

## Effect of Bone Graft with or without Platelet-Rich Fibrin Membrane in Vertical Bony Defects

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### ABSTRACT

**Introduction:** Alloplasts have been successfully utilised in periodontal regeneration with its osteoconductive effect. Platelet-rich fibrin (PRF) is a second-generation platelet concentrate, which has been found to release various growth factors, therefore, has the potential to be used as a regenerative treatment for periodontal defects.

**Objective:** The objective was to evaluate the effect of alloplastic bone graft material, 30%  $\beta$ -tricalcium phosphate and 70% Hydroxyapatite (30%  $\beta$ -TCP+70% HA) with and without PRF membrane in the treatment of vertical bony defects.

**Materials and Method:** Twenty patients (10 patients per group, one site per patient) were randomised into group A (30%  $\beta$ -TCP+70% HA and PRF membrane) and group B (30%  $\beta$ -TCP+70% HA). Pocket depths (PDs), clinical attachment levels (CALs) were recorded at baseline, three months, six months, and nine months post-operatively. Similarly, radiographic evaluations were done at baseline, three months, six months, and nine months to evaluate the defect fill.

**Result:** Significant reduction in all clinical and radiographic parameters were observed in both the treatment groups. Post-surgical measurements revealed a greater reduction in PD (3.8 mm), greater CAL gain (3.7 mm), and greater defect fill (1.45 mm) in group A as compared to group B with PD reduction (3.4 mm), CAL gain (3.2 mm) and, defect fill (1.23 mm), with no statistically significant difference between the groups.

**Conclusion:** PRF membrane in addition to alloplast (30%  $\beta$ -TCP+70% HA) did not provide additional benefit in terms of PD, CAL, and defect fill in the treatment of vertical bony defects in periodontitis patients.

**Keywords:** Bone graft; bony defect; periodontal regeneration; platelet-rich fibrin membrane.

### INTRODUCTION

Periodontal destruction compromises long-term prognosis of teeth by producing bony defects. Synthetic alloplastic materials are believed to promote the healing of bony defects through osteoconduction,<sup>1</sup> with advantages of availability, ease of manipulation, storage, and host acceptability.<sup>2</sup> The amount, frequency and predictability of periodontal regeneration have been promised with a combination of osteogenic material

and epithelial exclusion techniques.<sup>3</sup> Membrane delays apical migration of epithelium by excluding gingival connective tissue and allows granulation tissue derived from the periodontal ligament and osseous tissues to repopulate the space adjacent to the root surface.<sup>4</sup> Physical support is provided by filling material to the membrane maintaining its position. Thus, having the advantage of epithelial exclusion and scaffold effect of the bone graft.<sup>5</sup> Platelet-rich fibrin (PRF), known to accelerate wound healing through cell migration, proliferation,

with supportive effect on the immune system, can be formed as a regenerative membrane.<sup>6</sup> The rationale of using PRF membrane over bone graft is to prevent epithelial migration along with enhanced regeneration due to the release of various growth factors. The objective of this study is to compare the clinical and radiographic outcome of alloplastic material, 30%  $\beta$ -TCP+70% HA with and without PRF membrane in the treatment of vertical bony defects.

## MATERIALS AND METHOD

The study was conducted at the Department of Periodontology and Oral Implantology, People's Dental College and Hospital, Kathmandu, Nepal from June 2017 to June 2018. Ethical clearance was taken from the Institutional Review Board, Institute of Medicine and written informed consents were taken from all participants. It was a randomised clinical trial, where 20 systemically healthy patients (13 males, seven females) of age 25-50 years with vertical bony defects were enrolled. The inclusion criteria were maxillary and mandibular molars and premolars, interproximal probing depths (6-8 mm) when evaluated one week after initial therapy, two or three-walled vertical defects, Plaque index following initial therapy had to be <1.<sup>7</sup> Radiographic defect depth 3-4 mm as revealed by intraoral periapical radiograph (IOPA-R).

The exclusion criteria were patients contraindicated for periodontal surgery, patients under medications known to interfere with periodontal regeneration, pregnant, lactating mothers, smoking habit, grade II or III mobility, third molars, endodontic-periodontic lesions, patients undergoing orthodontic treatments, patients having antibiotics six months prior, and periodontal therapy in preceding 12 months.

Pre-surgical Therapy: Initial therapy consisted of patient education, motivation, and oral hygiene

instructions. Scaling, root planing was performed using Gracey curettes and an ultrasonic instrument. Patients were recalled after one week to confirm the suitability of the site for study. Patients were then randomised into group A (patients treated with bone graft and PRF membrane) and group B (patients treated with bone graft only) based on computer-generated random block.

Clinical and Radiographic parameters measured: An individual acrylic resin occlusal stent was fabricated to create fixed landmarks and standardise the location of the periodontal probe. Grooves were made on stents so that measurements could be made post-surgery using the same probe position and angulation as those made before surgery. Using groove as a guide, pocket depths (PDs) and clinical attachment levels (CALs) were measured (Figure 1). Measurements were performed using University of North Carolina-15 (UNC-15) (Hu Friedy, Chicago, IL, USA) periodontal probe.

Clinical variables recorded were Plaque Index (PI),<sup>7</sup> Gingival Index (GI)<sup>8</sup> and Oral Hygiene Index-Simplified (OHI-S index).<sup>9</sup>

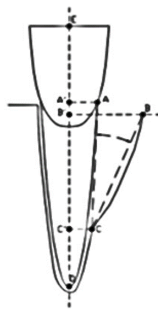
Long cone parallel technique with the metallic radiographic stent was used for radiographic standardisation (Figure 2). Two metallic wires (24 gauge, length 10 mm) were attached on an IOPA-R in vertical and horizontal directions. The bony defect was measured following the criteria described by Schei et al. (1959)<sup>10</sup> and Bjorn et al. (1969)<sup>11</sup> (Figure 3,4). If the distance between points B and C was found to be 3-4 mm, with pocket depth 6-8 mm in at least one of the interproximal aspects of the tooth (buccal or lingual), the defect was considered suitable for the analysis.<sup>12</sup> Following formula was used to evaluate the radiographic image distortion:



**Figure 1: Measurement of PD and CAL using occlusal stent and groove.**



**Figure 2: IOPA-R film with metallic stent.**



**Figure 3: Reference points for radiographic analysis.**

A'-B': Horizontal component of the defect.

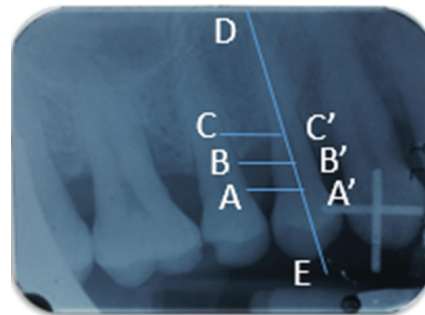
B'-C': Vertical component of the defect.

(Reference: J Clinical Periodontal. 2015; 42:853-859)<sup>12</sup>

$$\frac{\text{Actual defect height}}{\text{Radiographic defect height}} = \frac{\text{Actual stent height}}{\text{Radiographic stent height}}$$

Preparation of PRF membrane: PRF was first prepared according to the protocol given by Choukroun et al. 2001.<sup>13,14</sup> Prior to surgery, 10 ml of blood was drawn by venipuncture of the patient's antecubital vein. Blood sample was then transferred in the sterile glass test tubes (10 ml) and was centrifuged in centrifuging machine (ISO9001, ISO 13485, GMP, FDA, CE MARKING, Gemmy Industrial Corp. Taipei, Taiwan) at 3000 rpm for 10 minutes.

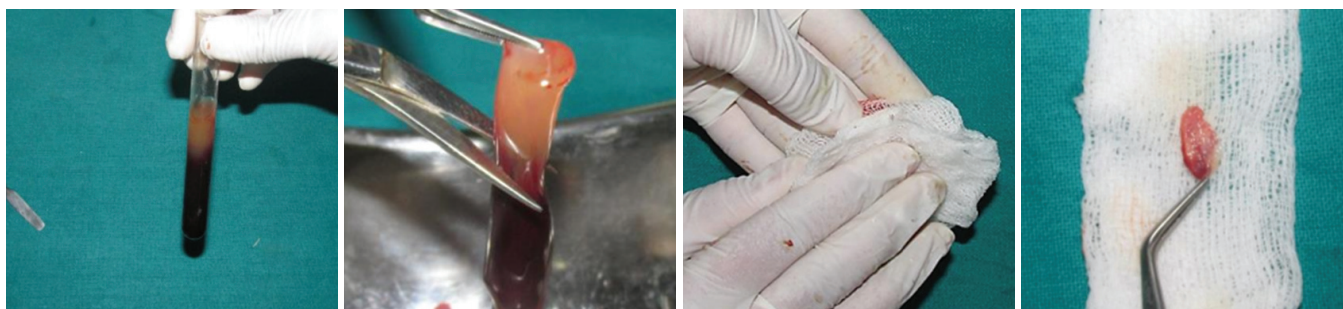
The centrifuged blood mass presented with a structured fibrin clot in the middle of the tube, between the red corpuscle layer on the bottom and the acellular plasma on top. The fibrin clot was then obtained preserving a small red blood cell (RBC) layer using a sterile tweezer and scissor. A stable fibrin membrane was prepared by squeezing serum out of wet gauze<sup>15</sup> (Figure 5).



**Figure 4: Reference line drawn on IOPA- R.**

Surgical Procedures: Prior surgery, extraoral antiseptics was maintained using povidone-iodine solution. Patients were asked to rinse with 0.2% chlorhexidine digluconate. The surgical site was anaesthetised by 2% lidocaine infiltration with epinephrine (1:100,000). Buccal and lingual sulcular incisions were made extending one tooth mesially and distally of the tooth being treated. Full-thickness mucoperiosteal flaps were elevated. Interproximal soft tissues were preserved as much as possible. With the help of Gracey curettes, defects were debrided and root surfaces were planed.

In group A, after defect debridement, black silk suture (4-0 Ethicon) was passed through buccal and lingual flap before the graft placement. Bone graft used was GENOSS OSTEON II (particle size: 0.2-0.5 mm, volume: 0.25 cc). Bone graft was mixed as per the manufacturer's instructions. The prepared bone graft was then placed to slightly overfill the defect to maximise regeneration and not to compromise the proper flap closure and vascular supply. PRF membrane was adapted over



**Figure 5: Preparation of platelet rich fibrin membrane.**



**Figure 6: Platelet rich fibrin membrane placed over grafted site in distal aspect of upper right first premolar.**



**Figure 7: Bone graft placed in distal aspect of upper left first premolar.**

the grafted defect (Figure 6). It was made sure that the membrane was extended over the periphery of the defect in the buccal and lingual direction. The mucoperiosteal flaps were secured in place with interrupted suture. The periodontal dressings were given.

In group B, surgical procedures were performed with the same surgical protocol, but only bone graft was placed after defect debridement (Figure 7). Capsule. Amoxicillin 500 mg thrice daily for five days, tablet. Ibuprofen 400 mg thrice daily for three days, Chlorhexidine mouth rinse (0.2%) twice daily for two weeks were prescribed. Periodontal dressing and sutures were removed two weeks postoperatively. GI, PI, OHI-S scores were recorded at baseline, one month, three months, six months, and nine months, postoperatively. PDs, CALs and

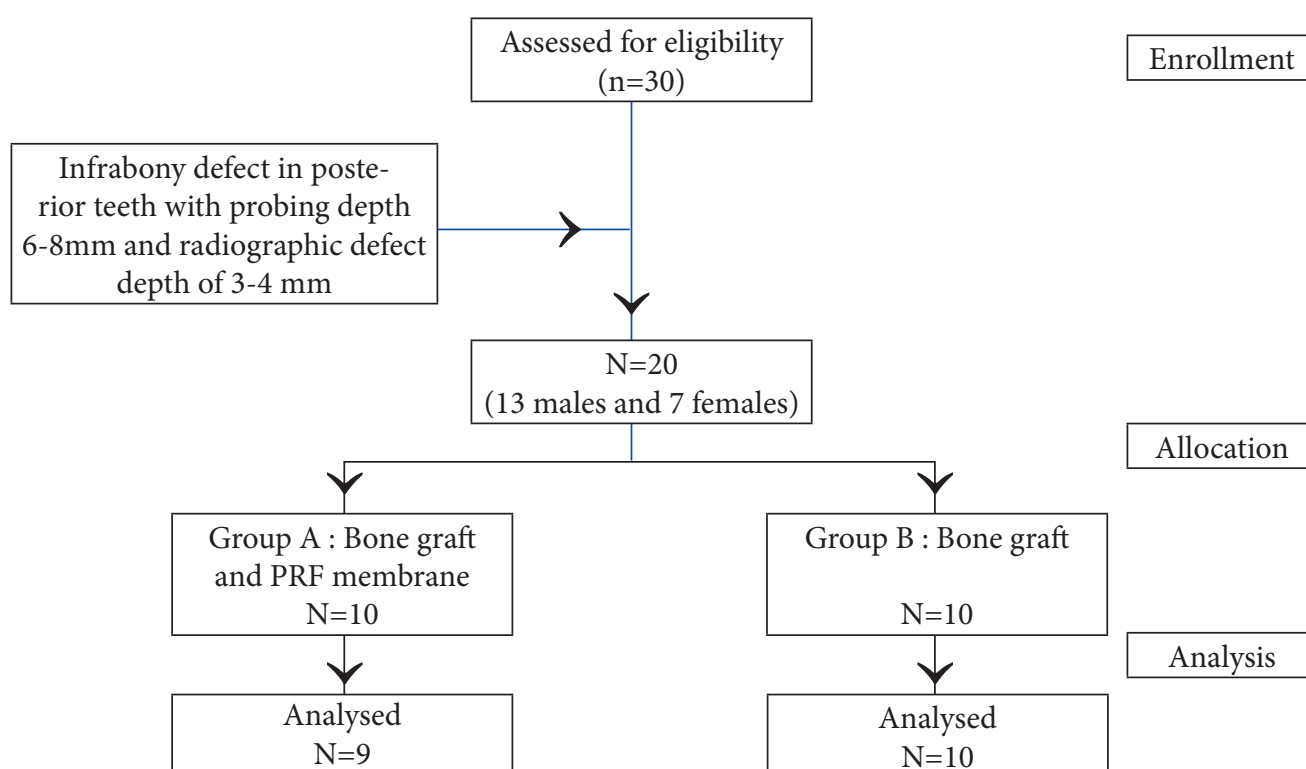
IOPA-R were recorded at baseline, three months, six months, and nine months.

Data was collected using SPSS Statistics for Windows, version 17.0 (SPSS Inc., Chicago, IL, USA). Clinical and radiographic outcomes between groups were compared at different time intervals with Independent T-test and Paired T-test. Level of significance and power of study at 5% and 80% respectively.

## RESULT

Out of 20 patients, nine patients completed the study in group A, while in group B, total 10 patients completed the study (Figure 8).

Changes in PD are reported in Table 1. Group A showed mean PD of  $7.40 \pm 0.84$  at baseline which



**Figure 8: Study flow chart.**

**Table 1: Comparison of probing depth between two groups.**

Probing depth	Group A (Mean±SD)	Group B (Mean±SD)	Mean diff.	95% CI of mean diff		P value
				Lower	Upper	
Baseline	7.40±0.84	7.20±0.78	0.200	-0.567	0.967	0.591
At 3 months	5.20±0.42	5.30±0.82	- 0.100	- 0.715	0.515	0.736
At 6 months	4.20±0.42	4.30±0.82	- 0.100	- 0.715	0.515	0.736
At 9 months	3.60±0.52	3.80±0.63	- 0.200	- 0.742	0.342	0.449

**Table 2: Comparison of clinical attachment level between two groups.**

Clinical attachment level	Group A (Mean±SD)	Group B (Mean±SD)	Mean diff.	95% CI of mean diff		P value
				Lower	Upper	
Baseline	7.40±0.84	7.20±0.78	0.200	-0.567	0.967	0.591
At 3 months	5.30±0.67	5.50±0.97	-.200	- 0.986	0.586	0.600
At 6 months	4.30±0.67	4.50±0.97	-.200	- 0.986	0.586	0.600
At 9 months	3.70±0.67	4.00±0.82	-.300	-0.1004	0.404	0.382

**Table 3: Comparison of radiographic vertical defect depth between two groups.**

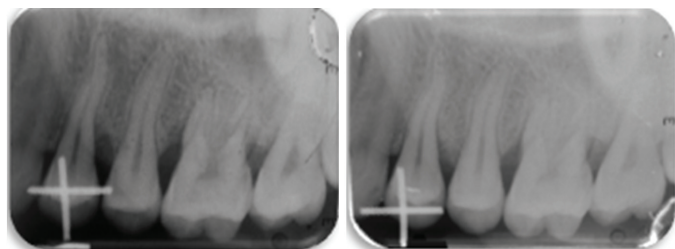
Vertical Defect	Group A (Mean±SD)	Group B (Mean±SD)	Mean diff.	95% CI of mean diff		P value
				Lower	Upper	
Baseline	3.55±0.43	3.45±0.43	0.10	- 0.311	0.511	0.616
At 3 months	2.69±0.39	2.66±0.33	0.03	- 0.314	0.374	0.857
At 6 months	2.35±0.41	2.25±0.35	0.10	- 0.260	0.460	0.567
At 9 months	2.10±0.39	2.22±0.31	-0.12	- 0.457	0.217	0.464

was reduced to 3.60±0.52 in nine months. In group B, mean PD of 7.20±0.78 at baseline was reduced to 3.80±0.63 in nine months. Mean PD reduction in group A and group B was 3.8 mm and 3.4 mm, respectively. The difference observed between the two groups was not statistically significant.

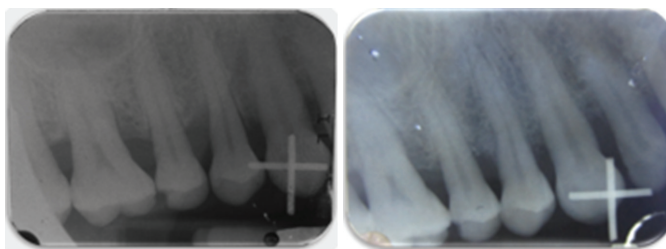
Similarly, changes in CAL for the two groups are reported in Table 2. Group A shows mean CAL 7.40±0.84 at baseline which was reduced to 3.70±0.67 in nine months. Similarly, in group B, mean CAL of 7.20±0.78 at baseline was reduced to 4.00±0.82 in nine months. The difference observed between the two groups was not statistically significant.

Changes in defect depth are reported in Table 3.

Group A showed mean defect depth 3.55±0.43 at baseline which reduced to 2.10±0.39 in nine months. In group B, mean defect depth was 3.45±0.43 at baseline which reduced to 2.22±0.31 in nine months. Reduction in defect depth in Group A was 0.86 mm, 1.20 mm, and 1.45 mm at three, six, and nine months respectively. Similarly, the reduction in Group B, was 0.79 mm, 1.20 mm, and 1.23 mm, respectively. At each follow up visit, the comparison of defect depth between two groups were not significantly different implying that both treatments were similar in terms of reducing defect depth (Figures 9,10). PI, GI and OHI-S scores were not significantly different between the two groups at baseline, one month, three months, six months, and nine months (Table 4,5,6).



**Figure 9: IOPA-R at baseline and nine months respectively in distal aspect of upper right first premolar (Group A).**



**Figure 10: IOPA-R at baseline and nine months respectively in distal aspect of upper left first premolar (Group B).**

**Table 4: Comparison of Plaque Index between two groups.**

Plaque Index	Group A (Mean±SD)	Group B (Mean±SD)	Mean diff.	95% CI of mean diff		P value
				Lower	Upper	
Baseline	0.77±0.14	0.78±0.10	-0.010	-0.1266	0.106	0.859
At 1 month	0.84±0.08	0.90±0.06	-0.060	-0.1314	0.011	0.095
At 3 months	0.95±0.07	0.96±0.08	-0.010	-0.083	0.063	0.777
At 6 months	1.14±0.12	1.03±0.14	0.110	-0.020	0.240	0.093
At 9 months	1.08±0.19	0.96±0.08	0.1200	-0.020	0.260	0.089

**Table 5: Comparison of Gingival Index between two groups.**

Gingival Index	Group A (Mean±SD)	Group B (Mean±SD)	Mean diff.	95% CI of mean diff		P value
				Lower	Upper	
Baseline	0.86±0.14	0.83±0.06	0.03	-0.075	0.135	0.556
At 1 month	0.94±0.08	1.00±0.06	- 0.06	-0.131	0.011	0.095
At 3 months	1.06±0.08	1.07±0.09	- 0.01	-0.094	0.074	0.806
At 6 months	1.25 ±0.11	1.13±0.14	0.12	-0.006	0.246	0.062
At 9 months	1.18±0.19	1.07±0.09	0.11	-0.033	0.253	0.123

**Table 6: Comparison of Oral Hygiene Index - Simplified between two groups.**

OHI-S	Group A (Mean±SD)	Group B (Mean±SD)	Mean diff.	95% CI of mean diff		P value
				Lower	Upper	
Baseline	0.910±0.09	1.03±0.14	-0.120	-0.240	0.005	0.051
At 1 month	1.16±0.09	1.22±0.06	- 0.06	-0.136	0.016	0.885
At 3 months	1.28±0.06	1.29±0.08	-0.01	-0.081	0.061	0.593
At 6 months	1.46±0.15	1.34±0.15	0.12	-0.024	0.264	0.452
At 9 months	1.42±0.21	1.30±0.08	0.12	-0.032	0.272	0.101

## DISCUSSION

The present study compared the efficacy of alloplastic bone graft (30% β-tricalcium phosphate+70% Hydroxyapatite) with and without PRF membrane in the treatment of vertical bony defects. Total of 20 patients (10 patients per group, one site per patient) were treated.

In both groups, the result demonstrated significant improvement in PD, CAL, and radiographic defect depth in nine months as compared to baseline. There was greater reduction in PD (3.8 mm), greater CAL gain (3.7 mm), and greater defect fill (1.45 mm) in bone graft with PRF membrane treated group as compared to bone graft treated group with PD reduction (3.4 mm), CAL gain (3.2 mm) and, defect fill (1.23 mm).

At nine months, the mean PD difference between the group was not statistically significant with 0.2 mm higher reduction in group A than group B. The

placement of graft material into the defect may have impeded penetration of periodontal probe without having gained in actual clinical attachment level.<sup>16</sup>

Similarly, after intervention at nine months, the mean difference in CAL between the groups was not statistically significant with 0.3 mm higher gain in group A than group B. The gain in CAL in both groups was attributed to the resolution of tissue inflammation, reformation of collagen fibers and a new attachment to the root surface and bone fill.<sup>17</sup>

Since defect fill is a desirable outcome of any periodontal regenerative therapy, radiographic evaluation for the changes in alveolar bone level was done. After nine months, the mean difference in bony defect between groups was not statistically significant with 0.12 mm higher in group A.

No postoperative complications were observed in any of the patients in either groups which supports the biocompatibility of alloplastic material and PRF membrane as highlighted in other studies.<sup>18,19</sup>

To our knowledge, there are very fewer studies reporting the comparison of bone graft and bone graft with PRF membrane in the treatment of vertical bony defects. Therefore, direct comparison with other studies is not possible.

The result of our study is in accordance with the study conducted by Kishore et al. who compared the regenerative potential of the  $\beta$ -tricalcium phosphate bone graft and bone graft with bioresorbable type I collagen membrane. Added benefit by combining alloplast and membrane was not observed statistically significant in terms of PD, CAL and defect fill.<sup>20</sup> In the same study, comparison between two groups revealed, the amount of PD reduction at six and nine months to be 0.6 mm and 0.1 mm respectively. In our study, while comparing group A and group B, the amount of PD reductions were 0.3 mm, and 0.2 mm respectively.

Similarly, on comparison between two groups, the amount of defect depth reduction at six and nine months were 0.3 mm and 0.2 mm respectively. Whereas in current study, comparing group A and Group B revealed, the amount of defect depth reduction to be 0 mm, 0.2 mm respectively.

Elgendy et al. conducted a study where nano-sized ceramics and nano-sized ceramics with PRF membrane were compared in intrabony defects. There was statistically significant reduction in PD, CAL gain, and increased bone density in the sites treated with nano-sized ceramics and PRF membrane as compared to nano-sized ceramics at six months, which was inconsistent with our study.<sup>21</sup>

In current study, the PRF membrane was used as a barrier membrane. The intended role of the PRF membrane was to contain the bone graft in the intrabony defect in the early phase of healing. It is unlikely, although not impossible, for the PRF membrane to have exerted a guided tissue regeneration effect on the wound. Similar to a natural clot, the PRF membrane is resorbed in approximately seven days,<sup>22</sup> which is a substantially shorter period of time than the four-six weeks necessary for guided tissue regeneration to occur.<sup>14,23</sup> Though PRF membrane release growth factors at initial healing phase, long-term release of growth factor from this preparation is impossible as well as there is no significant fibrin matrix to support growth factor release and cell migration.<sup>24</sup>

This might be the reasons for not having superior outcomes in bone graft and PRF membrane treated patients as compared to bone graft treated patient.

PI, GI, and OHI-S scores were monitored over a nine months healing period. There was a statistically significant increase in mean PI, GI and OHI-S scores in both groups. However, observed scores were under the range of fair oral hygiene and moderate gingivitis. Further, improvement in PI, GI score, oral hygiene maintenance regime, regular follow up visits, and reinforcement of oral hygiene instructions for patients throughout the study could have attributed to superior result in our study in both the groups than was obtained, as supported by other studies.<sup>25</sup> Further, long-term success following regenerative procedures in intrabony defects is directly related to compliance with maintenance visits and plaque control procedures.<sup>26</sup>

Since histologic examination, after surgical re-entry was not employed in our study, the nature of healing on the root surface was lacking, as obtained in another study.<sup>3</sup> Bone fill obtained from surgical re-entry is relevant to substantiate postoperative measurement data.<sup>27</sup> However, in current study, successive diagnostic quality IOPA-R was taken with parallel cone technique to minimise projection errors along with metallic stents for calculation of radiographic distortion.

Several defect characteristics have been related to responses following regenerative surgery. Deep intraosseous bony defects characterised by two and three-walled bony configurations show significant positive responses following GTR regenerative surgeries.<sup>28</sup> One recent study has reported much less fill in one wall component of treated defects versus the two and three-walled components.<sup>29</sup>

Radiographic defect angle was not taken into consideration in our study. However, there has been an impact on bone fill based on pre-surgical radiographic defect angle. There has been a positive impact on bone fill related to a narrower radiographic defect angle. Similarly, decreasing radiographic bone fill was related to increasing defect angle.<sup>30</sup> Since our results are based on a single-centred study with small sample sizes, further research is needed with histologic evidence of periodontal regeneration in defects treated with alloplast and PRF membrane with larger sample size, split-mouth design and multi-centred trial with a longer follow up period.

## CONCLUSION

The data from this study suggests that there is similar improvement in PD, CAL, and defect fill at sites treated with alloplastic bone graft material using PRF membrane and at sites treated with alloplastic bone graft material alone. It is observed that PRF membrane barrier in addition to alloplastic bone graft material did not provide additional benefit in terms of PD, CAL, and defect fill in treatment of vertical defects in periodontitis patients.

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**Conflict of Interest:** None.

JNDA

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