-rich fibrin (PRF) (8-10).

In bone regenerative procedures, bone grafts of autogenous origin remain the gold standard. Bone cell precursors present in the graft are osteogenic without inducing any adverse immunological response (11). The drawbacks of using autografts include an increase in the morbidity of the donor site and limited availability (9). These considerations have led to the introduction of alternative bone substitutes that are biocompatible, noninfectious and nonantigenic with good osteogenic potential (12). Recently, a new class of bone grafts with nanosized particles was introduced that demonstrated improved osteointegrative properties (13). Nanohydroxyapatite (nHA; SyboGraf® HA 6.1), a calcium phosphate-based bone substitute, exhibits favorable osteoconductive properties and supports bone regeneration (14). The high specific surface area and small size of nanoparticles increase osteoblastic adhesion and calcium deposition. It is biodegradable and resorbs slowly, allowing it to maintain space and support new bone growth over an extended period (15).

Among the various regenerative techniques, concentrated platelet products rich in transforming growth factors and platelet-derived growth factors have demonstrated superior potential for enhanced wound healing and regeneration. Numerous platelet concentrates have been developed, including platelet-rich plasma (PRP) and 2nd generation platelet concentrates, such as PRF (16). These concentrates form a membrane that is enriched with cells, such as platelets and leukocytes, which play a crucial role in regeneration and wound healing (17).

Various modifications to the centrifugation technique have led to the creation of different types of platelet concentrates. Forms, such as leukocyte-rich PRF, advanced PRF (A-PRF) and injectable PRF (i-PRF) are among the commonly employed platelet concentrates (18). The A-PRF proposed by Ghanaati *et al* (19) in 2014 was based on a low-speed centrifugation technique (1,500 rpm, 14 min), which resulted in the formation of a loose fibrin clot with increased cell density and even distribution of platelets, cytokines and neutrophilic granulocytes.

Furthermore, a liquid formulation of PRF (namely i-PRF) was proposed by Miron *et al* (20) in 2017, which clots outside the PRF tube. i-PRF contains a higher concentration of platelets and leukocytes, making it a rich source of growth factors. These are biologically active substances that are essential to encourage tissue regeneration through the movement and replication of osteoprogenitor cells. Furthermore, i-PRF is in a liquid state; thus, it facilitates gradual and extended growth factor release, making its regenerative effects improve with time.

Of note, two forms of PRF, namely i-PRF and A-PRF, were mixed together with nHA bone graft material to form a cohesive regenerative matrix termed the A-PRF block. This synthesis is a three-dimensional construct able to fit in the shape of bony defects (21,22). The biological activation of the nHA component by the presence of platelets in the block increases its regenerative power, leading to more active bone formation (23).

Although previous studies have demonstrated the regenerative potential of PRF and nHA in periodontal intrabony defects, direct comparisons between different PRF-based regenerative constructs remain limited. Mallappa *et al* (21) evaluated an A-PRF block in comparison with nHA alone and reported superior clinical and radiographic outcomes with the A-PRF block. However, the additional benefit of incorporating A-PRF into a regenerative construct already containing i-PRF and nHA has not been adequately investigated.

The present study differs in several key aspects. First, it compares A-PRF block (A-PRF + i-PRF + nHA) with i-PRF + nHA rather than A-PRF block with nHA alone, thereby allowing evaluation of the specific contribution of A-PRF within a biologically active regenerative matrix (21). Second, radiographic assessment was performed using cone-beam computed tomography (CBCT), which enabled three-dimensional evaluation of defect fill and defect resolution and provided a comprehensive assessment of regenerative outcomes. Third, only three-walled intrabony defects were included, thereby ensuring a relatively homogeneous defect morphology and reducing variability associated with defect configuration.

At 6 months, the A-PRF block group demonstrated greater reductions in probing pocket depth, greater clinical attachment gain, and superior radiographic defect resolution compared with the i-PRF + nHA group. These findings provide additional evidence supporting the use of A-PRF block as a regenerative approach for the management of periodontal intrabony defects.

Patients and methods

Study setting. The present study recruited participants from the Outpatient Department, Department of Periodontology, Manipal College of Dental Sciences (MCODS) Mangalore, India. Patient recruitment was carried out between December, 2023 to March, 2024. Both male and female participants who satisfied the following eligibility criteria were included in the study.

Study design. The present study was a prospective, assessor-blinded, parallel-arm randomized controlled clinical trial performed over 6 months. The present study was conducted and reported in accordance with the CONSORT guidelines for randomized controlled trials. A CONSORT flow diagram describing participant enrollment, allocation, follow-up and analysis is provided in Fig. S1.

Study population. The study population included 26 patients (12 males and 14 females) aged 20-60 years, all diagnosed with stage III, grade B periodontitis. Patients were randomly allocated into two groups: The test group (A-PRF block) and the control group (i-PRF + nHA) with 13 patients in each group (Table I).

Ethical considerations. The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee, Manipal College of

Table I. Baseline demographic and clinical characteristics of the study participants.

Characteristic	Control group (i-PRF + nHA) (n=13)	Test group (A-PRF block) (n=13)	P-value
Age (years), mean ± SD	51.38±9.04	46.85±3.36	0.103
Male, n (%)	7 (53.8%)	5 (38.5%)	
Female, n (%)	6 (46.2%)	8 (61.5%)	
Stage III grade B, n (%)	10 (76.9%)	11 (84.6%)	
Stage III, grade C, n (%)	3 (23.1%)	2 (15.4%)	
Chief complaint	Periodontal symptoms requiring treatment	Periodontal symptoms requiring treatment	
Medical history	Systemically healthy participants	Systemically healthy participants	
Drug history	No medications affecting periodontal healing	No medications affecting periodontal healing	
Smoking status	Non-smokers[a]	Non-smokers[a]	
Baseline periodontal status	Presence of intrabony periodontal defects	Presence of intrabony periodontal defects	
Intrabony defects, n	13	13	
Total participants, n	13	13	

[a]As per the study inclusion and exclusion criteria. Data are presented as the mean ± standard deviation or number (%), as appropriate. i-PRF, injectable platelet rich fibrin; A-PRF, advanced platelet-rich fibrin; nHA, nanohydroxyapatite.

Dental Sciences Mangalore (Protocol Ref No. 23018). The study was prospectively registered with the Clinical Trials Registry of India (CTRI) under protocol ID REF/2023/03/064312 on March 3, 2023. Written informed consent was obtained from all subjects involved in the study.

Patient inclusion criteria. The present study included patients aged between 20-60 years, irrespective of sex, who voluntarily agreed to participate by signing an informed consent form. A platelet count that fell within normal limits (1.5-3 lakhs/μl) was required for patients to be eligible for the study. Patients diagnosed with stage III periodontitis (grade B or C) according to the 2017 World Workshop Classification (24), presenting with a single three-walled intrabony defect ≥3 mm in depth and probing pocket depth ≥6 mm, were included. To ensure defect morphology standardization, only three-walled intrabony defects were selected. Only one defect per patient was included in the study to avoid clustering effects and ensure statistical independence of observations.

Additionally, all participants were required to have a full mouth plaque score of <25% to ensure adequate oral hygiene prior to the surgical procedure.

Patient exclusion criteria. Patients with aggressive periodontitis were excluded from the study, along with individuals known to have allergies to any of the materials used. Chronic smokers and those with systemic conditions, such as hypertension, uncontrolled diabetes mellitus, osteoporosis, autoimmune diseases, bleeding disorders, immunocompromised conditions, chronic inflammatory diseases, or any systemic condition that could affect periodontal wound healing or bone metabolism were excluded. Additionally, patients with bleeding and coagulation disorders, pregnant and lactating women, patients with parafunctional habits, those undergoing orthodontic treatment and immunocompromised patients were excluded.

Intervention: Pre-surgical phase. Prior to initiating the study, the patients were informed of the aim and design of the study, and informed consent forms were signed by the patients. Information concerning the chief complaint, history of present illness, medical and dental, family, personal history, drug and gingival and periodontal status was recorded in the case proforma.

Patients were examined under good illumination using a mouth mirror and a University of North Carolina-15 probe (UNC-15 probe). A brief history of the patient was recorded prior to initiating the treatment. Alginate impressions were then recorded for the maxillary and mandibular arches to fabricate a custom acrylic stent. The baseline measurements of clinical parameters, such as probing pocket depth (PPD), relative clinical attachment level (RCAL), plaque index (PI) and gingival index (GI) were performed. RCAL was measured by making grooves on the stent to standardize the placement of the UNC-15 probe (Figs. 1A, 2A, 3 and 4). Representative stent grooves used for reproducible RCAL measurements are illustrated in Fig. S2.

To evaluate intrabony defects radiographically, CBCT imaging was performed at baseline and at 6 months, pre- and post-operatively, respectively. All CBCT scans were obtained using the same CBCT unit (Planmeca ProMax 3DMid CBCT machine; Planmeca Oy) protocol throughout the study. Measurements were performed using a calibrated examiner. To assess reproducibility, 10 randomly selected scans were re-evaluated after 1 week and intraclass correlation coefficients were >0.85, demonstrating excellent measurement reliability. The sagittal section was standardized to a slice thickness of 1.0 mm. The cementoenamel junction (CEJ), base of defect (BOD) and alveolar crest (AC) of the adjacent tooth were identified. The intrabony defect fill (IDF) is measured as the distance from CEJ to BOD, and radiographic linear defect depth (RLDD) is measured from the CEJ to AC (Figs. 5 and 6).

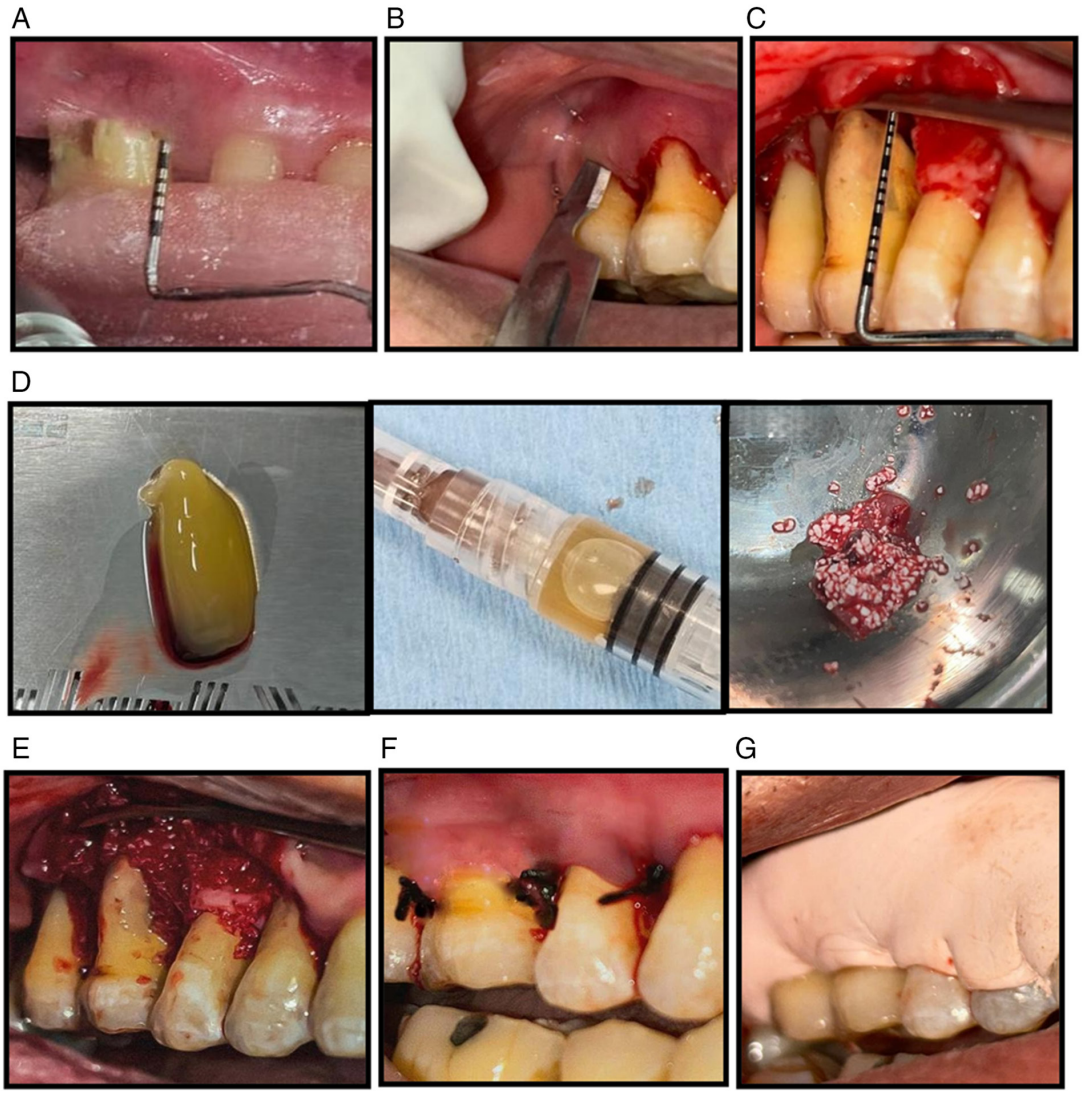

Figure 1. Test group surgical procedure. (A) Preoperative clinical measurement of relative clinical attachment level and probing pocket depth. (B) Intrasulcular incisions. (C) Intrasurgical measurement of the intrabony defect. (D) Preparation of the A-PRF block: (Left panel) harvested advanced platelet-rich fibrin (A-PRF) clot following centrifugation; (middle panel) injectable platelet-rich fibrin (i-PRF) collected in a syringe; (right panel) A-PRF block prepared by mixing diced A-PRF, i-PRF and nanohydroxyapatite (SyboGraf®) before placement into the periodontal intrabony defect. (E) Placement of the A-PRF block into the defect. (F) Suturing. (G) Periodontal dressing placement.

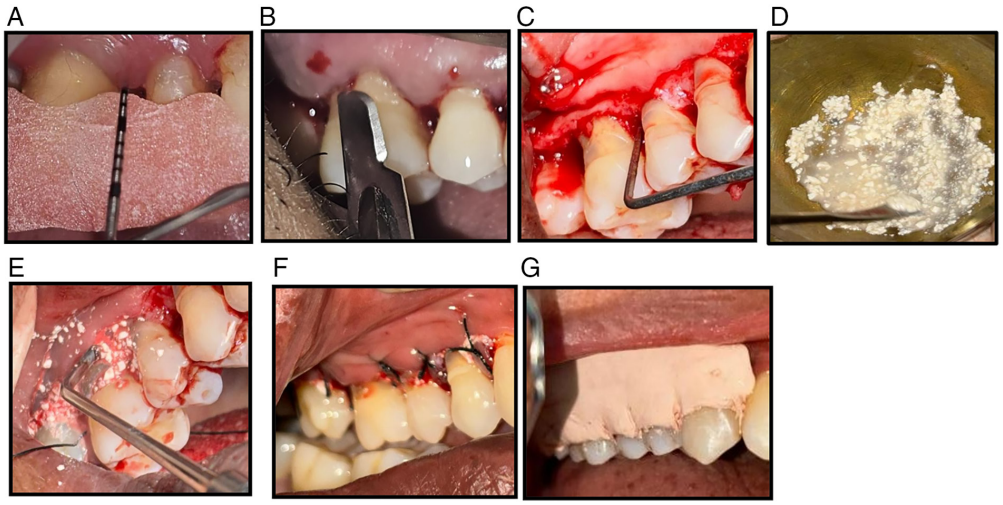

Figure 2. Control group surgical procedure. (A) Pre-operative clinical measurement of relative clinical attachment level and probing pocket depth. (B) Intrasulcular incisions. (C) Intrasurgical measurement of the intrabony defect. (D) Injectable PRF mixed with nanohydroxyapatite. (E) Placement of graft material into the defect. (F) Suturing. (G) Periodontal dressing placement.

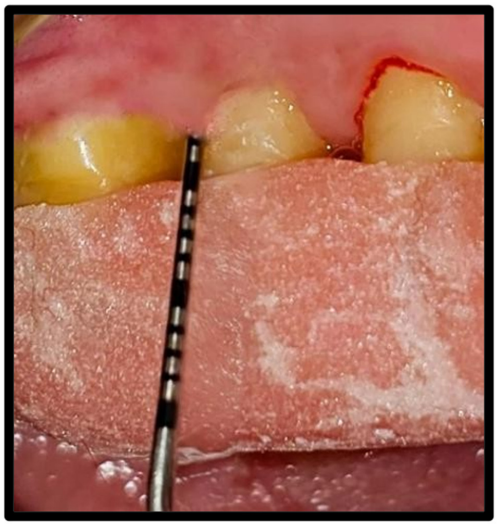

Figure 3. Test group clinical outcomes showing si6x-month post-operative evaluation.

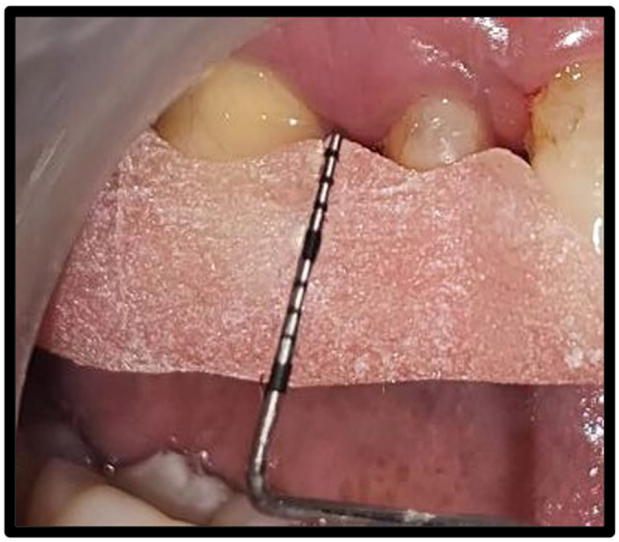

Figure 4. Control group clinical outcomes showing six-month post-operative evaluation.

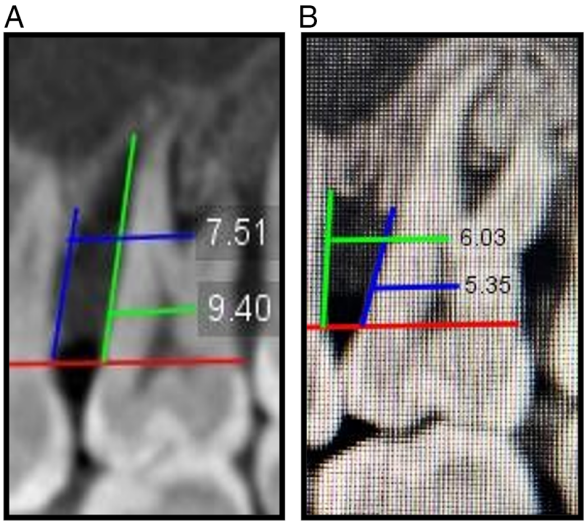

Figure 5. Test group cone-beam computed tomography analysis illustrating (A) pre-operative and (B) 6-month post-operative measurements. Intrabony defect fill and residual linear defect depth were assessed using native -beam computed tomography software.

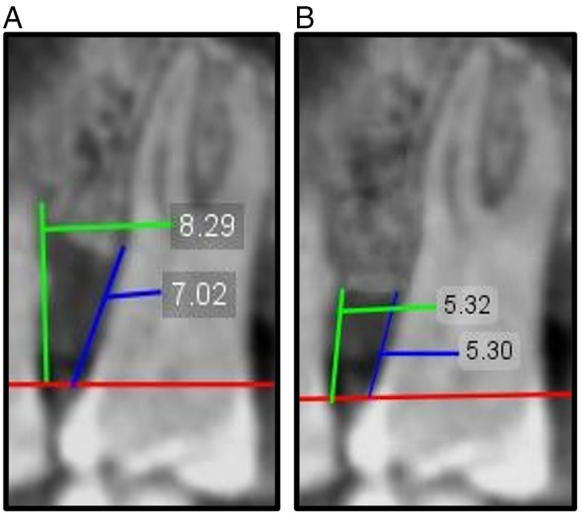

Figure 6. Control group cone-beam computed tomography analysis showing (A) pre-operative and (B) 6-month post-operative measurements. Intrabony defect fill and residual linear defect depth were assessed using native cone-beam computed tomography software.

Subsequently, all patients underwent phase I therapy, which included instructions on maintaining oral hygiene, both supra- and subgingival scaling using manual instruments and an ultrasonic device, followed by professional polishing with a rubber cup and a polishing paste.

Surgical intervention was performed at ~6-8 weeks following the completion of phase I therapy. The interval depended on individual patient healing and achievement of satisfactory plaque control (<25% plaque score) before surgery. The intrabony defect sites were split into two groups: The test group [open flap debridement (OFD) + A-PRF block] and the control group (OFD + i-PRF + nHA).

Intervention: Surgical phase. After 6-8 weeks, OFD was performed under local anesthesia (LA) using a full thickness mucoperiosteal flap. The bone defect was thoroughly debrided using Columbia universal curettes and Gracey curettes.

Test group. A-PRF was obtained in accordance with the method outlined by Ghanaati *et al* (19), where 10 ml venous blood were obtained from the antecubital vein of the patient into a glass tube and blood samples were centrifuged at 1,500 rpm (~200 x g) for 14 min using a Duo Quattro centrifuge (Process for PRF) at room temperature. The obtained fibrin clot was then placed inside an expression box to create the A-PRF membrane.

i-PRF was prepared in accordance with the method described in the study by Miron *et al* (20), where 9 ml venous blood were obtained in a glass tube and immediately centrifuged for 3 min at 700 rpm (~42-48 x g at the clot level) at room temperature using a Duo Quattro centrifuge (Process for PRF). Following centrifugation, the liquid i-PRF was drawn from the upper portion using a 2-cc syringe.

A standardized quantity of 0.5 cc nHA graft material was used for all defects to ensure consistency between the treatment groups. (SyboGraf®; particle size, 600-700 microns). It was placed into a bone well and combined with i-PRF. During the polymerization phase, small sections of the A-PRF membrane were placed into the mixture. This resulted in a cohesive, moldable graft termed the 'A-PRF block', which was subsequently inserted into the area of the defect.

Following grafting, the flap was repositioned, and interrupted sutures using 3-0 black braided silk (Ethicon®, Johnson & Johnson) were placed to achieve primary closure. Periodontal dressing was placed (Fig. 1).

Control group. In accordance with the protocol established by Miron *et al* (20), i-PRF was prepared by drawing 9 ml venous blood into a glass tube, which was then centrifuged for 3 min at 700 rpm (~42-48 x g at the clot level) at room temperature using a Duo Quattro centrifuge (Process for PRF). Following centrifugation, the liquid i-PRF was drawn using a 2-cc syringe from the upper portion of the tube. A 0.5 cc alloplastic nanohydroxyapatite graft (SyboGraf®; particle size, 600-700 microns) was added to a bone well and combined with i-PRF.

Following grafting, the flap was repositioned, interrupted sutures using 3-0 black braided silk (Ethicon®, Johnson & Johnson) were placed to achieve primary closure. Periodontal dressing was placed (Fig. 2).

Post-surgical care. The patients were given a prescription for amoxicillin (500 mg) to be taken thrice a day for 5 days, along with diclofenac sodium (50 mg) as an analgesic to be used as needed. Patients were instructed to begin rinsing with 10 ml of 0.2% chlorhexidine mouthwash 24 h following surgery and to continue this twice daily for 2 weeks. Mechanical plaque control was avoided at the surgical site during this period to prevent trauma to the healing tissues and grafted area. Sutures and periodontal dressing were removed at 14 days post-operatively. At the end of 6 months, all clinical and radiographic parameters were recorded (Figs. 3-6).

Clinical outcomes. The primary outcomes were PPD, RCAL, IDF and RLDD at baseline and 6 months. The PI and GI were the secondary study outcome variables assessed at baseline and 6 months.

Sample size. Sample size estimation was based on the primary outcome variable and data reported by Mallappa *et al* (21) Using a power of 95% and a significance level of 5%, the minimum required sample size was calculated as 13 defects per group, using the following equation:

$$N = \frac{2(Z_{1-\frac{\alpha}{2}} + Z_{1-\beta})^2 \sigma^2}{d^2}$$

where N is the sample size, σ is the pooled (average) standard deviation of the two groups, d is the minimum clinically relevant difference, Z(1-α/2) is the Z-score corresponding to the selected alpha error, and Z(1-β) is the Z-score corresponding to the selected study power.,

Randomization. Eligible sites were randomly allocated into the test group (A-PRF block) or the control group (i-PRF + nHA) using simple randomization. Allocation was performed using a coin toss at a 1:1 ratio immediately prior to surgery. Participants were informed about the study procedures, but were blinded to the treatment allocation. A total of 13 patients in the test group were treated with A-PRF block and 13 patients in the control group were treated with i-PRF + nHA.

Blinding. All clinical assessments were performed by a single calibrated examiner who was not an author of the study and was independent of the principal investigator., to reduce observer bias. Prior to study initiation, the examiner underwent calibration training for PI, GI, PPD, RCAL, IDF and RLDD. Intra-examiner reliability was evaluated using 10 randomly selected patients at baseline, with assessments repeated after 1 week. The results were analyzed using the intraclass correlation coefficient (ICC), which demonstrated a high level of agreement (ICC >0.85), confirming the scoring consistency and reliability of the examiner throughout the study. Due to the nature of the surgical procedures, operator blinding was not feasible.

Statistical analysis. The data were entered in Microsoft Excel and analyzed using SPSS version 29 (IBM Corp.). PPD, RCAL, PI and GI were the assessed clinical parameters at baseline and at 6 months. IDF and RLDD were the assessed radiographic parameters at baseline and at 6 months using CBCT imaging. Descriptive statistics are expressed as the mean ± standard deviation/median and interquartile range (IQR), depending on the data distribution. As PPD, RCAL and IDF did not follow a normal distribution (P-value <0.05), non-parametric tests were applied for their analysis. To compare the test group (OFD + A-PRF) and the control group (OFD + i-PRF + nHA), the independent t-test was used for normally distributed variables (RLDD, PI and GI), while the Mann-Whitney U test was used for nonnormally distributed variables (PPD, RCAL and IDF). Intragroup comparisons between baseline and 6-month values were performed using the Wilcoxon signed-rank test (PPD, RCAL and IDF) and paired samples t-test (RLDD, PI and GI). A P-value <0.05 was considered to indicate a statistically significant difference. Change scores were calculated as the difference between baseline and 6-month values for PPD, RCAL, IDF, RLDD, PI and GI, and intergroup comparisons of change scores were performed using the Mann-Whitney U test. To account for baseline differences, analysis of covariance (ANCOVA) was performed for each outcome, with the 6-month value as the dependent variable, treatment group as the fixed factor, and the corresponding baseline value as the covariate. All analyses were performed on a per-protocol basis. All 26 participants completed the 6-month follow-up, and no outcome data were missing; therefore, no imputation procedures were required.

Results

A total of 26 patients aged between 20 and 60 years of age with 26 intrabony defects were recruited for the present two-arm parallel randomized controlled clinical trial based on predetermined inclusion and exclusion criteria. The randomization of patients was performed into the two groups using simple randomization with the coin toss technique. All participants

Table II. Intergroup comparisons between the test group and control group.

Group	No. of patients	Mean	SD	t-test value	P-value
RLDD (baseline)					
Test	13	7.263	1.370	2.26	0.033
Control	13	8.44	1.276		
RLDD (6 months)					
Test	13	5.247	1.205	3.94	**<0.001**
Control	13	7.17	1.280		
PI (baseline)					
Test	13	1.231	0.218	2.51	0.019
Control	13	1.49	0.307		
PI (6 months)					
Test	13	0.831	0.193	3.65	**<0.001**
Control	13	1.11	0.193		
GI (baseline)					
Test	13	1.115	0.208	2.25	0.034
Control	13	1.33	0.275		
GI (6 months)					
Test	13	0.708	0.250	3.96	**<0.001**
Control	13	1.05	0.181		
Percentage bone fill (%)					
Test	13	38.93	5.67	6.67	**<0.001**
Control	13	24.64	5.24		

Data were analyzed using an independent samples t-test. Values are expressed as the mean ± standard deviation, unless otherwise stated. Values in bold font indicate a statistically significant difference (P<0.05). PPD, probing pocket depth; RLDD, radiographic linear defect depth; PI, plaque index; GI, gingival index.

completed the study, and there were no missing data points for analysis. Intergroup comparisons between the test group and control group were performed using the independent samples t-test for RLDD, PI and GI at baseline and at 6 months (Table II).

Although randomization was performed, statistically significant baseline differences were observed for RLDD, PI and GI, which may be attributed to the relatively small sample size. Therefore, both intergroup and intragroup comparisons were performed to appropriately evaluate treatment effects over time.

At baseline, the mean RLDD in the test group was 7.263±1.370 mm, while in the control group, it was 8.44±1.276 mm, exhibiting a statistically significant difference (P=0.033). At 6 months, the RLDD was further reduced in both groups, with the test group exhibiting a significantly lower mean value of 5.247±1.205 mm compared to 7.17±1.280 mm in the control group (P<0.001). At baseline, the mean PI was 1.231±0.218 in the test group and 1.49±0.307 in the control group, indicating a statistically significant difference (P=0.019). At 6 months, both groups exhibited improvement; however, the test group demonstrated a significantly lower PI (0.831±0.193) than the control group (1.11±0.193) (P<0.001). The mean GI at baseline was 1.115±0.208 in the test group and 1.33±0.275 in the control group, indicating a statistically significant difference (P=0.034). At 6 months, the GI was significantly reduced in the test group (0.708±0.250) compared to the control group (1.05±0.181) (P<0.001). Percentage bone fill was also evaluated to facilitate clinical interpretation of the radiographic findings. The mean percentage bone fill was 38.93% in the test group and 24.64% in the control group, indicating greater radiographic defect resolution in sites treated with the A-PRF block (Table II).

Intergroup comparisons between the test group and control group were performed using the Mann-Whitney U test for PPD, RCAL and IDF (Table III). At baseline, no statistically significant differences were observed between the test and control groups for any of the measured parameters. The median PPD was 8.00 mm (IQR, 1.00) in both groups (P=0.274), RCAL was 11.00 mm (IQR, 1.00) in the test group and 12.00 mm (IQR, 2.00) in the control group (P=0.235), and IDF was 9.40 mm (IQR, 1.39) in the test group and 10.10 mm (IQR, 1.89) in the control group (P=0.169). At the 6-month follow-up, PPD (median, 3.00 mm; IQR, 1.00) in the test group was significantly lower than that in the control group (median, 4.00 mml IQR, 1.00) (P<0.001). Similarly, RCAL was 6.00 mm (IQR, 1.00) in the test group and 9.00 mm (IQR, 1.00) in the control group (P<0.001). The IDF was 5.78 mm (IQR, 1.68) in the test group, which was significantly lower than the value of 7.73 mm (IQR, 1.87) in the control group (P=0.002) (Table III).

To further evaluate the treatment effects, change scores (Δ) from baseline to 6 months were calculated and compared between the groups (Table IV). The test group demonstrated

Table III. Intergroup comparisons between the test group and control group.

Group	No. of patients	Median	IQR	Mann-Whitney U test value	P-value
PPD (baseline)					
Test	13	8	1.00	63.50	0.274
Control	13	8	1.00		
PPD (6 months)					
Test	13	3	1.00	17.00	**<0.001**
Control	13	4	1.00		
RCAL (baseline)					
Test	13	11	1.00	61.50	0.235
Control	13	12	2.00		
RCAL (6 months)					
Test	13	6	1.00	8.00	**<0.001**
Control	13	9	1.00		
IDF (baseline)					
Test	13	9.40	1.39	57.00	0.169
Control	13	10.1	1.89		
IDF (6 months)					
Test	13	5.78	1.68	27.00	0.002
Control	13	7.73	1.87		

Data were analyzed using the Mann-Whitney U test. Values in bold font indicate a statistically significant difference (P<0.05). IQR, interquartile range; PPD, probing pocket depth; RCAL, relative clinical attachment level; IDF, intrabony defect fill.

significantly greater improvements in PPD, RCAL and IDF compared with the control group. The between-group difference in change scores was significant for PPD (P=0.007), RCAL (P<0.001) and IDF (P<0.001). No statistically significant differences were observed between groups for RLDD (P=0.113), PI (P=0.436) or GI (P=0.436).

As statistically significant baseline differences were observed for RLDD, PI and GI, additional baseline-adjusted analyses were performed using ANCOVA (Table IV). Following adjustment for corresponding baseline values, a significant treatment group effect remained for all evaluated outcomes. The group effect was significant for PPD (F=36.8, P<0.001, η^2p=0.616), RCAL (F=85.7, P<0.001, η^2p=0.788), IDF (F=50.7, P<0.001, η^2p=0.688), RLDD (F=10.1, P=0.004, η^2p=0.305), PI (F=5.45, P=0.029, η^2p=0.192) and GI (F=8.02, P=0.009, η^2p=0.259). These findings indicate that the superior outcomes observed in the test group remained statistically significant even after controlling for baseline values (Table V).

The intragroup comparison of the test group revealed a significant reduction in the median and IQR for PPD from baseline (8.00 mm; IQR, 1.00) to 6 months (3.00 mm; IQR, 1.00) (P<0.001). RCAL improved significantly from a median and IQR of 11.00 mm (IQR, 1.00) at baseline to 6.00 mm (IQR, 1.00) at 6 months (P<0.001). Similarly, IDF demonstrated a statistically significant reduction from 9.40 mm (IQR, 1.39) at baseline to 5.78 mm (IQR, 1.68) at 6 months (P<0.001) (Fig. 7A).

RLDD exhibited a statistically significant reduction from a mean of 7.26±1.37 mm at baseline to 5.24±1.20 mm at 6 months (P<0.001). The PI also demonstrated a significant improvement, decreasing from 1.23±0.21 mm at baseline to 0.83±0.19 mm at 6 months (P<0.001). Similarly, GI decreased significantly from 1.11±0.20 mm at baseline to 0.70±0.25 mm at 6 months (P<0.001) (Fig. 7B).

The intragroup comparison of the control group revealed a statistically significant reduction in the median and IQR for PPD from 8.00 mm (IQR, 1.00) at baseline to 4.00 mm (IQR, 1.00) at 6 months (P<0.001). Similarly, the RCAL exhibited a significant improvement from the median, and the IQR decreased from 12.00 mm (IQR, 2.00) at baseline to 9.00 mm (IQR, 1.00) at 6 months (P<0.001). IDF also improved significantly, decreasing from 10.14 mm (IQR, 1.89) at baseline to 7.73 mm (IQR, 1.87) at 6 months (P=0.002) (Fig. 8A).

RLDD exhibited a statistically significant reduction from a baseline mean of 8.44±1.28 mm to 7.17±1.28 mm at 6 months (P<0.001). Similarly, PI decreased significantly from 1.49±0.30 mm at baseline to 1.11±0.19 mm (P<0.001). GI also exhibited a statistically significant improvement, decreasing from 1.33±0.27 mm at baseline to 1.05±0.18 mm at 6 months (P<0.001) (Fig. 8B).

Discussion

PRF, a 2nd generation platelet concentrate, differs from earlier platelet concentrates, such as PRP, in multiple ways. Unlike PRP, PRF does not require the use of anticoagulants or gelling agents, as demonstrated by Dohan *et al* (25). The naturally formed PRF clot exhibits a dense and intricate 3D fibrin network that not only traps platelets, but also incorporates leukocytes. PRF produces a dense fibrin mesh that delays the natural clearance of PRF in the body, allowing the sustained release of platelet and

Table IV. Intergroup comparisons of change scores (Δ) between baseline and 6 months for clinical and radiographic parameters.

Parameter	Test vs. control Δ difference	95% CI	Mann-Whitney U test value	P-value	Interpretation
PPD difference	-1.000	-2.000 to -3.26e-5	36.00	0.007	Significant
RCAL difference	-2.000	-3.000 to -1.000	7.50	<0.001	Significant
IDF difference	-1.180	-1.570 to -0.780	3.00	<0.001	Significant
RLDD difference	-0.590	-1.340 to 0.080	53.00	0.113	Not significant
PI difference	-4.39e-5	-0.200 to 0.1001	69.00	0.436	Not significant
GI difference	-4.39e-5	-0.200 to 0.1001	69.00	0.436	Not significant

Data were analyzed using the Mann-Whitney U test. Values represent differences between baseline and 6-month measurements. Mann-Whitney U statistics, P-values and 95% confidence intervals are presented. PPD, probing pocket depth; RCAL, relative clinical attachment level; IDF, intrabony defect fill; RLDD, radiographic linear defect depth; PI, plaque index; GI, gingival index.

Table V. Analysis of covariance (ANCOVA) for 6-month outcomes adjusted for baseline values.

Outcome at 6 months	Baseline covariate	Group F	Group P-value	Partial η^2	Interpretation
PPD	Baseline PPD	36.8	<0.001	0.616	Significant following adjustment
RCAL	Baseline RCAL	85.7	<0.001	0.788	Significant following adjustment
IDF	Baseline IDF	50.7	<0.001	0.688	Significant following adjustment
RLDD	Baseline RLDD	10.1	0.004	0.305	Significant following adjustment
PI	Baseline PI	5.45	0.029	0.192	Significant following adjustment
GI	Baseline GI	8.02	0.009	0.259	Significant following adjustment

Six-month measurements were entered as dependent variables, the treatment group as the fixed factor and the corresponding baseline values as covariates. F-statistics, P-values and partial eta squared (η^2p) are presented. PPD, probing pocket depth; RCAL, relative clinical attachment level; IDF, intrabony defect fill; RLDD, radiographic linear defect depth; PI, plaque index; GI, gingival index.

leukocyte growth factors to occur at the site of tissue damage, as demonstrated by Dohan *et al* (26). Furthermore, it can be enriched with leukocytes, which can potentially increase its antimicrobial effects that can be used in the control of wound infections, as demonstrated by Cieslik-Bielecka *et al* (27). Such unique structural and biological properties bring PRF to the forefront as a highly useful tool in periodontal regeneration encouragement and wound healing.

The development of novel technologies in centrifugation led to the emergence of various forms of platelet concentrate, such as leukocyte platelet-rich fibrin (L-PRF), A-PRF, and i-PRF. A-PRF was developed by Ghanaati *et al* (19) in 2014; this was created through reduced-speed centrifugation at 1,500 rpm over 14 min. This approach resulted in a fibrin clot with a looser structure, higher cellular content and uniform distribution of platelets, cytokines and neutrophil granulocytic cells. Subsequently, in 2017, Miron *et al* (20) developed i-PRF, the liquid platelet-rich form of fibrin that clots outside of the collection tube. This liquid preparation is notably enriched with growth factors, owing to a higher concentration of leukocytes and platelets suspended within it.

A novel PRF formulation, referred to as the PRF block, is produced by mixing liquid fibrinogen with a bone graft (combining L-PRF, i-PRF and the graft material). Cortelleni *et al* (28) in 2018 were the first to apply this approach during horizontal ridge augmentation, reporting improved bone regeneration. In the present study, an A-PRF block was prepared by combining A-PRF, i-PRF and nHA (SyboGraf®). This mixture could yield better regenerative results than the combination of i-PRF with nHA alone in treating periodontal intrabony defects.

The gold standard for bone regeneration remains autogenous bone grafts due to their osteogenic properties and absence of immunological reactions (Rosenberg and Rose, 1998) (29). However, their application is constrained due to complications at the donor site and limited supply, as demonstrated by Cohen *et al* (30). These challenges have led to the development of alternative bone substitutes that are biocompatible, noninfectious and nonantigenic, while offering strong osteogenic potential. Nanocrystalline hydroxyapatite (NcHA) (SyboGraf®) serves as an osteoconductive material with a high density of surface molecules, facilitating the rapid healing of critical-sized defects, as previously described by Murugan and Ramakrishna (13). It binds to bone and enhances the expression of BMPs, encouraging the migration and differentiation of osteoprogenitor cells. The small particle size and large surface area of nHA improve osteoblast adhesion and calcium deposition. Additionally, it is bioresorbable and has

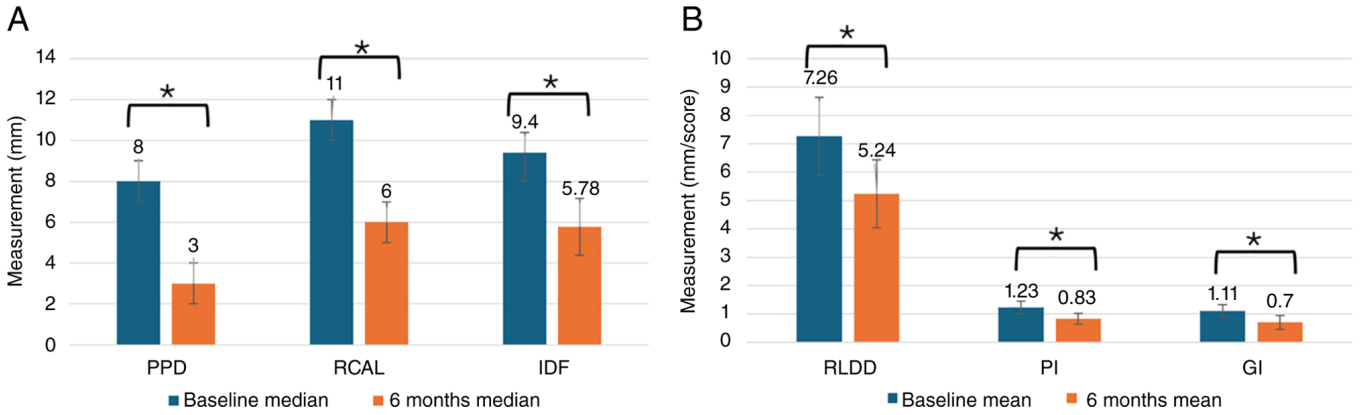

Figure 7. Intragroup comparison of clinical and radiographic parameters in the test group at baseline and 6 months. (A) Median and interquartile range values for PPD, RCAL and IDF. Data are presented as the median and interquartile range. Error bars represent the interquartile range. *P<0.05, indicates a statistically significant difference between baseline and 6-month measurements. (B) Mean ± standard deviation values for RLDD, PI and GI. Data are presented as the mean and standard deviation. Error bars represent the standard deviation. *P<0.05, indicates a statistically significant difference between baseline and 6-month measurements. PPD, probing pocket depth; RCAL, relative clinical attachment level; IDF, intrabony defect fill; RLDD, radiographic linear defect depth; PI, plaque index; GI, gingival index.

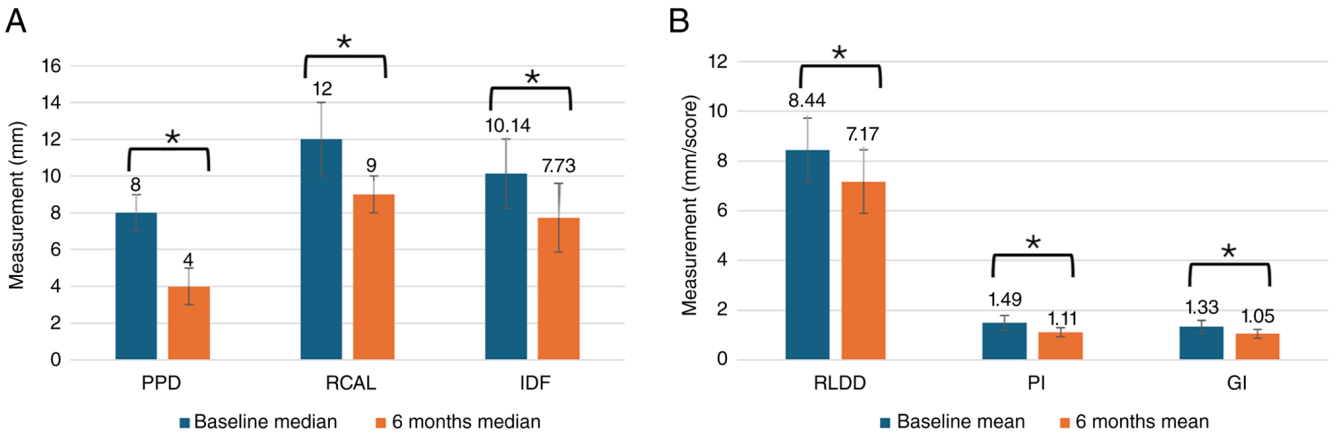

Figure 8. Intragroup comparison of clinical and radiographic parameters in the control group at baseline and six months. (A) Median and interquartile range values for PPD, RCAL and IDF. Data are presented as the median and interquartile range). Error bars represent the interquartile range. *P<0.05, indicates a statistically significant difference between baseline and 6-month measurements. (B) Mean ± standard deviation values for RLDD, PI and GI. Data are presented as the mean and standard deviation. Error bars represent the standard deviation. *P<0.05, indicates a statistically significant difference between baseline and 6-month measurements. PPD, probing pocket depth; RCAL, relative clinical attachment level; IDF, intrabony defect fill; RLDD, radiographic linear defect depth; PI, plaque index; GI, gingival index.

an extended resorption period, allowing for effective space maintenance to support new bone formation, as demonstrated by Thorwarth *et al* (15).

The present study aimed to assess, both clinically and radiographically, the regenerative capability of the A-PRF block compared to i-PRF combined with nHA in the management of periodontal intrabony defects. The present study demonstrated superior clinical outcomes in the A-PRF block group compared with the i-PRF + nHA group. Greater reductions in PPD and greater gains in the clinical attachment level were observed in the test group at 6 months post-operatively. Although the observed differences between groups were statistically significant, they were also clinically meaningful. The additional reduction in PPD and gain in the clinical attachment level observed in the A-PRF block group may contribute to improved periodontal stability, greater defect resolution and potentially enhanced long-term tooth retention. The observed clinical attachment gain was >1 mm, indicating a favorable clinical response following regenerative therapy. The additional clinical attachment gain observed in the present study therefore appears to be of practical significance in addition to statistical significance. These findings are consistent with those of previous studies demonstrating the regenerative potential of PRF and nHA in periodontal intrabony defects. Singh *et al* (31) reported improved clinical outcomes with NcHA and a collagen membrane compared with open flap debridement alone, while Pradeep *et al* (32) demonstrated enhanced regeneration when PRF was combined with NcHA in three-walled intrabony defects. Mallappa *et al* (21) further reported superior clinical and radiographic outcomes with an A-PRF block compared with nanohydroxyapatite alone. The findings of the present study support and extend these observations, suggesting that incorporation of A-PRF into an i-PRF and nHA construct may further enhance periodontal regeneration. Periodontal regeneration and soft

tissue healing achieved at the base of the periodontal pocket may account for the observed reduction in probing pocket depth and gain in clinical attachment level.

Radiographically, the A-PRF block group demonstrated superior defect fill, defect resolution and bone gain compared with the i-PRF + nHA group. These findings are in agreement with those of previous studies reporting enhanced regenerative outcomes when PRF was combined with bone graft materials. Elgendy and Shady (33) observed improved clinical and radiographic outcomes with the combination of nanocrystalline hydroxyapatite and PRF compared with NcHA alone. Similarly, Pradeep *et al* (32) reported a greater intrabony defect fill when PRF was combined with porous hydroxyapatite, while Nair *et al* (34) demonstrated favourable clinical outcomes with i-PRF and nHA in grade II furcation defects. The present study further suggests that the incorporation of A-PRF into this regenerative construct may provide additional benefits in the management of periodontal intrabony defects. The superior outcomes observed with the A-PRF block may be particularly advantageous in three-walled intrabony defects as the remaining bony walls provide natural containment and stability for the graft material. The fibrin scaffold created by A-PRF may enhance cell migration, angiogenesis and sustained release of growth factors within this contained environment. Furthermore, the osteoconductive properties of nHA may be better utilized when graft stability is maintained by the surrounding defect walls. Whether similar outcomes would be achieved in one- or two-walled defects remains uncertain, as such defects provide less intrinsic support and space maintenance. Future studies are thus warranted to evaluate the performance of A-PRF block in defects with different morphologies.

The improved clinical and radiographical outcomes observed in the present study can be due to the porosity of A-PRF, which is achieved through using the low-speed centrifugation concept (LSCC). Within 7-10 days, the fibrin matrix degrades slowly, and within this time, there is a continuous release of neutrophilic granulocytes and cytokines of platelet origin. LSCC uses balanced access of these items across the matrix, as demonstrated by Chatterjee and Debnath (35). Laboratory experiments have shown that the interconnected fibrin network in PRF blocks can induce the proliferation of osteoblasts, the release of significant amounts of growth factors, enhanced wound closure and tissue remodeling (18,19,23). Furthermore, the blocked segmented membrane structure of A-PRF serves as a natural biological scaffold prompting osteoprogenitor cell migration into the core of the graft. This attribute is probably one of the keys to the high regenerative results behind our studies.

The i-PRF will polymerize in the presence of the bone graft and A-PRF to create a stiff, yet flexible lump that may be contoured and shaped to perfectly fit into the defect region. The mixture provides two key advantages: First, it increases the osteoinductive capability of the graft due to the slow delivery of significant growth factors, such as BMP-2 and platelet-derived growth factor. Second, the combination of A-PRF and i-PRF, together with the graft in the present study, formed a biological glue, and this provided a significant clinical benefit.

With its low resorption rate, the nHA bone graft (SyboGraf®) preserves the space required for new bone to grow. Pilloni *et al* (36) explored the role of nHA in the process of differentiating and maturing alveolar bone cells in an *in vitro* study. Their results suggested that NcHA markedly facilitates osteoblast attachment and maturation, which indicates that nHA can induce bone formation by activating the local osteoprogenitor lineage during the regeneration of alveolar bone. In addition, the higher secretion of BMPs on nHA surfaces can be of value in recruiting progenitor cells during the healing process when applied in nHA scaffolds. Previous studies have reported that nanohydroxyapatite (nHA) is a promising graft material for periodontal regeneration because of its favorable osteoconductive properties and its ability to support periodontal ligament and cementum regeneration (12,31,36). Koduru *et al* (37) further demonstrated that nHA resulted in greater intrabony defect fill than bioactive calcium phosphosilicate putty (bioactive glass). Considering these favorable regenerative properties, nanohydroxyapatite (SyboGraf®) was selected as the graft material in the present study.

nHA was added to i-PRF in the present study. In the i-PRF scaffold, platelet-rich clots surround the bone graft powders, essentially making them part of the fibrin network. Shah *et al* (23) reported that the platelets present in i-PRF gradually release growth factors at the site of implantation, highlighting this method as a simple, cost-effective chairside approach to bioactivate bone graft materials. This also contributes to an increase in graft volume, allowing clinicians to cover a larger area using a graft that is autologous, cost-effective, and most importantly, biologically active.

Cortellini *et al* (28) demonstrated that an L-PRF block created using liquid fibrinogen, chopped L-PRF membranes, and a particulate biomaterial achieved a linear horizontal bone gain of 4.6 mm and a volumetric gain of 1.05 cm^3 with minimal resorption. Similarly, Edrees *et al* (38) found that both L-PRF and PRF block affect the alveolar ridge split with simultaneous implant placement, with PRF block yielding better bone density. Likewise, in the present study, an A-PRF block comprising A-PRF, i-PRF, and nHA was utilized for the management of intrabony defects. The combined regenerative potential of A-PRF and i-PRF, along with the osteoconductive properties of nHA, appears to provide a more effective solution for the treatment of intrabony defects in comparison to i-PRF with nHA.

The magnitude of clinical improvement observed in the present study compares favorably with that reported by Mallappa *et al* (21), who demonstrated a PPD reduction of 3.5 mm, a relative attachment gain of 3.3 mm and a defect fill of 3.2 mm following treatment with an A-PRF block. In comparison, the present study demonstrated a PPD reduction of 4.15 mm, a clinical attachment gain of 5.92 mm and a defect fill of 3.71 mm in the test group. These findings further support the regenerative potential of A-PRF block and suggest that its combination with i-PRF and nanohydroxyapatite may provide favorable clinical and radiographic outcomes in periodontal intrabony defects.

The favorable outcomes observed in the present study may also be influenced by the morphology of the treated defects. Only three-walled intrabony defects were included, which are

generally considered to possess greater regenerative potential due to the presence of a contained bony environment, improved blood supply and enhanced graft stability. The cohesive fibrin architecture of the A-PRF block may further facilitate stabilization of the graft material within such contained defects, while serving as a scaffold for cellular migration and angiogenesis. Therefore, the regenerative benefits observed with the A-PRF block may be particularly evident in three-walled defects. Whether similar outcomes would be achieved in less favorable one- or two-walled defects remains uncertain and warrants further investigation. Furthermore, radiographic assessment in the present study was performed using CBCT, allowing three-dimensional evaluation of defect fill and defect resolution, which may provide a more accurate assessment of regenerative outcomes than conventional two-dimensional radiographic techniques.

In addition to statistical significance, the observed differences appear to be clinically meaningful. In periodontal regenerative therapy, reductions in PPD and gains in clinical attachment level of at least 1 mm are generally considered clinically meaningful, as they may contribute to improved periodontal stability and facilitate long-term maintenance. In the present study, the test group demonstrated an additional 1 mm reduction in PPD and a ~2 mm greater clinical attachment gain compared with the control group. Such improvements may contribute to improved periodontal stability by reducing residual pocket depth, facilitating plaque control and enhancing attachment support around the treated tooth. Furthermore, the greater radiographic defect resolution observed in the test group suggests a more favorable regenerative response within the intrabony defect. While longer-term studies are required to determine the effects of these improvements on tooth survival and long-term periodontal stability, the magnitude of change observed in the present study is likely to be of clinical relevance.

Although the present study demonstrated significant improvements with the A-PRF block compared to i-PRF with nHA in both clinical and radiographic parameters, certain methodological limitations should be considered. The small sample size may affect the reproducibility of the results. Additionally, the study utilized a two-arm parallel randomized controlled trial design. A split-mouth design could have minimized the influence of individual patient factors, such as wound healing response and age, while providing a more robust estimate of the treatment effect with a smaller sample size. The presence of baseline differences between groups for certain parameters represents a limitation of the study and may be related to the modest sample size, despite appropriate randomization. Furthermore, the present study was conducted at a single center and the follow-up period was limited to 6 months, which may restrict the generalizability of the findings and preclude assessment of long-term regenerative stability. The present study evaluated clinical and radiographic parameters to assess the effects of regenerative treatment. However, the biological processes underlying the regenerative outcomes of A-PRF block could be better understood through histologic analysis of the treated sites, as histologic assessment remains the most accurate method for determining the type and quality of regenerated tissue. In addition, simple coin-toss randomization may have contributed to baseline imbalance between groups despite random allocation.

In conclusion, the present study aimed to assess and compare the radiographic and clinical efficacy of A-PRF (i-PRF + A-PRF + nHA) vs. i-PRF (i-PRF + nHA) in the treatment of periodontal intrabony defects. Both groups exhibited significant improvements in PPD, RCAL and defect resolution at 6 months. However, the test group demonstrated superior outcomes in PPD reduction, RCAL gain, IDF and RLDD compared to the control group. Within the limitations of this 6-month randomized clinical trial, A-PRF block demonstrated favorable clinical and radiographic outcomes compared with i-PRF combined with nanohydroxyapatite. However, further multi-center studies with larger sample sizes and longer follow-up periods are required to confirm these findings.

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Availability of data and materials

The data generated in the present study may be requested from the corresponding author.

Authors' contributions

SG contributed to the conception and design of the study, performed the investigation, data collection, statistical analysis, drafted the manuscript and critical revision of the manuscript. NJS contributed to the conception and design of the study, validation, supervision, interpretation of data, and critically revised the manuscript. SS contributed to the investigation, data analysis, interpretation of data, manuscript drafting and critical revision of the manuscript. HK contributed to data curation, statistical analysis, and interpretation of data. DK contributed to the conception and design of the study, interpretation of data, and critical revision of the manuscript. PJS contributed to the conception and design of the study, interpretation of data, and critical revision of the manuscript. SG, SS and NJS confirm the authenticity of all the raw data. All authors have read and approved the final manuscript and agree to be accountable for all aspects of the work.

Ethics approval and consent to participate

The present study was conducted in accordance with the Declaration of Helsinki and was approved by the Institutional Ethics Committee, Manipal College of Dental Sciences Mangalore (Protocol Ref No. 23018). The study was prospectively registered with the Clinical Trials Registry of India (CTRI) under protocol ID REF/2023/03/064312 on March 3, 2023. Written informed consent was obtained from all subjects involved in the study.

Patient consent for publication

Written informed consent was obtained from all subjects involved in the study.

Competing interests

The authors declare that they have no competing interests.

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