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Research Article

COMPARATIVE EVALUATION OF ALLOPLAST (BIPHASIC CALCIUM PHOSPHATE) AND DEMINERALIZED FREEZE-DRIED BONE ALLOGRAFT (DFDBA) IN THE TREATMENT OF PERIODONTAL INTRABONY DEFECTS-A CLINICAL AND RADIOGRAPHIC STUDY

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ABSTRACT

Several studies demonstrated that the use of graft material has better clinical results for the treatment of intrabony defects. The purpose of this study is to compare the clinical and radiographic efficacy of alloplast (Biphasic calcium phosphate) and demineralized freeze-dried bone allograft (DFDBA) in treatment of periodontal intraosseous defects. A split mouth study was conducted in 20 subjects diagnosed with chronic periodontitis presenting atleast two intrabony defects in either arch. One quadrant (Site A) received alloplast (Biphasic calcium phosphate) and the contralateral defect (Site B) received DFDBA. The results of study showed that clinically Site B (DFDBA) showed greater reduction in pocket depth and gain in clinical attachment level than site A (Biphasic calcium phosphate), however it was not statistically significant. Site A (Biphasic calcium phosphate) showed slightly more gingival marginal recession than Site B (DFDBA). Radiographically Site B (DFDBA) showed greater bone fill compared to Site A (Biphasic calcium phosphate) which was statistically significant.

INTRODUCTION

The key to tissue regeneration is to stimulate a cascade of healing events which, if coordinated, can result in completion of integrated tissue formation. (Bansal M *et al*, 2014)

Deep intraosseous defects represent a major challenge for the clinician. Sites with deep intraosseous lesions have been shown to be at higher risk of disease progression in subjects who had not received systematic periodontal therapy.(Trombelli L, 2002)

Several studies demonstrated that the use of graft material has better clinical results for the treatment of intrabony defects. Autogenous bone grafts, demineralized freeze-dried bone allografts (DFDBAs), alloplasts, xenografts have demonstrated regenerative potential.

Demineralised freezed dried bone allograft (DFDBA) have repeatedly demonstrated significant improvement in soft and hard clinical tissue parameters for the treatment of intraosseous periodontal defects. The demineralization process of the graft exposes the bone inductive proteins located in the bone matrix such as BMP-2 and BMP-7 which are capable of inducing mesenchymal cells to differentiate into osteoblasts in vivo.

However, incomplete resorption of these materials has frequently been reported and although statistically negligible, risk of transmitting diseases still exist from the use of allografts and xenografts. Therefore the use of alloplastic materials, which are synthetic, inorganic, and biocompatible bone-graft substitutes, may be an alternative for the treatment of intrabony periodontal defects. (Nery EB *et al*, 1992)

Synthetic hydroxyapatite (HA) and tricalcium phosphate (TCP) are also the most commonly used bone grafts substitutes at present. Biphasic calcium phosphate (BCP) which is a combination of hydroxyapatite and tricalcium phosphate has drawn considerable attention. The dense hydroxyapatite ceramics when used as bone implant is almost non-resorbable and bio-inert .While the porous β-TCP containing ceramics displays affinity for high speed biological degradation. They are bioactive and bioresorbable material. (Ellegard B *et al*, 1973). Hence the purpose of this study is to compare the clinical and radiographic efficacy of alloplast biphasic calcium

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phosphate with demineralized freeze-dried bone allograft (DFDBA) in treatment of periodontal intraosseous defects.

MATERIALS AND METHODS

A split mouth study was conducted in 20 subjects diagnosed with chronic periodontitis presenting atleast two intrabony defects in either arch. They were selected from the Out Patient Department of Periodontology of the institute. Ethical committee approval was taken prior to conducting the study. In 20 subjects with chronic periodontitis 40 sites with bilaterally similar intrabony defects in either arch were randomly selected as a part of split mouth study design. One quadrant(Site A) received alloplast (Bone Medik®, DM Biphasic calcium phosphate) and the contralateral defect (Site B) received DFDBA (obtained from TATA MEMORIAL HOSPITAL TISSUE BANK.)

The patients were selected on the basis of following inclusion criteria. Subjects within the age group of 30 – 50 years with atleast 20 teeth present, Systemically healthy subjects of either sex, not under any medication affecting the periodontal treatment outcome, Subjects with atleast 2 periodontal pockets on either side of the same arch with probing depth of ≥ 5 mm after phase I therapy and with atleast 2 similar intrabony defects in the same arch, Subjects willing to be a part of the study without any objection. The exclusion criteria were medically compromised subjects, pregnant, lactating females and those on oral contraceptives, smokers (AHA guidelines) and tobacco chewers, subjects with any parafunctional habits such as mouth breathing, bruxism, who have undergone any periodontal flap surgery within the last six months, uncooperative subjects.

Clinical Parameter

The following clinical parameters were recorded at baseline, 3 months, 6 months and 9 months interval. Gingival Index (Loe H. and Silness J, 1963), Plaque Index (Silness and Loe,1964), Probing depth, Clinical attachment level, Gingival recession .

Clinical parameters were measured using UNC-15 probe. Customised acrylic occlusal stents were fabricated for each patient to record accurate clinical parameters. This technique provided a fixed reference point and fixed angulation for measurements at each site. (Molinari JA, 1992)

Radiographic Parameter

Periapical radiographs were standardized using IOPA grid and parallelling angle technique using round collimation. Bone fill was evaluated radiographically at baseline and 9 months. All intraoral radiograph scanned and stored in JPEG format were transferred and digitized using radiovisiography. For measurement, calibrated measurement tool was used. The following anatomical landmarks of the intrabony defects were identified based on criteria set by Schei *et al* (1959), Bjorn *et al*(1969)

1. The cement-enamel junction (CEJ) of the tooth associated with intrabony defects.
2. The most coronal position of the alveolar bone crest of the intrabony defect when it touches the root surface of the adjacent tooth, i.e top of the alveolar crest(AC)
3. The most apical extension of the intrabony defect where the periodontal ligament space still retains its normal width, i.e bottom of the defect(BD)

Based on these landmarks on radiovisiography calibrated line was drawn from alveolar crest (AC) to base of defect (BD) (Laurell L,1998)

i.e AC-BD: Distance from alveolar crest to base of the defect (to check bone fill).

Patients meeting the inclusion criteria were selected for the study. Phase I therapy was carried out. Oral hygiene instructions were given to all the subjects participating in the study.

Subjects were recalled 21 days post Phase I therapy during which the clinical parameters (i.e gingival index, plaque index, gingival recession radiographic parameters were assessed.

Surgical Procedure

The surgical procedure was performed using local anesthesia. Sulcular incisions were made for the teeth indicated. Conventional mucoperiosteal flap was raised on the buccal and lingual/palatal aspects of the teeth. A vertical releasing incision extending into the alveolar mucosa was made only when necessary for proper access to the defects. Thorough debridement, scaling and root planing was accomplished.

Thereafter one defect (Site A) received alloplast (Bone Medik®, DM Biphasic calcium phosphate) and the contralateral defect (Site B) received DFDBA (obtained from TATA MEMORIAL HOSPITAL TISSUE BANK.)

After grafting, flaps were approximated for closure and then sutured with 3-0 black- braided silk suture (Mersilk)™ using simple interrupted sutures followed by application of non-eugenol periodontal dressing. Appropriate post- operative instructions were given to the subjects. Suitable antibiotics, analgesics and anti inflammatory drugs were prescribed. Subjects were advised to rinse with chlorhexidine gluconate 0.2% twice daily for two weeks. Subjects were motivated to maintain the oral hygiene and were recalled at 3 months, 6 months and 9 months post surgery to assess clinical parameters. At recall visits oral hygiene instructions were reinforced.

Statistical Analysis

Descriptive statistics were expressed as mean ± standard deviation (SD) for each group. Between and within group differences in the various clinical parameters over a period of 9 months of time period was analyzed using Paired and Unpaired t test, Repeated measures ANOVA test of significance with Bonferroni correction. In the above test, p value less than or equal to 0.05 ($p \leq 0.05$) was taken to be statistically significant. All analyses were performed using SPSS software version 17.

RESULTS

Among the 20 patients (n=20) selected for the study there were 13 males (65%) and 7 females (35%) with mean age 39.75±6.46years (Table no 1, Table no2, Figure no 1, Figure no 2). Clinical parameters plaque index, gingival index, probing depth, clinical attachment level and gingival marginal level

were evaluated at baseline,3 months, 6months,9months and in addition bone fill was evaluated at baseline to 9months.

Table no 1 Gender distribution of the study participants

	Number	Percentage
Males	13	65 %
Females	7	35 %

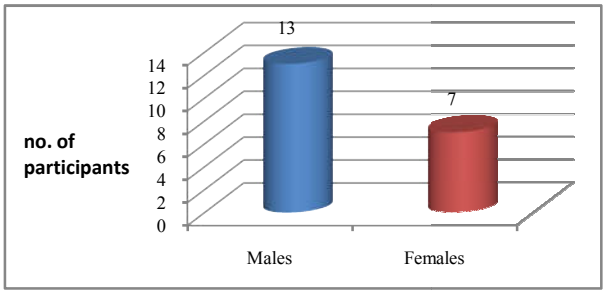
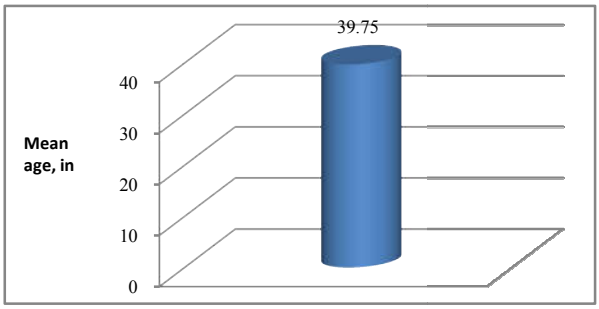


Figure no 1 Gender distribution of the study participants

Table no 2 Age distribution of the study participants

	Mean	Standard deviation
Age (in years)	39.75	6.46



Clinical parameters

Plaque index

The Plaque Index score was measured at baseline, 3months, 6 months and 9 months The mean plaque index score at baseline was 1.47±0.53 which reduced to 0.82±0.33 at 9 months.

Table no 3 Plaque Index over a period of 9 months

Plaque index score	Baseline	3 months	6 months	9 months	P value (Repeated measures ANOVA)
Mean	1.47	1.29	1.03	0.82	<0.001*
SD	0.53	0.45	0.39	0.33	
Mean Change		0.18	0.44	0.65	
P value (post hoc Bonferroni test)		<0.001*	<0.001*	<0.001*	

*p<0.05 is statistically significant

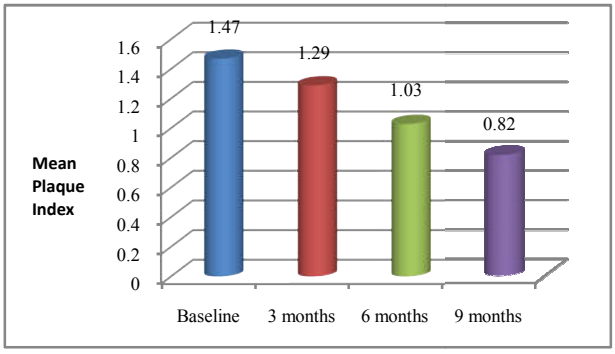


Figure no.3 Plaque Index over a period of 9 months

The mean reduction in plaque index score from baseline to 9 months was 0.65 which was statistically significant. (p <0.001)(Table no 3, Figure no 3)

Gingival Index

The Gingival Index score was measured at baseline, 3months, 6 months and 9 months The mean gingival index score at baseline was 1.59±0.24 which reduced 1.01±0.24 at 9 months. The mean reduction in gingival index score from baseline to 9 months was 0.58 which was statistically significant (p < 0.001)(Table no 4,Figure no 4).

Table no 4 Gingival Index over a period of 9 months

Gingival index score	Baseline	3 months	6 months	9 months	P value (Repeated measures ANOVA)
Mean	1.59	1.38	1.21	1.01	<0.001*
SD	0.24	0.23	0.26	0.24	
Mean Change		0.21	0.39	0.58	
P value (post hoc Bonferroni test)		0.003*	<0.001*	<0.001*	

*p<0.05 is statistically significant

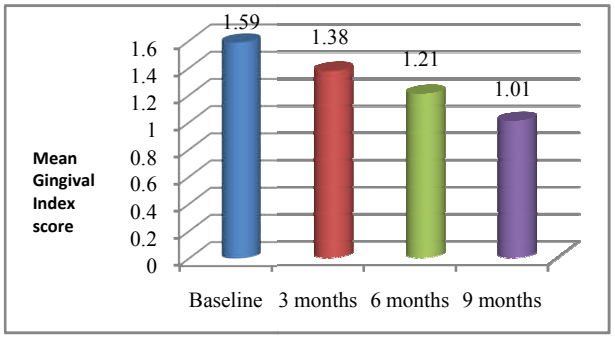


Figure no.4 Gingival Index over a period of 9 months

Probing depth

Statistically significant (p<0.001) reduction in probing depth was seen in both the groups over the period of 9 months.

Table no 5 Intergroup comparison of change in Probing depth over a period of 9 months

Change in probing depth, mm (Mean ± SD)	Baseline-3 month	Baseline-6 months	Baseline-9 months
Site A	2.85 ± 0.88	3.40 ± 1.05	3.60 ± 1.42
Site B	3.15 ± 1.14	3.80 ± 1.24	3.95 ± 1.28
P value (Unpaired t test)	0.356	0.277	0.367

*p<0.05 is statistically significant

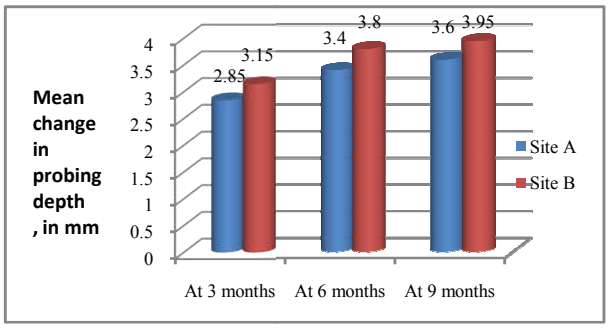


Figure no.5 Intergroup comparison of change in Probing depth over a period of 9 months

However intergroup comparison showed greater reduction in probing depth in Site B (DFDBA), mean change 3.95±1.28mm compared to Site A (biphasic calcium phosphate), mean change 3.60±1.42mm) over a period of 9 months, but this was not statistically significant .(p =0.367)(Table no 5, Figure no 5)

Clinical attachment level

Statistically significant gain in clinical attachment level over the period of 9 months was seen in both the groups. Intergroup comparison showed greater gain in clinical attachment in Site B(DFDBA) 3.80±1.79mm compared to 3.55±1.32mm at Site A(biphasic calcium phosphate) but this was not statistically significant. (p=0.618)(Table no 6, Figure no 6)

Table no 6 Intergroup comparison of change in clinical attachment level over a period of 9 months

Change in clinical attachment level, mm (Mean ± SD)	Baseline-3 months	Baseline-6 months	Baseline-9 months
Site A	2.80 ± 1.24	3.40 ± 1.31	3.55 ± 1.32
Site B	3.00 ± 1.69	3.70 ± 1.84	3.80 ± 1.79
P value (Unpaired t test)	0.671	0.556	0.618

*p<0.05 is statistically significant

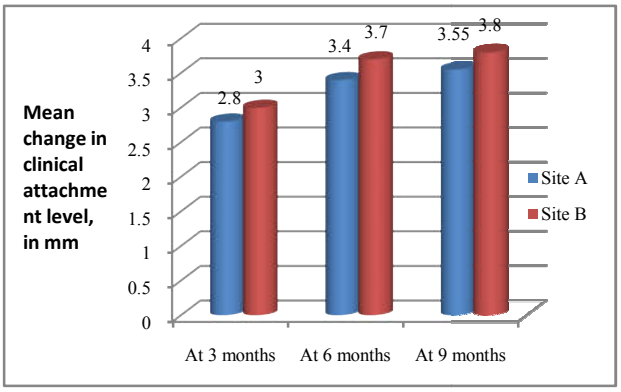


Figure no.6 Intergroup comparison of change in clinical attachment level over a period of 9 months

Gingival marginal level

Gingival marginal recession at Site A (biphasic calcium phosphate) was 0.65±0.59mm at 3 months which remained constant at 6 and 9 months. Gingival marginal recession at Site B(DFDBA) was 0.55±0.83mm at 3 months which remained constant at 6 and 9 months. Gingival marginal recession at site A was slightly greater than Site B over the period of 9 months but this was not statistically significant. (p =0.662)(Table no 7, Figure no 7)

Table no 7 Intergroup comparison of change in gingival marginal level over a period of 9 months

Change in Gingival Marginal level, mm (Mean ± SD)	Baseline-3 months	Baseline-6 months	Baseline-9 months
Site A	-0.65 ± 0.59	-0.65 ± 0.59	-0.65 ± 0.59
Site B	-0.55 ± 0.83	-0.55 ± 0.83	-0.55 ± 0.83
P value (Unpaired t test)	0.662	0.662	0.662

*p<0.05 is statistically significant

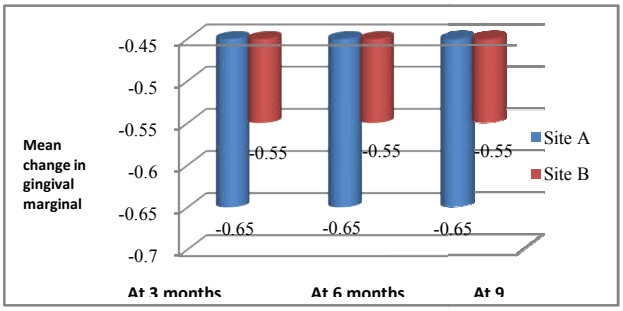


Figure no.7 Intergroup comparison of change in gingival marginal level over a period of 9 months

Bon fill over the period of 9 months

The bonefill at 9 months was 1.97±0.54mm at Site A (biphasic calcium phosphate) and 2.57±0.67mm at Site B (DFDBA) Site B showed greater bone fill as compared to Site A. This reduction in defect depth was statistically significant (p <0.003). (Table no 8, Figure no 8)

Table no 8 Intergroup comparison of bone fill over a period of 9 months

Change in AC – BD (Bonefill) (Mean ± SD)	Baseline – 9 months
Site A	1.97 ± 0.54
Site B	2.57 ± 0.67
P value (Unpaired t test)	0.003*

*p<0.05 is statistically significant

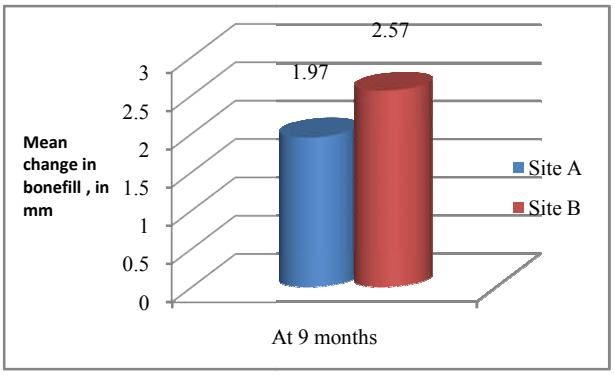
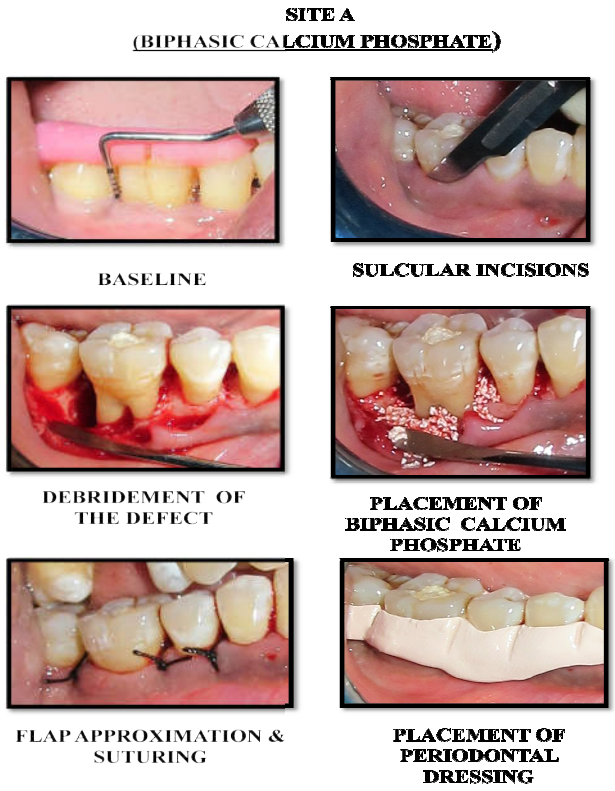
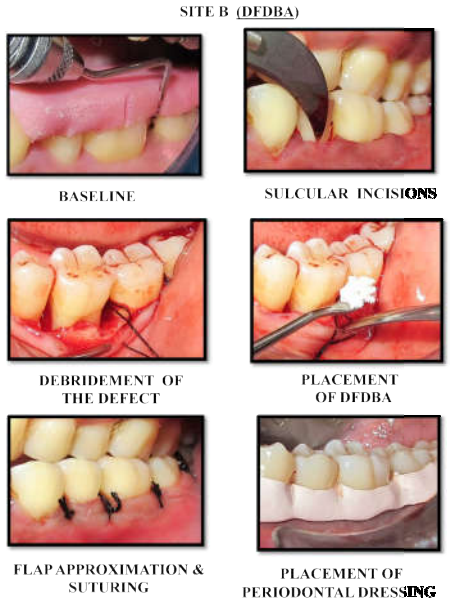
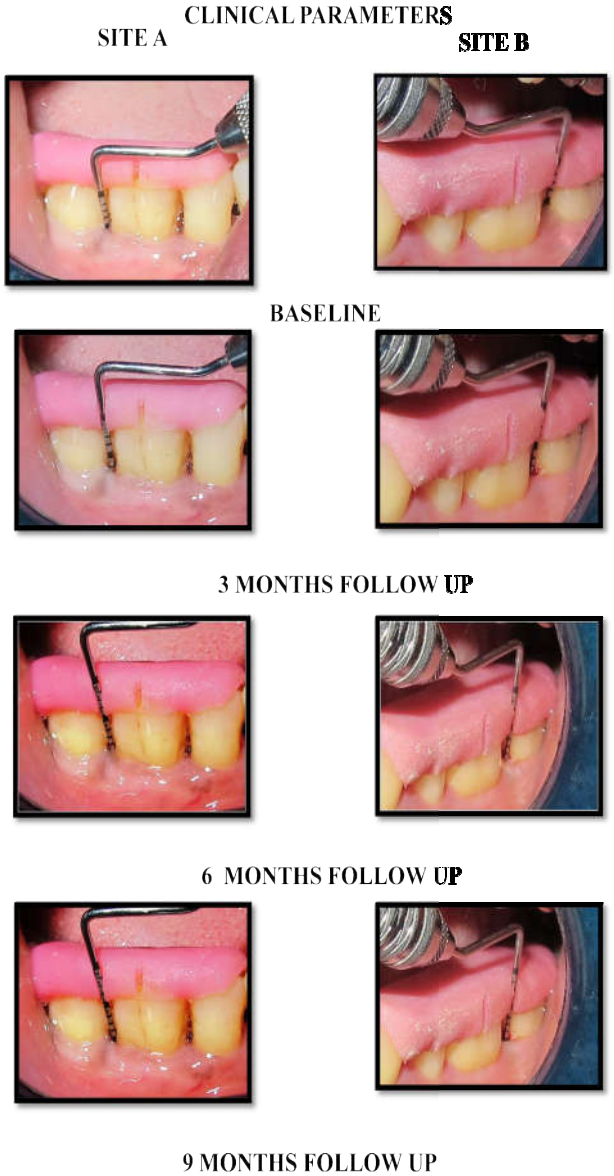
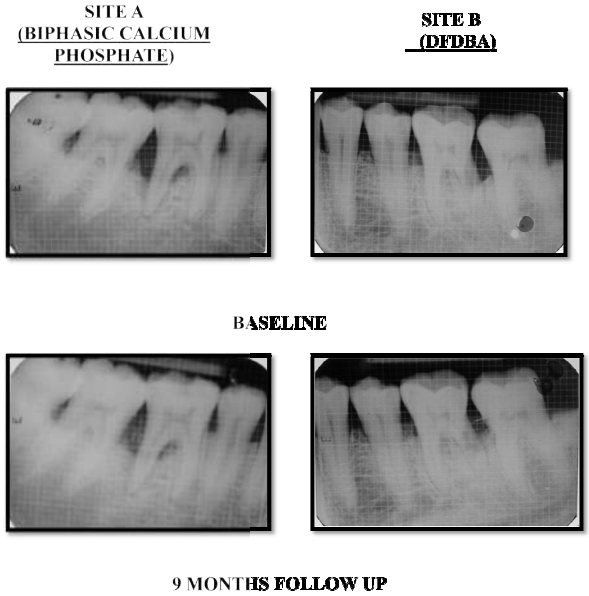


Figure no 8 Intergroup comparison of bone fill over a period of 6 months









DISCUSSION

Periodontal intrabony defects can be treated through use of bone grafts, barrier membranes. Effective alternative to autogenous grafts are allografts such as demineralized freeze-dried bone allograft (DFDBA). It is harvested from donors, which then undergoes a rigorous tissue banking protocol to ensure a sterile graft preparation. Despite both meticulous screening of donors and processing of human derived tissues, chances of antigenecity and disease transfer remain. (Bright RW *et al*, 1977)

Synthetically produced bone-substitute materials (i.e.alloplasts) overcome some of the disadvantages of autogenous and allogenic bone grafts. (Stavropoulos A *et al*, 2010)

Synthetic hydroxyapatite (HA) & tricalcium phosphate (TCP) are the most commonly used bone graft substitutes at present. Recently a combination of Hydroxyapatite (HA) and β-tricalcium phosphate (β-TCP) has been used in periodontal regeneration called biphasic calcium phosphate.

Stein JM *et al*.(2009) found that the clinical benefits of Biphasic calcium phosphate were similar to those of autogenous bone, and showed improved results when compared to OFD alone. Keeping in mind the advantages of alloplastic materials, a study was designed to evaluate their efficacy in the treatment of intrabony defects. To the best of our knowledge, so far no study has comparatively evaluated the use of biphasic calcium phosphate (BCP) and DFDBA in a split mouth design.

Although a study was conducted by Miron RJ *et al*.(2016) in animal model to evaluate osteoinductive potential of a biphasic calcium phosphate bone graft(BCP) in comparison with autografts, xenografts, and DFDBA. They reported ectopic bone formation at DFDBA and in BCP grafts sites.

A split mouth design was selected to avoid natural variation between individuals and to limit patient based and defect based factors. While measuring all the soft tissue parameters a fixed reference point at the apical edge of the custom made acrylic stent was used.

Effects on bone i.e bone fill were measured radiographically. Although histologic evaluation and surgery re-entry are accurate and could have been done, they were not considered due to ethical considerations.

Di Battista P *et al*.(1995) showed that radiographs were better predictors of volumetric bone fill rather than either PD or CAL even though, at best they represented a 2 dimensional picture of a 3 dimensional defect. Radiographic monitoring of alveolar bone changes following regenerative procedures was considered as a good alternative to direct bone measurements. Oral health status was assessed by taking full-mouth plaque index and gingival index.

The biphasic calcium phosphate used in the study was a mixture of 60%hydroxyapatite and 40% β-tricalcium phosphate. DFDBA used in the study was from TATA Memorial Hospital Tissue Bank with a particle size of 500 to 1040µm.

Both plaque and gingival index showed significant improvement from baseline over a period of 9 months. This could be attributed to an improvement in the home care and reinforcement of oral hygiene instructions at each recall visit.
In the present study mean probing depth reduction obtained at Site A (Biphasic calcium phosphate) was 3.60±1.42mm at 9 months which was statistically significant (p<0.001).These results were in accordance with the studies done by **Stein** JM *et al*.(2009), Pandit N *et al*.(2010), Lee MJ *et al*.(2012), Kaushal S *et al*.(2014) and Bansal R *et al*.(2014). Whereas at Site B (DFDBA) probing depth reduction was 3.95±1.28mm at 9 months which was statistically significant (p<0.001).These results were in accordance with the studies done by Rummelhart JM *et al*.(1989), Hoidal MJ *et al*.(2008), Katuri KK *et al*.(2013), Gothi R *et al*.(2015) and Dave D *et al*.(2015).

Although Site B (DFDBA) showed more reduction in probing depth than Site A (Biphasic calcium phosphate). The difference was not statistically significant.

The gain in clinical attachment level Site A (Biphasic calcium phosphate) 3.55±1.32mm at 9 months from baseline which was statistically significant (p<0.001).

These results were in accordance with the studies done by Stein *et al*.(2009), Pandit *et al*.(2010) , Lee MJ *et al*.(2012), Kaushal S *et al*(2014) and Bansal R *et al*.(2014) Whereas at Site B (DFDBA) the gain in clinical attachment level was 3.80 ± 1.79mm at 9 months from baseline which was statistically significant (p<0.001).These results were in accordance with the studies done by Rummelhart JM *et al*.(1989) , Hoidal MJ *et al*.(2008), Katuri KK *et al*.(2013), Dave D *et al*.(2015) and Gothi R *et al*.(2015) .

Site B (DFDBA) showed greater gain in clinical attachment level than Site A (biphasic calcium phosphate) clinically. Although these results were not statistically significant. Monitoring the gingival marginal level helps to explain the overall clinical picture and the factors affecting the regenerative response. Apical shift of the gingival margin is likely to reduce the regenerative capacity thus affecting the final outcome.

At Site A (biphasic calcium phosphate) the reduction in gingival marginal level from baseline was 0.65±0.59mm at 3 months which remained constant over the study period of 9 months and was statistically significant (p<0.001). These results were in accordance with the studies done by Stein JM *et al*.(2009), Lee MJ *et al*.(2012).

Whereas at Site B (DFDBA) the reduction in gingival marginal level from baseline was 0.55±0.83 mm at 3 months which remained constant over the study period of 9 months and was statistically significant(p<0.001).These results were in accordance with the studies done by Dave D *et al*.(2015)

Clinically Site B (DFDBA) showed less gingival recession as compared to Site A (biphasic calcium phosphate). Although this was not statistically significant. This study suggest that both biphasic calcium phosphate and DFDBA used as regenerative graft materials yield generally favorable clinical results in periodontal intrabony defects and that there are essentially no differences in results between the two materials when clinical parameters were measured at 9months.

The bone fill at Site A (Biphasic calcium phosphate) was 1.97±0.54mm at 9 months which was in accordance with the studies done by Stein JM *et al*.(2009), Pandit N *et al*.(2010) , Lee MJ *et al*.(2012), Kaushal S *et al*.(2014) and Bansal R *et al*.(2014).

Studies have shown that the estimated ratio of 60% HA to 40% β-TCP provides osteoconductive property. Ellinger RF *et al*.(1986) Nery EB *et al*.(1992) in his study evaluated different ratios of HA/TCP in periodontal osseous defects and concluded that higher HA ratio showed increased new bone formation histologically.

The dissolution rate of biphasic calcium phosphate is a function of the ratio of β- TCP to HA as well as the mechanical and structural characteristics of the granules including the density and microporosity.

Hashimoto-Uoshima M *et al*.(1995) found that biphasic calcium phosphate granules degraded by phagocytic process and were replaced by new bone tissue. In a similar study it was found that almost all biphasic calcium phosphate was resorbed and the implanted area was filled with compact new bone tissue. It is a valid assumption that macrophages containing biphasic calcium phosphate crystals ultimately disappear and are replaced with new bone. Such a bone-formation process is desirable for periodontal tissue. Shetty and Han *et al*.(1991) suggested that an ideal synthetic bone substitute should serve as a scaffold for bone formation and slowly resorb to permit replacement by new bone. The biphasic calcium phosphate ceramics satisfy this requirement The bone fill at Site B(DFDBA) was 2.57±0.67mm at 9 months which was in accordance with the studies done by Rummelhart *et al*.(1989), Hoidal *et al*.(2008), Katuri KK *et al*.(2013), Gothi *et al*.(2015) and Dave D *et al*.(2015).

In the present study Site B (DFDBA) showed a greater bonefill as compared to Site A (biphasic calcium phosphate) over the period of 9 months. This reduction in defect depth was statistically significant (p<0.003).

CONCLUSION

The study was conducted with small sample size, difference between treatment groups may have existed if the sample size had been larger. This was difficult as our study was a split mouth study requiring almost identical defect. In this study conventional radiographic technique were used for assessment. However Surgical re-entry or 3D imaging like CBCT would have been a better estimation of the amount of bone fill.

The most reliable outcome for assessing for periodontal regeneration is histological analysis, however due to ethical consideration no histologic evidence was obtained.

Biphasic calcium phosphate acts as good scaffold, it should be combined with biologics mediators like bone morphogenic proteins ,platelet rich fibrin, growth factors. It needs to be evaluated using Guided tissue regeneration(GTR).It can also be combined with recombinant growth factors e.g rhPDGF (Recombinant platelet derived growth factor) or rhBMP (Recombinant bone morphogenic proteins).

Radiographic parameters should be evaluated using 3D imaging like CBCT for better estimation of the bone fill. Future long term follow up, multicentre, prospective, longitudinal randomized controlled trials are needed to confirm the finding of the study.

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