

**Comparison of bovine bone mineral to demineralized freeze- dried bone allograft in the treatment of periodontal infrabony defects: A Clinico-Radiographic Study**

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**Abstract**

**Background:** The present study was undertaken to compare and correlate bone regeneration potential of Demineralized Freeze-Dried Bone Allograft and Bovine Bone Mineral in the treatment of human infrabony defects.

**Materials and Methods:** 14 patients (aged 20-60 years) with an average age of  $41.8 \pm 2.3$  years with 28 surgical

sites present bilaterally were selected as part of split-mouth study design. Group-A was grafted with DFDBA while Group-B was grafted with bovine bone mineral. Various clinical and radiographic parameters viz. Plaque index (PI), Gingival index (GI), Probing pocket depth (PPD), clinical attachment level(CAL), Defect depth (DD) and linear bone fill (LBF) were recorded preoperatively, 3- and 6-months postoperatively.

**Results:** Both Group-A & Group-B sites exhibited a highly significant reduction in probing pocket depth, and gain in clinical attachment level at 3- & 6-months post-operatively while defect depth and linear bone fill showed improvement at the end of 6-months. Intergroup comparison showed no statistical difference between the materials for PI, GI, PPD, CAL, DD parameters while for sites grafted with bovine bone mineral showed a significant difference in the linear bone fill than the DFDBA group

**Conclusions:** Within the limits of this study, both the materials bovine bone mineral and DFDBA have shown promising results for the treatment of periodontal infrabony defects thus warranting long term studies with a larger sample size to explore their true regenerative potential to the maximum.

**Keywords:** Bovine bone mineral, demineralized freeze-dried bone allograft, infrabony defects, bone grafts.

### **Introduction**

Periodontitis, being one of the most common reasons for the loss of teeth, is the inflammation of the supporting periodontal tissues such as periodontal ligament, cementum and alveolar bone that extends from the marginal gingiva into these deeper structures.<sup>1</sup> Periodontal disease not only results in reduction of the alveolar bone height, but it also causes alteration in the morphologic features of bone leading to an array of osseous defects.<sup>2</sup> The disease is characterized by connective tissue attachment loss, apical migration of the junctional epithelium leading to periodontal pocket formation, progressive increase in the tooth mobility due to bone loss, ultimately causing tooth loss.<sup>3</sup> The alveolar bone deformities can include horizontal defects, periodontal infrabony defects, furcation defects, so on and so forth.

An infrabony defect is defined as a periodontal defect surrounded by one, two, or three bony walls or a combination of these.

The periodontal therapy aims to avert attachment loss and predictably re-establish the periodontal supporting structures that were lost because of disease or trauma such that the architecture and function of the lost structures can be re-stored.<sup>4</sup>

Bone grafting is one of the therapeutic modalities employed to fulfill the ideal goal of periodontal therapy-reconstruction of periodontal tissues that had been lost by disease process. Alveolar bone replacement materials for periodontal regeneration are categorized into one of four categories: Autogenous bone; allogeneic bone substitutes, such as freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA); xenogeneic; and alloplastic.

Ideal grafting material for regenerative procedures is autogenous bone graft but the major disadvantage with this graft is the need for secondary surgical site to procure donor material and frequent lack of intra-oral donor site to obtain sufficient quantities of graft for multiple or deep osseous defects.<sup>5</sup>

Hence to overcome these disadvantages, bone allografts were introduced as an alternative. There are two forms-Freeze-dried bone allograft (FDBA) and demineralized freeze-dried bone allograft (DFDBA). DFDBA, used for periodontal therapy in humans since 1975, not only provides osteoconductive surface, but also acts as a source of osteoinductive factors. Availability in adequate quantities, predictable results and elimination of an additional donor site for surgery are some of the few advantageous properties of DFDBA.<sup>1</sup>

A bone graft substitute based upon the inorganic component of bone, a natural derived porous bone mineral of bovine origin has been used in periodontal

therapy. Its morphology, porosity, internal surface, crystalline structure and chemical composition are reported to more closely resemble human cancellous bone as compared to DFDBA.<sup>2</sup>

Thus, the present study was undertaken to compare and correlate bone regeneration potential of Demineralized Freeze-Dried Bone Allograft and Bovine Bone Mineral in the treatment of human infrabony defects.

### **Materials and methods**

A split mouth randomized controlled clinical study was designed with 14 patients (aged 20-60 years) with an average age of  $41.8 \pm 2.3$  years having 28 surgical sites suffering from chronic/aggressive periodontitis with residual probing pocket depth of more than 6 mm following Phase I therapy and radiographically exhibiting infrabony defects of  $\geq 4$  mm present bilaterally were selected from Out Patient Department of Periodontology, Sardar Patel Postgraduate of Dental and Medical Sciences, Lucknow.

### **Pre-surgical therapy**

All subjects received a full diagnostic work up that included clinical examination, history recording and intra oral periapical radiograph with grid according to area of interest. Radiographs were standardized by using paralleling technique and holder, placing the grid in between, which revealed presence of interproximal angular bone loss and helped measure the dimensions of the defect. For measurement of the bone defect, the distance from the cemento-enamel junction to the base of the defect was measured on radiographic image. (Figure 6 & 11)

Subjects were instructed and demonstrated with oral hygiene measures. Non-surgical periodontal therapy by means of thorough scaling and root planing was performed. Patients were recalled and re-examination was done after 28 days after Phase I therapy.

Routine haematological investigations were performed for all the selected subjects. Surgical therapy was planned in patients with persistent periodontal pockets with probing depth of  $\geq 6$  mm after the initial phase I therapy.

### **Surgical procedure**

Prior to beginning with the surgical procedure, extraoral antisepsis was performed for all patients by painting the povidone iodine solution around the mouth. Intraoral antisepsis was performed using 10 ml 0.2% chlorhexidine digluconate solution for 1 minute as pre-procedural mouth rinse.

Following this, the local anesthetic solution was injected for the required block. Once the desired anesthetic effect was achieved, sulcular incisions were made using BP handles and surgical blades. Full thickness mucoperiosteal flaps were reflected with the help of periosteal elevators and a bloodless field was tried to achieve. Precise defect location was found and meticulous defect debridement with root planing was carried out with the help of curettes. The direct examination after debridement confirmed the presence of three-walled/ two-walled intraosseous defect.

To manipulate the graft for the ease of handling and condensability, the graft was mixed with saline/ patient's own blood in a glass dappen dish before condensing it into the defect site. An incremental filling of the defect was performed and the graft was condensed approximately 0.5mm apical to the alveolar crest. (Figure 2, 3, 4, 7, 8, 9)

In Group A patients, post defect debridement, the infrabony defect was filled with Demineralized freeze-dried bone allograft (DFDBA). (Figure 1)

In Group B patients, post defect debridement, the infrabony defect was filled with Bovine bone mineral (Bio-Oss®). (Figure 1)

The flap was re-adapted to its original position and suturing was done using 3-0 non-resorbable silk suture (MERSILK). Periodontal Dressing (Coe-Pak™) used to cover the surgical area. (Figure 5,10)

### Clinical Parameters

All subjects were subjected to assessment of following parameters at baseline, 3 months and 6 months of study: Plaque Index (PI) (Silness and Loe, 1964)<sup>6</sup>, Gingival Index (GI) (Loe and Silness, 1963)<sup>7</sup>, Periodontal Probing Depth (PPD) with the help of occlusal stent (from gingival margin to base of the pocket), Clinical Attachment Level (CAL) with the help of occlusal stent (cemento-enamel junction to the base of the defect), Defect Depth (DD), Linear Bone fill (LBF) using Intra Oral Periapical Radiograph with grid (at baseline and 6-month post-surgery) and the results obtained were statistically analyzed.

### Results

Observations revealed that both the materials were well-abided by all the patients with no adverse tissue reaction, infection or delayed healing reported during the study period.

The mean values of all the parameters namely, PI, GI, PPD, CAL, DD at baseline and 3-months & 6-months post-operatively are enumerated in Table I.

Intragroup comparison showing the mean reduction or gain in postoperative PI, GI, PPD, CAL, DD for both Group-A and Group-B from baseline to 3-months & 6-months post-operatively is summarized in Table II & III.

Table IV describes the intergroup comparison of PI, GI, PPD, CAL, DD for Group A and Group B at varied timelines i.e. at baseline, at 3-months & 6-months post-operatively.

A comparison in the mean scores of linear bone fill assessed radiographically for both Group A and Group B at 6-months post-operatively is presented in Table V.

Groups were compared by independent Student's t test. Groups were also compared by repeated measures two factor (groups and periods) analysis of variance (ANOVA) and the significance of mean difference within (intra) and between (inter) the groups were done by Tukey HSD (honestly significant difference) post hoc test after ascertaining normality by

Shapiro-Wilk's test and homogeneity of variance between groups by Levene's test. Discrete (categorical) groups were compared by chi-square ( $\chi^2$ ) test. A two-tailed ( $\alpha=2$ )  $p<0.05$  was considered statistically significant.

### Discussion

Studies in the recent past have shown a relative efficacy of xenograft material, that is, bovine bone mineral (Bio-Oss®) with respect to DFDBA individually. Hence the present study was carried out to evaluate and compare the regenerative potentials of these grafting materials in the treatment of infrabony defects. Periodic oral prophylaxis was performed so as to avoid formation of plaque, calculus deposits and debris on the grafted site as they could hamper the final outcome.

The selection of two- and three-walled infrabony defects was based on the results obtained from controlled clinical trials providing evidence that three-walled osseous defects allow better containment, stability, and increased blood supply to the graft as mentioned by Camargo PM et al. (2000).<sup>8</sup>

The intragroup comparison for Group A showed change in the plaque index scores from baseline to 3-months post-surgery i.e.  $0.972 \pm 0.02$  to  $0.974 \pm 0.02$  and to 6 month follow up period i.e.  $0.98 \pm 0.03$  respectively. This change in the plaque index scores were found to be statistically non-significant. Similar changes were observed in Group B wherein a non-statistically significant change was observed from baseline to 3-months post-surgery i.e. from  $0.973 \pm 0.03$  to  $0.98 \pm 0.02$

and remained constant at 6 month follow up period i.e.  $0.98 \pm 0.02$ . For each period, intergroup comparison showed that the difference in the mean plaque score for both the groups from baseline to 3 and 6 months were similar and statistically non-significant. The results obtained in the study were similar to the findings of Piemontese et al. (2008)<sup>9</sup> and Gothi et al. (2015).<sup>10</sup>

The intragroup comparison for Group A statistically non-significant reduction in the gingival index scores were observed from baseline to 3 months and 6 months. This was found to be from  $0.90 \pm 0.02$  at baseline to  $0.94 \pm 0.02$  at 3-months post-surgery and  $0.92 \pm 0.02$  at 6 month follow up period and in Group B wherein the reduction in the gingival score from baseline  $0.90 \pm 0.02$  to  $0.94 \pm 0.02$  at 3-month post-surgery and  $0.92 \pm 0.03$  at 6 month follow up was found to be statistically non-significant. For each period, intergroup comparison showed that the difference in the mean gingival index score for both the groups from baseline to 3 and 6 months were similar and statistically non-significant. The result obtained in the gingival index score and its maintenance in the present study was similar to the studies conducted by Caton JG et al. (2000)<sup>11</sup> and Gothi et al. (2015).<sup>10</sup>

A mean reduction of PPD from baseline  $8.00 \pm 0.33$  mm to  $4.79 \pm 0.30$  mm at 3 months postoperatively and  $2.93 \pm 0.29$  mm at 6 months follow up period was observed for Group A, which were found to be statistically significant, implying thereby, that substantial pocket depth reduction had taken place post-surgery. Similar trend of result has been found by Lovelace et al. (1998)<sup>12</sup> and Katuri et al. (2013)<sup>13</sup> in their study where the authors evaluated and compared the effectiveness of DFDBA against other grafting material in the treatment of infrabony defects and reported significant decrease in probing pocket depth post-surgery using DFDBA.

Group-B showed a mean reduction of PPD from  $8.43 \pm 0.25$  mm at baseline to  $4.50 \pm 0.25$  after 3 months postoperatively and was  $2.57 \pm 0.25$  after 6 months. The changes in between both the periods were statistically significant. On inter-group comparison, the difference in mean PPD did not differ significantly. These findings compare favorably with those found by Hutchens (1999)<sup>14</sup> and Yukna et al. (2000)<sup>15</sup> where the investigators reported significant decrease in PPD post-surgery using bovine bone mineral.

Through the result of this study, it can be established that the defects treated with bovine bone mineral, although statistically not significant, showed an average of 1 mm more probing pocket depth reduction. The net decrease (i.e. mean change from baseline to after 6 months) in mean PPD of Bio-Oss<sup>®</sup> group (69.5%) was found 6.1% higher as compared to DFDBA group (63.4%). This was found to be in accordance with the study conducted by Richardson et al. (1999)<sup>16</sup> wherein the authors also reported a similar trend in the PPD reduction when treated with bovine derived xenograft.

The DFDBA group showed substantial statistically significant gain in CAL from baseline ( $5.43 \pm 0.29$  mm) to at 3 months ( $3.21 \pm 0.24$  mm) postoperatively and to  $1.29 \pm 0.27$  mm at 6-months follow-up time period. The findings of the present study were in accordance with those of Pearson et al. (1981)<sup>17</sup> and Oreamuno et al. (1990).<sup>18</sup>

The Bio-Oss<sup>®</sup> group showed gain in CAL from baseline  $5.93 \pm 0.27$  mm to  $3.00 \pm 0.26$  mm at 3 months postoperatively and to  $0.93 \pm 0.16$  mm at 6 months follow up period, which was proved to be a substantial gain, statistically. On intergroup comparison, for each period, the difference in mean CAL showed similar ( $p > 0.05$ ) CAL between two groups at all periods i.e. did not differ

significantly. These findings are similar to those of Hutchens (1999)<sup>14</sup> and Yukna et al. (2000).<sup>15</sup>

Through the result of this study, it can be established that the defects treated with bovine bone mineral, although statistically not significant, showed an average of 1.1 mm more gain in the attachment levels. The net gain (i.e. mean change from baseline to after 6 months) in mean CAL of Bio-Oss<sup>®</sup> group (84.3%) was found 8.0% higher as compared to DFDBA group (76.3%). This was found to be in accordance with the study conducted by Richardson et al. (1999)<sup>16</sup> wherein the authors also reported a similar pattern in the mean attachment level gain when treated with bovine derived xenograft.

Group A showed a mean decrease in DD from baseline ( $5.21 \pm 0.24$  mm) to 6 months ( $3.07 \pm 0.25$  mm) post-surgery that was assessed radiographically using grid and was found to be a substantial gain, statistically. Group B group showed a mean decrease in DD from baseline ( $5.50 \pm 0.27$  mm) to 6 months ( $2.87 \pm 0.21$  mm) postoperatively, which was proved to be a substantial gain, statistically. On intergroup comparison, for each period, showed similar DD between two groups at both baseline and after 6 months i.e. did not differ significantly. From the result of this study, it could be inferred that, although not statistically significant, bovine bone mineral (Bio-Oss<sup>®</sup>) showed an average of 1 mm more defect depth resolution when used in treatment of infrabony defects as compared to DFDBA. The net decrease (i.e. mean change from baseline to after 6 months) in mean DD of Bio-Oss<sup>®</sup> group (48.1%) was found 7.0% higher as compared to DFDBA group (41.1%).

A mean linear bone fill (LBF) of  $2.14 \pm 0.10$  mm at 6 months postoperatively was obtained with the DFDBA group. The result was found to be in conformity with those of Richardson et al. (1999)<sup>16</sup>. Concurrent to the

findings of Yukna et al. (2000)<sup>15</sup>, in our study a mean linear bone fill of  $2.64 \pm 0.17$  mm at 6 months follow up period, assessed radiographically using grid, was obtained with the Bio-Oss<sup>®</sup> group. The mean LBF of Bio-Oss<sup>®</sup> group was slightly higher than DFDBA group. Comparing the mean LBF of two groups, showed significantly different and higher (18.9%) LBF of Bio-Oss<sup>®</sup> group as compared to DFDBA group. It was also observed that the rate of linear bone fill progressed in an almost uniform/similar rate over the entire study span.

The results of this study are in accordance with the studies of Richardson et al. (1999)<sup>16</sup> and Blaggana V et al. (2014)<sup>1</sup>, which compared DFDBA and Bio-Oss<sup>®</sup> in the treatment of infrabony defects, in all respects except the mean linear bone fill. In our study, the mean linear bone fill was found to be more significant with the Bio-Oss<sup>®</sup> group than with DFDBA.

### Conclusion

The results demonstrated that when compared to baseline data a significant improvement in defect parameters was seen with each material. Intergroup comparison showed no statistical difference between the materials for PI, GI, PPD, CAL, DD parameters while for sites grafted with Bove bone mineral showed a significant difference in the linear bone fill than the DFDBA group.

Within the limits of this study, both the materials bovine bone mineral (Bio-Oss<sup>®</sup>) and DFDBA have shown promising results for the treatment of periodontal infrabony defects thus warranting long term studies with a larger sample size to explore their true regenerative potential to the maximum.

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**Legend Figure and Tables**



Fig. 1: DFDBA and Bovine bone mineral (Bio-Oss®) as the regenerative



Fig. 2: Sulcular Incision placed (Group A)



Fig. 3: Flap reflection and debridement showing the osseous defect (Group B)



Fig. 4: DFDBA placed in the defect site (Group A)



Fig. 5: Suturing done at the surgical site (Group)



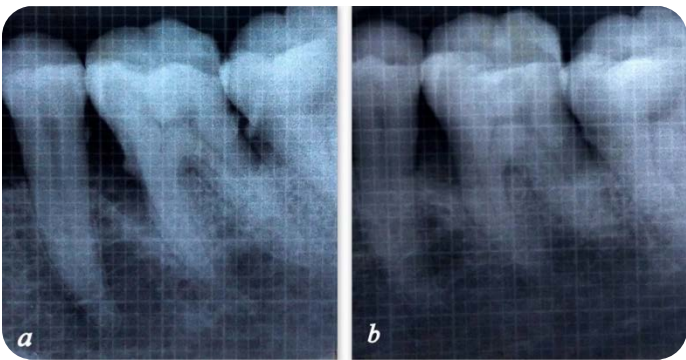


Fig. 6: Osseous defect fill after DFDBA placement (Group A) a- Baseline; b- 6-months post-operative

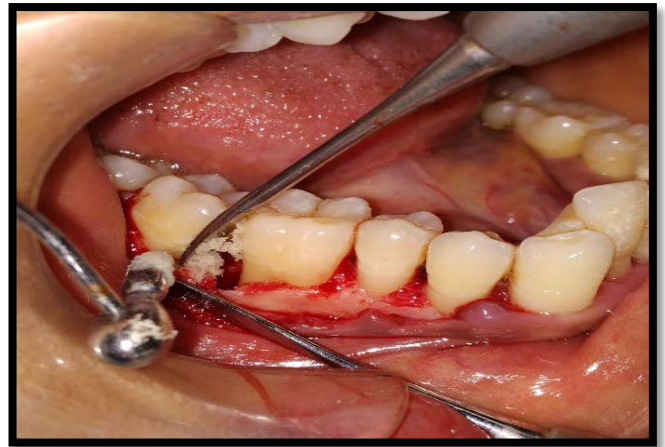


Fig 9: Bovine bone mineral (Bio-Oss®) placed in the defect site (Group B)



Fig. 7: Sulcular Incision placed (Group B)



Fig 10: Suturing done at the surgical site (Group B)

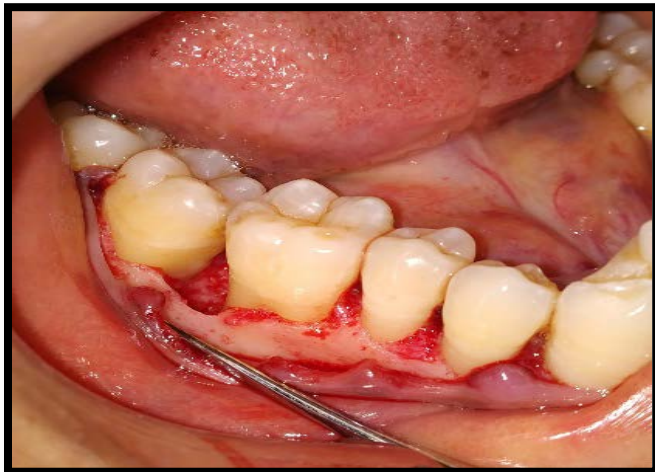


Fig. 8: Flap reflection and debridement showing the osseous defect (Group B)

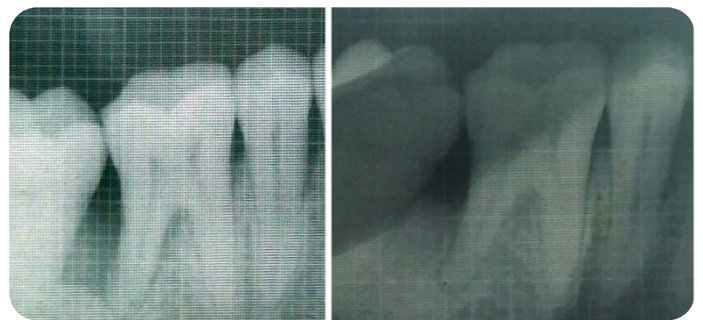


Fig 11: Osseous defect fill after Bio-Oss placement (Group B) a-Baseline; b- 6-months post-operative

Table 1: Mean scores of all the parameters namely PI, GI, PPD, CAL, DD (Mean ± SD) (in mm)

Parameters/ Timeline	Group A			Group B		
	Baseline	3 Months	6 Months	Baseline	3 Months	6 Months
PI	0.97±0.03	0.97±0.02	0.98±0.03	0.97±0.03	0.98±0.02	0.98±0.02
GI	0.90±0.02	0.94±0.02	0.92±0.02	0.90±0.02	0.94±0.02	0.92±0.03
PPD	8.00±0.33	4.79±0.30	2.93±0.29	8.43±0.25	4.50±0.25	2.67±0.25
CAL	5.43±0.29	3.21±0.24	1.29±0.27	5.93±0.27	3.00±0.26	0.93±0.16
DD	5.21±0.24	N/A	3.07±0.25	5.50±0.27	N/A	2.86±0.21

PI- Plaque index; GI- Gingival index; PPD- Probing pocket depth; CAL- Clinical attachment level; DD- Defect depth

Table 2: Intragroup comparison of all the parameters in Group A between baseline against 3-months & 6-months post-operatively (in mm)

Parameters/ Timeline	Group A					
	Baseline v/s 3 months		Baseline v/s 6 months		3 months v/s 6 months	
	Mean Difference	P Value	Mean Difference	P Value	Mean Difference	P Value
PI	0.002	0.984	0.01	0.983	0.012	0.996
GI	0.04	0.061	0.02	0.138	0.02	0.932
PPD	3.21	<0.001	5.07	<0.001	1.86	<0.001
CAL	2.21	<0.001	4.14	<0.001	1.93	<0.001
DD	N/A		2.14	<0.001	N/A	

PI- Plaque index; GI- Gingival index; PPD- Probing pocket depth; CAL- Clinical attachment level; DD- Defect depth

Table 3: Intragroup comparison of all the parameters in Group B between baseline against 3-months & 6-months post-operatively (in mm)

Parameters/ Timeline	Group B					
	Baseline v/s 3 months		Baseline v/s 6 months		3 months v/s 6 months	
	Mean difference	P value	Mean difference	P value	Mean difference	P value
PI	0.02	0.955	0.03	0.938	0.01	0.985
GI	0.04	0.078	0.02	0.272	0.002	0.988
PPD	3.93	<0.001	5.86	<0.001	1.93	<0.001
CAL	2.93	<0.001	5.00	<0.001	2.07	<0.001
DD	N/A	--	2.64	<0.001	N/A	--

PI- Plaque index; GI- Gingival index; PPD- Probing pocket depth; CAL- Clinical attachment level; DD- Defect depth

Table 4: Intergroup comparison between Group A and Group B for all the parameters at varied timelines of baseline, 3-months & 6-months post-operatively (in mm).

Parameters/ Timeline	Group A v/s Group B					
	Baseline		3 Months		6 Months	
	Mean Difference	p value	Mean Difference	p value	Mean Difference	p value
PI	0.001	0.976	0.001	0.996	0.014	0.928
GI	0.003	0.944	0.002	0.726	0.002	0.843
PPD	0.43	0.885	0.29	0.978	0.36	0.943
CAL	0.50	0.720	0.21	0.990	0.36	0.913
DD	0.29	0.837	N/A	--	0.21	0.923

PI- Plaque index; GI- Gingival index; PPD- Probing pocket depth; CAL- Clinical attachment level; DD- Defect depth

Table 5: Mean value of linear bone fill (LBF) of two groups after 6 months (Mean ± SE) (in mm)

Group A	Group B	T Value	P Value
2.14 ± 0.10	2.64 ± 0.17	2.56	0.017*