Original Article

Efficacy of decalcified freeze-dried bone allograft in the regeneration of small osseous defect: A comparative study

ABSTRACT

Aim: To access the efficacy of decalcified freeze-dried bone allograft (DFDBA) in the regeneration of bone following small osseous defect in minor oral surgery.

Objectives: To evaluate the ability of DFDBA to enhance the rate of wound healing and assess radiographic bone density, pain, and infection preoperatively and postoperatively.

Materials and Methods: Twenty patients with cysts were assessed. Ten patients were filled with DFDBA (Group 1) and ten without bone graft (Group 2), respectively. Radiographic bone density was assessed on preoperative, intraoperative, and postoperative radiographs on 1st day, 3rd month, and at 6th month using Adobe Photoshop CS6 - Grayscale histogram.

Results: Bone density in Group 1 was found to be significantly higher than in Group 2 on 3rd and 6th month postoperatively with a $P = 0.024$ and $P = 0.016$ which was statistically significant. The percentage increase in bone density between both the group was determined and yielded no difference over a period of time, but the difference in percentage increase was markedly higher in Group 1 compared to Group 2 at all the time intervals.

Conclusion: Bone formed as depicted by bone density is significantly higher when DFDBA is used in small bony defects.

Keywords: Allograft, osseous defect, periapical pathology

INTRODUCTION

Bony surgeries for treatment of pathologies leave behind bony defects ranging from simple one-wall defects to composite defects. Their dimensions may vary from few millimeters to few centimeters. With the progression of time, maxillofacial surgeons have got a variety of bone grafting options ranging from alloplastic material to vascularized bone graft.

Carious lesions leading to bacterial invasion of pulp tissue, leading to necrosis of the pulp are the common causes of periapical pathology.[1] Bacteria in the periapical region lead to periapical abscess, granuloma, or periapical cyst. Destruction in the periapical region leads to destruction of bone known as an "osseous defect."[1]

Decalcified freeze-dried bone allograft (DFDBA) was first used in dentistry and medicine in 1965, but for the treatment of periodontal defects in humans, it was utilized in 1975 for the first time. DFDBA provides osteoconductive and osteoinductive factors. It induces the host undifferentiated mesenchymal cell to differentiate into osteoblasts with subsequent formation of new bone. It contains bone morphogenic proteins (BMPs) such as BMP 2, 4, and 7, which help stimulate osteoinduction.

The advantages of allogeneic grafts include availability in adequate quantities, predictable results, and the elimination

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of an additional donor site surgery. The disadvantages of allografts include host incompatibility, potentially contaminated specimens resulting in recipient site infections, and potential transmission of disease from donor to recipient of the allograft and impractical or biologically ineffective usefulness.

There is currently no evidence to indicate which of these materials promote greater bone growth in the osseous defect healing.[2] This study is undertaken to evaluate the efficacy of locally available DFDBA (TATA Tissue Bank, Mumbai, Maharashtra, India) in the healing of small periapical osseous defects so that the prognosis of the affected teeth can be improved by increasing bony support by regenerative procedures. While the dimensions of the ridge are crucial for subsequent implant placement, intuitively the quantity of vital bone that forms in the healing extraction socket is also important. Therefore, DFDBA has been taken as material in this study due to its osteoconductive as well as osteoinductive properties.

MATERIALS AND METHODS

This study was conducted in the Department of Oral and Maxillofacial Surgery and Oral Implantology, I.T.S. Centre for Dental Studies and Research, Muradnagar, Ghaziabad, Uttar Pradesh, India. Sample sizes of twenty patients were signed and divided into two groups. Group 1: ten patients in which bone graft was used (DFDBA) and Group 2: ten patients in which bone graft was not used.

Pathology was assessed clinically, and investigations were undertaken such as routine blood investigation and radiograph intraoral periapical (radiovisiography [RVG]) of standard values (70 kV, 8 mA, 0.8 exposure time). All healthy persons between the age group of 18–70 years with no contraindication for surgery were included with small osseous defects at the maximum dimension of approximately <1 cm.

All the patients were operated under local anesthesia. During the surgery, intraoperative defect details and size of the defect at its maximum dimension, and depth was noted using a caliper. Surgery procedure was dependent on the type of defect of each group [Figures 1 and 2].

The graft material used in Group 1 was DFDBA (TATA Tissue Bank, Mumbai, Maharashtra, India), with a particle size of 1000 μm. The graft material was mixed with autologous blood of the same patient then carefully packed with light pressure into the defect; sutured was placed to ensure complete soft tissue coverage of the graft [Figure 1c and d].

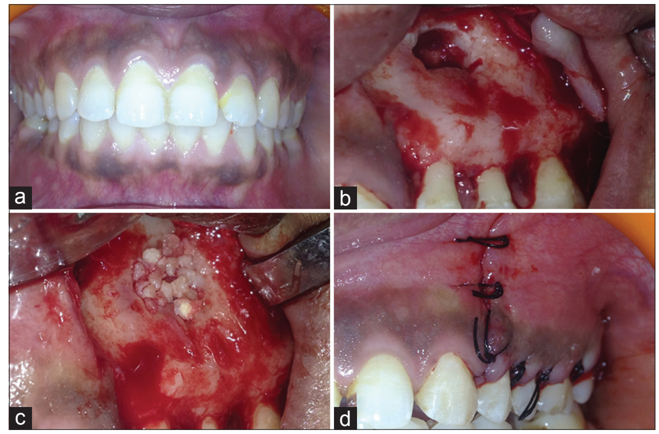


Figure 1: Periapical cyst affecting a 19-year-old female, treated by enucleation and bone grafting. (a) Preoperative picture. (b) After flap reflection and curettage of periapical soft tissue lesion. (c) Defect filled using decalcified freeze-dried bone allograft. (d) Sutures given

All patients in the study routinely received a postoperative dose of oral antibiotics in the form of capsule Cloxam 500 mg and tablet metronidazole 400 mg three times daily for 5 days and analgesics in a combination of tablet ibuprofen 400 mg and paracetamol 325 mg three times daily for 3 days.

Clinical evaluation was done for pain (visual analog scale [VAS]) and sign of infection (pus discharge and persistent postoperative swelling) on preoperative and postoperative 1st day, 1st week, 3rd month, and 6th month.

Radiographic bone density was assessed on preoperative, intraoperative, and postoperative radiographs on the 1st day, 3rd month, and at 6th month [Figures 3 and 4]. Radiograph was taken with the help of RVG and analyzed using Adobe Photoshop CS6 - Grayscale histogram (ITS Dental College, Ghaziabad, U.P., India) [Figure 5].

RESULTS

The intergroup comparison of pain intensity as measured in VAS was statistically analyzed using independent sample test, and *P* values were found to be statistically not significant, thereby implicating that there was no difference in the intensity of pain in both group either preoperative or postoperative periods.

When Chi-square tests were applied to find the statistical significance of infection in both the group, it was found to be not significant.

Bone density analysis

As the bone graft and blood clot due to its inherent calcium content produce a certain amount of radio-opacity. For all practical purposes, we took into consideration values

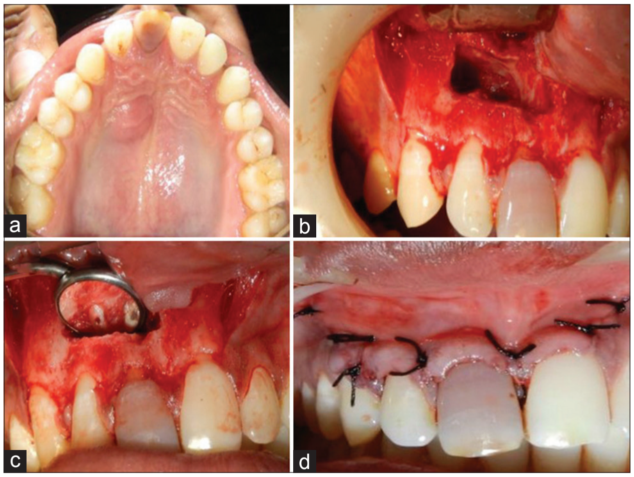


Figure 2: Periapical cyst affecting a 20-year-old male, treated by enucleation and without bone grafting. (a) Preoperative picture. (b) After flap reflection and curettage of periapical soft tissue lesion. (c) Root-end resection. (d) Sutures given

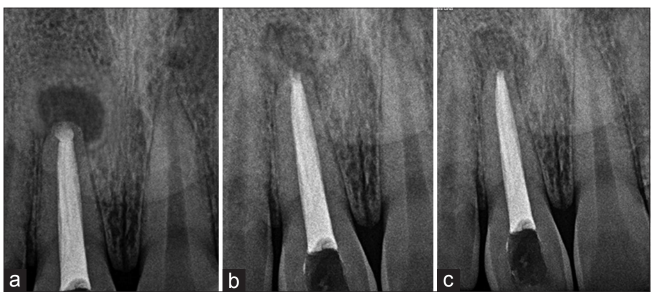


Figure 4: (a) Intraoperative radiograph (radiovisiography). (b) 3rd month radiogragh. (c) 6th month radiograph of a patient treated without bone graft

between 1st postoperative days to postoperative 90 days, 1st postoperative day to postoperative 180 days, and postoperative 90 days to postoperative 180 days for analyzing the increase in bone density.

The difference between the mean gray scale histogram values was synthesized between the gray scale values corresponding to day 1 subtracted (−) day 90 value, day 1 subtracted (−) day 180 value, and day 90 subtracted (−) day 180 value. When the values were subjected to statistical analysis using Bonferroni method, the resultant $P = 0.007$, 0.001, and 0.002. All were found to be statistically significant, thereby implicating there was a significant amount of increase in bone density, which was measured by the increase in gray scale histogram values [Table 1].

Differential mean gray scale histogram values between the various time intervals when subjected to Greenhouse-Geisser test that showed $P = 0.000$ thereby showing statistically significant increase in bone density with time.

Similarly, when the values were subjected to statistical analysis using Bonferroni method, the resultant $P = 0.038$, 0.040, and

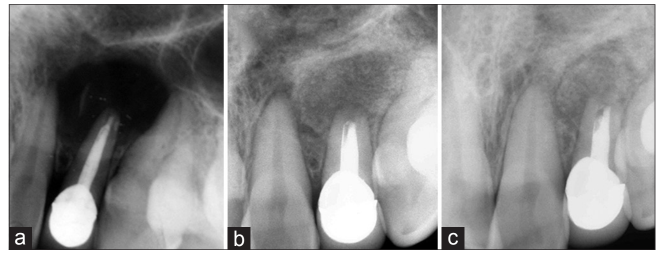


Figure 3: (a) Intraoperative radiograph (radiovisiography). (b) 3rd month radiograph. (c) 6th month radiograph of a patient treated with decalcified freeze-dried bone allograft

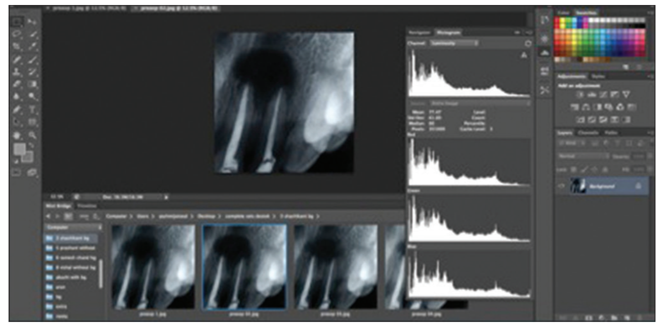


Figure 5: Adobe Photoshop CS6 software

Table 1: Intragroup comparison of mean gray scale histogram values at different time intervals of Group 1

Comparison between mean gray scale histogram values		Mean difference	SE	*P*
Day 1	Day 90	−30.600	6.576	0.007
58.8000	89.4000			
Day 1	Day 180	−52.000	8.269	0.001
58.8000	110.8000			
Day 90	Day 180	−21.400*	3.816	0.002
89.4000	110.8000			

SE: Standard error

0.387. All were found to be statistically significant except from day 90 to day 180, thereby implicating there was a significant amount of increase in bone density till day 90, which was measured by the increase in gray scale histogram values [Table 2].

When statistical analysis was done using independent sample test at various time intervals such as preoperative and postoperative (day 1, day 90, and day 180), the *P* value obtained were 0.16, 0.024, and 0.016, respectively. Out of which, the *P* value at day 90 and day 180 which was 0.024 and 0.016 and it was found to be statistically significant thereby implicating there was a significant amount of increase in bone density in Group 1 as compared to Group 2 at time interval of postoperative day 90 and day 180 [Table 3].

To determine the percentage increase in the mean gray scale histogram values in Group 1 and Group 2 individually between various time intervals, formula: Days 180–90 = (Day 180–Day 90) × 100/Day 90, Day 190 = (Day 90–Day 1) × 100/Day 1, and Days 180–1 = (Day 180–Day 1) × 100/Day 1 was applied. These values were subjected to statistical analysis using Mann–Whitney test and *P* value of days 1–90, days 1–180, and days 90–180 was derived and none were statistically significant [Table 4] thereby implicating the percentage difference in the increase of bone density in both the groups between various time intervals was not significant enough [Graphs 1 and 2].

DISCUSSION

This study was undertaken to evaluate the efficacy of locally available DFDBA (TATA Tissue Bank, Mumbai, Maharashtra, India) in the healing assessment of periapical osseous defects.

A study done by Arenaz-Búa *et al*.[3] compared mandibular bone regeneration by applying autologous bone, platelet-rich plasma, and two biomaterials (synthetic calcium hydroxyapatite and demineralized bone matrix), and Mishra *et al*.,[4] in 2010, evaluated decalcified freeze-dried allograft or hydroxyapatite and a combination of both as bone autograft substitutes in the healing of osseous jaw defects. Both evaluated pain and infection in relation to allograft and did not find any significant difference. In our study, pain intensity was not found to be significant thereby implicating that there was no difference in the intensity of pain between both the groups either preoperatively or postoperatively.

Similarly, the rate of infection in the study was not significant thereby implicating that there was no difference in the rate of infection between both the groups. Thus, it can be concluded that DFDBA though being allograft does not lead to increase in infection rate.

Various modalities have been used to analyze bone density such as DentaScan, bone scintigraphy,[4] and Adobe Photoshop CS6-Gray scale histogram used by Ezoddini-Ardakanil *et al*.[5] in 2011 on socket repair after dental extraction. In our study, we too used Adobe Photoshop CS6-Gray scale histogram for analyzing bone density.

Guillemin *et al*.[6] assessed radiographic changes in alveolar bone density and revealed that placement of DFDBA into defects produced in itself significant increase in radiographic density. On evaluating the bone density in this study, we too found that there was a significant increase in bone density over a period of time and as early at 90 days and continues to significant increase between 90 and 180 days in grafted site, whereas in nongrafted site, there is increase in bone density as early at 90 days but exhibits no significant increase from 90 to 180 days.

Table 2: Intragroup comparison of mean gray scale histogram values at different time intervals of Group 2

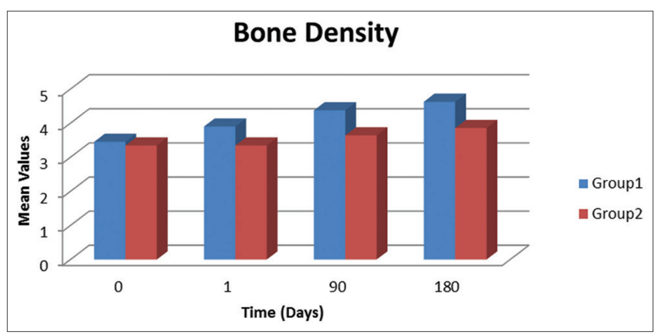
Comparison between mean gray scale histogram values		Mean difference	SE	*P*
Day 1 40.5000	Day 90 48.3000	−7.800	2.200	0.038
Day 1 40.5000	Day 180 61.8000	−21.300	6.088	0.040
Day 90 48.3000	Day 180 61.8000	−13.500	6.410	0.387 NS

NS: Not significant, SE: Standard error

Table 3: Intergroup comparison of mean logarithm gray scale histogram values

Group statistics						
Time	Group	*n*	Mean	SD	SE mean	*P*
Day 1	1	10	3.8988	0.66224	0.20942	0.16
	2	10	3.3436	0.98175	0.31046	
Day 90	1	10	4.3747	0.53158	0.16810	0.024
	2	10	3.6394	0.78243	0.24742	
Day 180	1	10	4.6251	0.45114	0.14266	0.016
	2	10	3.8542	0.80353	0.25410	

SE: Standard error, SD: Standard deviation

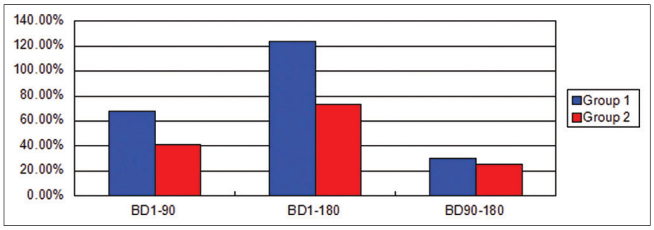


Graph 1: Intergroup comparison of mean logarithm gray scale histogram values

Table 4: Median and *P* value obtained using Mann–Whitney test

Time	Group 1 (*n*=10)		Group 2 (*n*=10)		*P*
	Median	Minimum-maximun	Median	Minimum-maximun	
Day 1-90	52.41	16.42-185.7	20	10-150	0.15 (NS)
Day 1-180	85.20	23.97-371.3	59.6	9.23-175	0.20 (NS)
Day 90-180	27.59	2.74-65	19.15	4.41-60.8	0.71 (NS)

NS: Not significant



Graph 2: Percentage increase in the mean gray scale histogram values between both the groups at various time intervals

Bodner[7] evaluated radiographically, the changes that occur in jaw defects after enucleation of cysts and either grafting with demineralized freeze-dried bone allograft (DFDBA) or packing with absorbable gelatin sponge (Gelfoam) in 32 patients divided into two groups and concluded that density in DFDBA group was significantly ($P < 0.05$ to $P < 0.01$) greater than nongrafted group at 6 and 12 months.

Similarly, in our study, bone density in Group 1 was found to be significantly higher than in Group 2 on 90th day and 180th day, thereby implicating the quality of bone formed as depicted by bone density is significantly higher when DFDBA is used in small bony defects as compared to nongrafted small bony defect. The percentage increase in bone density between both the group was determined and yielded no difference over a period of time, but the difference in percentage increase was markedly higher in Group 1 compared to Group 2 at all the time intervals.

Various authors have compared DFDBA with other bone grafts such as hydroxyapatite,[4] bioactive glass[8] where DFDBA showed better radiographic results. DFBDA when compared with FDBA[9] showed no significant difference, whereas in a study by Wood and Mealey[10] showed significantly greater new bone formation with DFDBA than FDBA.

The osteoinductive potential of DFDBA is related to the amount of BMPs although FDBA has the same BMP content in its organic matrix; it does not have this same osteoinductive capability. Demineralization by osteoclasts is necessary to release BMPs from the mineralized matrix, and since there are no osteoclasts in extraskeletal sites, the BMPs remain trapped in the mineralized particles; thus, no ectopic bone formation is induced, thereby proving that DFDBA is a good graft material. Although possibilities of disease transfer, immunogenicity exits but no cases have been reported till yet.[10]

DFDBA can be used in certain scenario where early bone formation is desired such as immediate implant, sinus lift procedure either with immediate implant or delayed implant placement which can lead to functional rehabilitation. In cases where limited time is required between third molar extraction and performing sagittal split ramus osteotomy and also in socket preservation cases.

CONCLUSION

This study attempted to use DFDBA to promote osseous regeneration in osseous defects. Demineralized freeze-dried bone allograft by virtue of its osteoinductive and osteoconductive properties supports the formation of new bone. The osteoinductive property has been attributed to the exposure of the BMPs and growth factors following the acid demineralization process of the allograft. These BMPs and growth factors permit rapid revascularization and hard tissue in growth in the osseous defects thereby promoting regeneration.

Management of osseous defects remains a challenge to the clinician. Therefore, the study has been concerned with clinical and radiographic observation using DFDBA, and the study states a significant increase in bone density over a period of time as early at 90 days. Future studies can be done using advanced digital imaging facilities available.

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Conflicts of interest

There are no conflicts of interest.

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