

Comparison of Regenerative Potential of Platelet-rich Fibrin Alone and in Combination with Bovine Bone Graft in Intraosseous Defect by Single Flap Approach: A Clinical and Radiographic Study

Bhaumik Thakkar, Sarath Chandran, Shivalal Vishnoi, Priyadarshini Nadig, Ruchi Raval, Priyanka Doshi

Department of Periodontics and Implantology, Manubhai Patel Dental College & Hospital, Vadodara, Gujarat, India

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ABSTRACT

Aim: To compare the regenerative potential of platelet-rich fibrin alone and in combination with bovine bone graft in intraosseous defect by the single flap approach. **Materials and Methods:** A total of 32 sites of intrabony defects were selected and were treated with platelet-rich fibrin (PRF) alone or in combination with bovine bone graft. Clinical parameters [Gingival index (GI), probing depth (PD), clinical attachment level (CAL), Gingival recession, and radiographic parameters (defect fill, alveolar crest level, and defect depth)] were recorded at baseline, 3 months, and 6 months. **Results:** Statistical analysis was done by independent and paired *t*-test. There were statistically significant changes in GI, PD reduction, CAL gain, defect fill, alveolar crest level changes, and defect depth resolution from baseline, 3 months, and 6 months in both the groups ($P < 0.001$). On intergroup comparison, Group II showed statistically significant changes in a reduction in pocket depth and defect depth resolution at $P < 0.001$. **Conclusion:** PRF in combination with bovine bone graft was more effective in the treatment of intrabony defects.

KEYWORDS: Collagen membrane, periodontitis, platelet-rich fibrin, regeneration, single flap approach

INTRODUCTION

Periodontitis is defined as an inflammation of the supporting tissues of the teeth which leads to loss of bone and periodontal ligament (PDL), characterized by extension of inflammation from gingiva to adjacent bone and ligament.^[1] In the 1950s, periodontitis was treated mostly by extraction of the affected tooth, for most of the world's population. Root surface debridement by scaling and root planing came into use in the first half of the present century and is the most common form of periodontal treatment.

An infrabony defect is defined as a "Periodontal defect within the bone surrounded by one, two or three bony walls or combination thereof."^[1] The clinical application of bone grafts is commonly used in regeneration of bone by applying regenerative material in osseous

defects. Hegedus^[2] clinically applied the use of bone grafting materials in periodontal regenerative therapy. Application of autogenous grafts in intraosseous bone defects can be traced back to Nabers and O'Leary,^[3] where cortical bone chips were used as grafting material. Since then, various techniques and materials have been used for regenerative therapy. Using bone graft materials can induce regeneration of bone height or volume with improvements in the clinical parameters. Further use of barrier membranes with bone grafts enhances regeneration by preventing migration of

Address for correspondence: Dr. Bhaumik Thakkar, Department of Periodontics and Implantology, C1/6B, Kumbhnath Society, Near Aavkar Hall, Ghodasar Road, Maninagar, Ahmedabad, Gujarat 380008, India. E-mail: brthakkar1991@gmail.com

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gingival epithelium at the healing site. Also, local application of various growth factors enhances the possibility of regeneration.

Trombelli *et al.*^[4] proposed a new minimally invasive surgical technique, the single flap approach (SFA), in adjunct with GTR membrane and bone grafting biomaterial. The procedure is specifically indicated to treat the intraosseous defects prevalent on buccal aspect. SFA is the elevation of a flap on buccal aspect to access the buccal defect, leaving the palatal side intact.

Human histological studies have evidently found that treatment of intrabony defects with a bovine bone-derived xenograft (BDX) alone or in adjunct with autogenous bone material, guided tissue regeneration (GTR), or enamel matrix protein derivative (EMD) could result in regeneration of loss periodontium (i.e., formation of new cementum, new PDL, and new alveolar bone).^[5]

Due to the re-entry of a second surgery to remove nonabsorbable membranes, a demand for bioabsorbable membranes with comparable, clinical outcomes became evident. Type I collagen is a primary component found in periodontal connective tissue. Low immunogenic property of collagen attracts and activates gingival fibroblastic cells which are hemostatic.^[6] A higher level of adherence to collagen membrane surface is seen significantly by osteoblasts.^[7]

Platelet-rich fibrin (PRF) is an immune and platelet concentrate, containing all the constituents of a blood sample which are favorable to healing and immunity.^[8] It appears neither like fibrin glue nor like classical platelet mix. It is simply centrifuged blood without any addition. PRF consists of a fibrin matrix polymerized in a tetra molecular structure, with incorporation of platelets, leukocytes, cytokines, and circulating stem cells.^[9,10]

Therefore, the objective of our study was to compare the regenerative potential of PRF alone and in combination with bovine bone graft in intraosseous defect by the SFA.

MATERIALS AND METHODS

The study was a randomized control trial carried out between March 2017 and October 2018. The patients for this study were randomly selected from the outpatient Department of Periodontics & Implantology in Manubhai Patel Dental College, Vadodara after fulfilling the inclusion and exclusion criteria. Written informed consent was obtained from patients and the ethical clearance was obtained from the institutional

ethical committee (BUETHICS/MPDC_114/PERIO-15/17). Thirty-two surgical sites were selected from those diagnosed as having chronic periodontitis, with clinical and radiographic evidence of intrabony defects and indicated for regenerative periodontal therapy.

INCLUSION CRITERIA

1. Male and female subjects of age between 18 and 60 years.
2. Interproximal probing depth ≥ 5 mm following phase I therapy.
3. The sites should exhibit clinical and radiographic evidence of intrabony defects ≥ 3 mm deep.
4. Clinical attachment loss at least ≥ 5 mm
5. No systemic conditions that would contraindicate routine periodontal procedures.
6. Good patient compliance and good oral hygiene maintenance.

EXCLUSION CRITERIA

1. Subjects who have received periodontal flap/regenerative therapy within the last 1 year.
2. Systemic disease that contraindicates periodontal surgery.
3. Medications affecting periodontal status.
4. Pregnant and lactating patients
5. Heavy smokers and tobacco chewers.
6. Patients who demonstrate poor oral hygiene maintenance.
7. Teeth with Grade II mobility or more.
8. Inadequate endodontic treatment and/or restoration were excluded.
9. Patients allergic to any drugs which can affect treatment outcome.

STUDY DESIGN

A thorough medical and dental history was recorded followed by a complete oral, clinical, and radiographic examination.

Initial therapy was performed on all patients, which consisted of full mouth scaling and root planing, oral hygiene instruction, and an occlusal adjustment when indicated. After 4 to 6 weeks of initial therapy, only those patients who met the following criteria at re-evaluation were continued into the surgical phase of treatment.

1. Residual probing depth ≥ 5 mm
2. Clinical attachment loss at least ≥ 5 mm
3. Optimal oral hygiene maintenance

A split-mouth randomized controlled trial was then carried out. A total of 32 sites with intrabony defects in chronic periodontitis patients were randomly assigned

into two groups, based on the treatment modality rendered to them, they are:

Group I ($n = 16$): Those to be treated with PRF and guided tissue regeneration membrane by SFA.

Group II ($n = 16$): Those to be treated with PRF mixed with bovine bone graft covered with Guided Tissue Regenerative membrane by SFA

An SFA was indicated in every patient and, after proper debridement, the intrabony defects were randomly divided.

CLINICAL ASSESSMENT

1. Gingival index (GI)^[11]
2. Gingival marginal position (GMP)
3. Probing pocket depth (PPD)^[12]
4. Clinical attachment level (CAL)^[13]

Study casts were made for fabrication of customized acrylic occlusal stents which were used as a fixed reference point (FRP) for clinical assessment at baseline, 3 months, and 6 months recordings. The recordings were made using the University of North Carolina 15 probe (Hu-Friedy UNC-15).

RADIOGRAPHIC ASSESSMENT

- A. Distance from CEJ to base of the defect
- B. Distance from CEJ to the alveolar crest
- C. Distance from the alveolar crest to the base of the defect

The intraoral periapical radiographs (IOPA) were taken using long cone paralleling technique and film holder. A standardized X-ray grid was placed in front of the IOPA film while taking the radiograph of the selected teeth.

SURGICAL PROCEDURE

The indicated operative site was anesthetized with 2% lignocaine HCl with adrenaline (1:80,000) using block and infiltration techniques. Sulcular incisions were performed using the Bard-Parker handle with a blade no. 12, including only buccal aspect following the gingival margin of the teeth. In the interproximal area (i.e., at the level of the interdental papilla) overlying the intraosseous defect, an oblique or horizontal incision was made following the profile of the underlying bone crest.^[14]

After reflection of the flap with complete visibility of the underlying osseous defect, a thorough granular debridement of soft tissue was performed using area-specific Gracey curettes and universal curettes (Hu-friedy). For Group I, the defects were filled with PRF and GTR membrane (Periocol®-GTR

25 × 30 mm (PGMS 16J110) Eucare Pharmaceuticals (P) Limited, Chennai-India. www.eucare.in] was placed [Figure 1]. For group II, the defects were filled with bovine bone graft [Cerabone®, grain size 0.5–1.0 mm (16JA10090), Botiss Dental, Berlin, Germany] and IMP [by Lifecare Devices Private Limited, Vasai (W), Thane, Maharashtra] mixed with PRF and covered with GTR membrane [Periocol®-GTR (PGMS 16J110) Eucare Pharmaceuticals (P) Limited, Chennai, India, www.eucare.in] [Figure 2].

The reflected mucoperiosteal flaps were approximated using 4-0 silk sutures after regenerative therapy. The interrupted simple loop sutures were taken to achieve primary closure of the interdental space. All surgically treated patients received systemic antibiotic therapy (amoxicillin 500 mg thrice daily) for 7 days and analgesic (aceclofenac 100 mg + paracetamol 325 mg twice daily) for 3 days to prevent postoperative pain and edema and discomfort.

Postoperative instructions were given to all the patients and were recalled to the department after 24 h and then

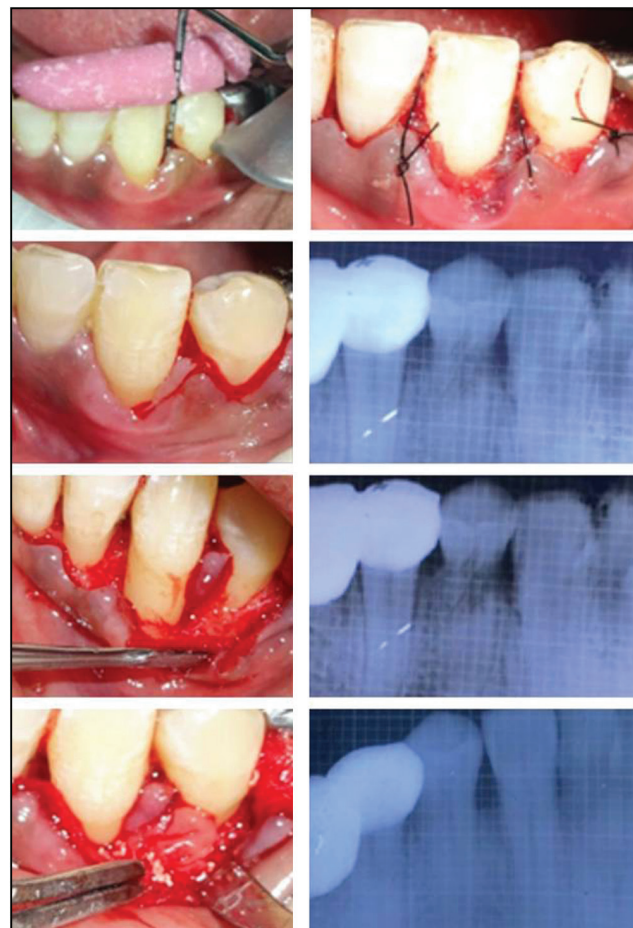


Figure 1: Clinical and radiographic images of Group I patient at Baseline, 3 months and 6 months

after 7 days sutures were removed. Any indication of swelling, infection, displacement of flap, hematoma, and necrosis was assessed at 1 week postsurgery.

Patients were reassessed weekly for 1-month postsurgery. At 3 and 6 months, all clinical and radiographic assessments carried out preoperatively were re-assessed.

STATISTICAL ANALYSIS

A paired *t*-test was done to compare the average value at baseline, 3, and 6 months (Intragroup comparison). An independent *t*-test was done to compare average values between the two groups (intergroup comparison). Throughout the results, the significance level is fixed at 5%, i.e., if $P < 0.05$, it is considered as a significant result. The data were analyzed using the STATA MP-13 software. [$P \leq 0.05$ = significant (S), $P \leq 0.001$ = highly significant (HS), $P > 0.05$ = not significant (NS)].

RESULTS

This randomized controlled clinical trial was designed to compare the regenerative potential of PRF alone and in combination with bovine bone graft in intraosseous defect by the SFA.

A total of 32 intrabony defects fulfilling the inclusion criteria were randomized into two groups. All defect site patients turned up for the 3-month and 6-month follow-up [Figure 3].

Throughout the study, there were no infectious episodes and no other adverse complications in any of the groups. No graft material could be detected outside the defects either at the time of suture removal or at subsequent recall visits in any of the groups.

There was a highly significant reduction in the Gingival index (GI) score from baseline to 3 months to 6 months for both the groups ($P < 0.001$). Improvement in

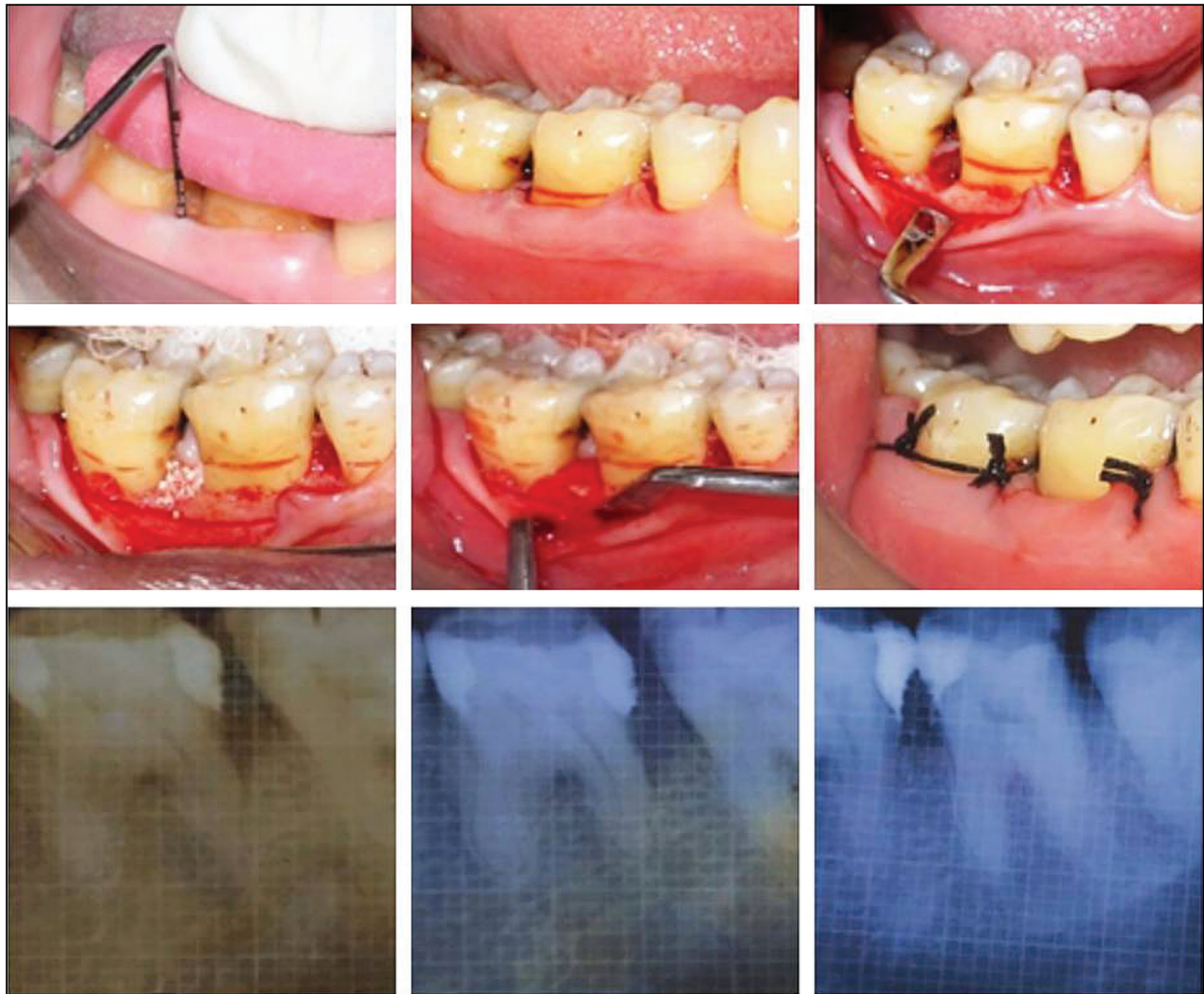


Figure 2: Clinical and radiographic images of Group II patient at Baseline, 3 months and 6 months

the gingival condition was contributed due to the maintenance of optimum oral hygiene by the patient and frequent oral prophylaxis performed.

For Group I (PRF alone), there was a statistically significant difference in PD reduction (2.94 ± 1.2), CAL gain (2.19 ± 0.9), gingival recession (-0.8 ± 1.2), defect fill (1.25 ± 0.5), change in alveolar crest level (-1 ± 0.5), and defect depth resolution (2.25 ± 0.8) when compared from baseline to 3 months to 6 months.

For Group II (PRF + bovine bone graft), there was a statistically significant difference in PD reduction (3.63 ± 1.5), CAL gain (2.19 ± 0.9), gingival recession (GR) (-0.75 ± 0.8), defect fill (2.31 ± 0.8), and defect depth resolution (3.44 ± 1.03) when compared from baseline to 3 months to 6 months.

The clinical parameters on intergroup comparison, that is when Group I (PRF alone) was compared to Group II (PRF + bovine bone graft), there was a significant difference in pocket depth (PD) reduction in Group II at 3 months to 6 months with P value 0.033. There was no significant difference in CAL and GR level between the two groups when compared at baseline, 3 months, and 6 months [Table 1].

The radiographic parameters for intergroup comparison showed a significant difference between two groups for defect fill (A) with higher values in Group II at P value < 0.001 . There was no significant difference in changes in the alveolar crest level (B) between the two groups. Group II showed higher significant values with P value < 0.001 for defect depth resolution (C) when compared with Group I [Table 2].

DISCUSSION

The primary rationale of periodontal therapy is to arrest and restore the functional healthy dentition. A convincing number of clinical studies have shown that these goals are attainable, provided that one adheres to certain fundamental principles. Moreover, these results can be achieved by a number of different therapeutic approaches like bone grafts, GTR, root conditioning, tissue-engineered products such as EMP's (enamel matrix proteins), growth factors either alone or in adjunct.

Growth factors obtained from platelets have an advantage over other factors which are native or recombinant factors, because a large number of growth factors are easily available in significant amount upon

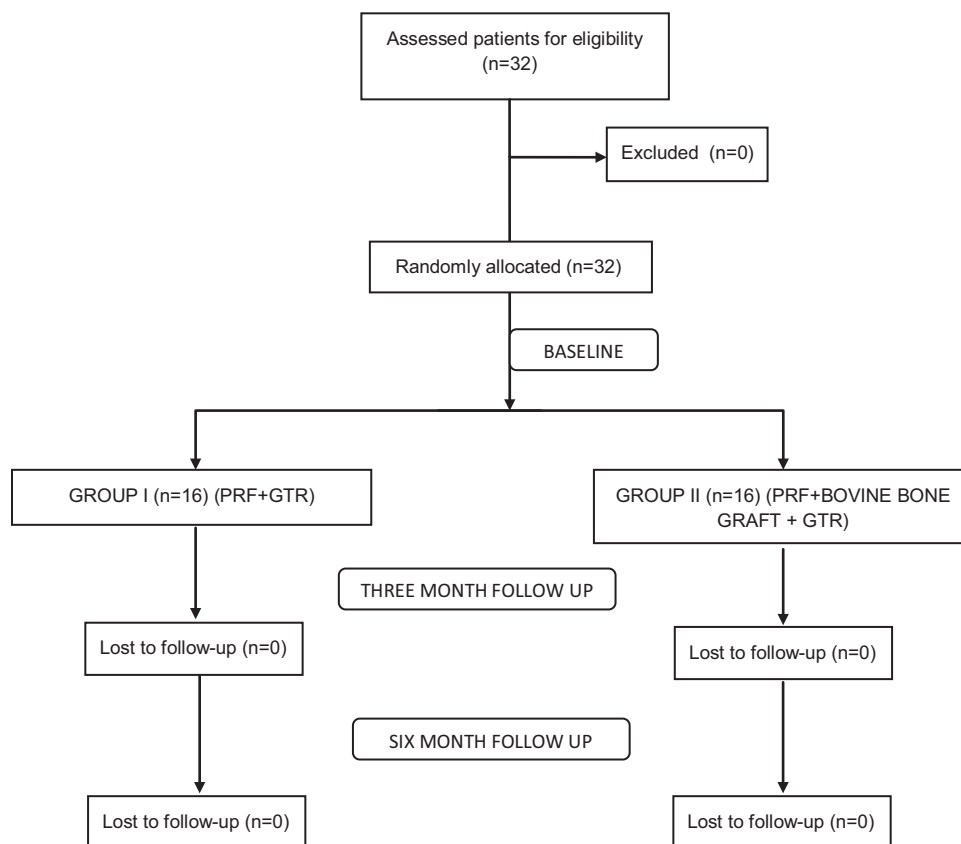


Figure 3: Study flow chart. n = number of patients

Table 1: Clinical parameters measured at baseline, 3 months, and 6 months

	Group no.	N	Mean	Std. deviation	T	Df	P-value
PD-0	I	16	6.69	1.138	-2.802	30	0.009
	II	16	8.13	1.708			
PD-3	I	16	4.44	0.814	-2.997	30	0.005
	II	16	5.63	1.36			
PD-6	I	16	3.75	0.931	-1.732	30	0.094
	II	16	4.5	1.461			
PD0-PD3	I	16	2.25	1.065	-0.62	30	0.54
	II	16	2.5	1.211			
PD0-PD6	I	16	2.94	1.237	-1.415	30	0.167
	II	16	3.63	1.5			
PD3-PD6	I	16	0.69	0.602	-2.236	30	0.033
	II	16	1.13	0.5			
CAL-0	I	16	7.44	1.59	-1.52	30	0.139
	II	16	8.44	2.097			
CAL-3	I	16	6.06	1.482	-1.229	30	0.229
	II	16	6.81	1.94			
CAL-6	I	16	5.25	1.438	-0.109	30	0.914
	II	16	5.31	1.778			
CAL0-CAL3	I	16	1.38	0.719	-0.835	30	0.41
	II	16	1.63	0.957			
CAL0-CAL6	I	16	2.19	0.981	-1.872	23.61	0.074
	II	16	3.13	1.746			
CAL3-CAL6	I	16	0.81	0.75	-1.635	22.013	0.116
	II	16	1.5	1.506			
GR-0	I	16	0.81	1.167	0.727	25.124	0.474
	II	16	0.56	0.727			
GR-3	I	16	1.31	1.195	1.214	26.306	0.236
	II	16	0.88	0.806			
GR-6	I	16	1.63	1.408	0.737	26.254	0.468
	II	16	1.31	0.946			
GR0-GR3	I	16	-0.5	0.966	-0.627	30	0.535
	II	16	-0.31	0.704			
GR0-GR6	I	16	-0.81	1.223	-0.167	30	0.868
	II	16	-0.75	0.856			
GR3-GR6	I	16	-0.31	0.602	0.574	30	0.57
	II	16	-0.44	0.629			

PD = pocket depth, CAL = clinical attachment level, GR = gingival recession

Bold values in the table represent statistical value according to the data acquired.

platelet activation. Growth factors may interact with each other, forming a cascade of different signal proteins with multiple pathways, ultimately leading to the activation of gene expression and then protein production.^[15]

While considering the technicality of periodontal reconstructive procedures for intraosseous defects, two major factors like elimination or reduction of postsurgical infection, blood clot contamination, and the implanted biomaterial, and the second is to minimize the postoperative gingival recession on the treated tooth. In addition, esthetic impairments could lead to loss of the interdental papilla which may result in food impaction.^[14] To overcome these

surgical complications, in the present clinical study, the SFA technique was carried out.^[4] The case series demonstrated that an SFA for periodontal regeneration with a collagen membrane and bone grafts is minimally invasive, resulting in significantly decreased PD with minimum GR and gain in CAL at the defect sites for 15 months.^[16]

Deproteinized bovine bone minerals (DBBMs) are inherently osteoconductive in nature which has a potential appeal to act as a bone graft substitute. Xenogenic bone material represents an unlimited availability for transplantation into human host.

In humans, intrabony defects treated with bovine bone grafts and collagen membranes showed clinical and

Table 2: Radiographic parameters measured at baseline, 3 months, 6 months

	Group no.	N	Mean	Std. deviation	T	Df	P-value
A0	I	16	6.56	1.711	-2.007	30	0.054
	II	16	7.69	1.448			
A3	I	16	5.56	1.59	-2.092	30	0.045
	II	16	6.69	1.448			
A6	I	16	5.31	1.662	-0.113	30	0.911
	II	16	5.38	1.455			
A0-A3	I	16	1	0.516	0	15	1
	II	16	1	0			
A0-A6	I	16	1.25	0.577	-4.06	26.011	<0.001
	II	16	2.31	0.873			
A3 -A6	I	16	0.25	0.447	-4.332	22.362	<0.001
	II	16	1.31	0.873			
B0	I	16	3.31	0.793	-0.159	30	0.875
	II	16	3.38	1.36			
B3	I	16	3.688	0.8732	-0.727	30	0.473
	II	16	3.938	1.0626			
B6	I	16	4.31	1.014	-0.575	30	0.57
	II	16	4.56	1.413			
B0-B3	I	16	-0.38	0.5	1.048	30	0.303
	II	16	-0.56	0.512			
B0-B6	I	16	-1	0.516	1	30	0.325
	II	16	-1.19	0.544			
B3-B6	I	16	-0.63	0.5	0	30	1
	II	16	-0.63	0.619			
C0	I	16	3.19	1.424	-2.295	30	0.029
	II	16	4.25	1.183			
C3	I	16	1.94	0.998	-2.591	30	0.015
	II	16	2.81	0.911			
C6	I	16	0.94	0.998	0.384	30	0.703
	II	16	0.81	0.834			
C0-C3	I	16	1.25	0.683	-0.878	30	0.387
	II	16	1.44	0.512			
C0-C6	I	16	2.25	0.856	-3.545	30	0.001
	II	16	3.44	1.031			
C3-C6	I	16	1	0.516	-4.899	30	<0.001
	II	16	2	0.632			

A = CEJ to base of the defect, B = CEJ to alveolar crest of defect, C = defect depth resolution

Bold values in the table represent statistical value according to the data acquired.

radiographic improvement at the defect site.^[17] In our present clinical trial, we have assessed clinically and radiographically the efficacy of bovine bone graft with PRF in intrabony defect.

Guided tissue regeneration allows regeneration of bone structure, PDL, and cementum around teeth. Resorbable collagen materials carry advantages, including hemostasis, chemotaxis for the formation of PDL fibroblasts and gingival fibroblasts, weak immunogenic function, easier manipulation, and ability to increase tissue thickness. Our present clinical trial comprised of using a resorbable collagen membrane for both the groups; Group I and Group II.

The clinical parameters are the indirect measurement of the amount of regeneration occurring at the sites.

Radiographic bone measurements after regenerative procedures are a noninvasive, painless method to identify bone measurements. The conventional radiographic method is the simplest and most cost-effective radiographic technique but, due to a two-dimensional approach, it has limited sensitivity and potential geometric distortion. One approach designed to avoid these potential errors is taking standardized radiographs to establish reproducible source tooth film geometry. In the present study, radiographic evaluation of the defect fill was done using intraoral periapical radiographs using a long cone paralleling technique with a standardized x-ray grid. Using such a grid provides an accurate way of assessing changes in bone height of less than 1 mm.

CONSIDERING THE RESULTS CLINICALLY AND RADIOGRAPHICALLY OBTAINED FROM THE PRESENT STUDY

For Group I, there was a statistically significant difference in PD reduction (2.94 ± 1.2), CAL gain (2.19 ± 0.9), gingival recession (-0.8 ± 1.2), defect fill (1.25 ± 0.5), change in alveolar crest level (-1 ± 0.5), and defect depth resolution (2.25 ± 0.8) when compared from baseline to 3 months to 6 months. Sharma *et al.*^[18] treated intrabony defects with PRF + open flap debridement and open flap debridement alone. They revealed significant pocket depth reduction (4.55 ± 1.87) and gain in CAL (3.31 ± 1.76) in intrabony defects treated with PRF only compared to open flap debridement alone (3.21 ± 1.64) reduction in pocket depth and (2.77 ± 1.44) gain in CAL.

Panda *et al.*^[19] treated 16 patients with 32 intrabony defects by resorbable collagen membrane adjunct to PRF in Groups 1 and 2 with collagen membrane alone. They revealed statistically significant improvement for probing depth ($P < 0.002$), clinical attachment level ($P < 0.001$), and radiographic defect depth ($P < 0.001$) after 9 months for Group 1 as compared with Group 2. Radiographic defect depth reduction was $58.19 \pm 13.24\%$ in Group 1 as compared with a $24.86 \pm 9.94\%$ reduction in Group 2. Ahmad *et al.*^[20] treated 36 patients with single intrabony defects by platelet-rich fibrin + minimal invasive surgical technique in Group 1 and minimally invasive surgical technique in Group 2. The study revealed an increase in probing pocket depth 4.12 ± 0.95 mm, gain in CAL 4.062 ± 1.63 mm, gain in bone level.

For Group II, there was a statistically significant difference in PD reduction (3.63 ± 1.5), CAL gain (2.19 ± 0.9), gingival recession (-0.75 ± 0.8), defect fill (2.31 ± 0.8), and defect depth resolution (3.44 ± 1.03) when compared from baseline to 3 months to 6 months which are in accordance with the study done by Lekovic *et al.*^[21] who revealed a significantly greater reduction in pocket depth in the PRF + bovine bone graft group (4.47 ± 0.78) when compared with the PRF group (3.35 ± 0.68 mm). The PRF + bovine bone graft group presented with significantly greater attachment gain (3.82 ± 0.78 mm) than the PRF group (2.24 ± 0.73 mm) which is due to bovine bone graft maintains the space for tissue formation to occur and works as a scaffold for the growth of mineralized tissue.

In our study, there were significant radiographic changes for defect fill, defect depth resolution was seen in Group II (PRF+ Bovine bone) when compared to Group I (PRF) at baseline, 3 months, and 6 months which attributes to the physical characteristic of bone grafting

material. This could be due to bovine bone that can enhance bone formation due to its osteoconductivity and has been used alone or in combination with other agents in the regenerative treatment of intrabony and furcation defects.

CONCLUSION

From the present study, we hereby draw the following conclusion:

- 1) Both the groups showed significant improvement in all clinical and radiographic parameters over the baseline measurement to 3 months to 6 months.
- 2) All regenerative materials were well tolerated by the patients and none of the patients exhibited pain, swelling, discomfort, or any other allergic reaction or abnormal wound healing characteristics.
- 3) Bovine bone graft material being largely and easily available, it was well tolerated by the patient which also exhibits osteoconductive activity, not being technique sensitive can be considered efficient for the treatment of infrabony defects.
- 4) On intergroup comparison, the combination of PRF and bovine bone graft showed significant bone fill and defect depth resolution than PRF placed alone.

LIMITATIONS

Within the limits of the present study, it can be concluded that the combination of PRF and bovine bone graft is effective in improving the clinical and radiological parameters.

However, the data from the study were derived from a 6-month observation period, which is probably a short-time interval when one considers the long-term success of regenerative procedures. Long-term studies with a larger sample size are required to evaluate whether or not the results obtained in the present study are sustainable over a long period of time. Also, histological examination being the ideal method of assessment of regenerative potential is needed.

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FINANCIAL SUPPORT AND SPONSORSHIP

Nil.

CONFLICTS OF INTEREST

There are no conflicts of interest.

AUTHORS CONTRIBUTIONS

Not applicable.

ETHICAL POLICY AND INSTITUTIONAL REVIEW BOARD STATEMENT

The study was approved by BUETHICS committee.

PATIENT CONSENT STATEMENT

The authors certify that they have obtained all appropriate patient consent forms. In the form, the patient(s) has/have given his/her/their consent for his/her/their images and other clinical information to be reported in the journal. The patients understand that their names and initials will not be published and due efforts will be made to conceal their identity, but anonymity cannot be guaranteed.

DATA AVAILABILITY STATEMENT

Not applicable.

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