Clinical and Radiological Evaluation of an Osseous Xenograft for the Treatment of Infrabony Defects

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ABSTRACT

Objective: To evaluate the effectiveness of demineralized bone matrix (DMBM), an osteoconductive and osteoinductive graft material, as a bone graft for the treatment of osseous defects, both clinically and radiographically.

Materials and Methods: The effectiveness of DMBM was assessed at 40 sites in 30 patients who had infrabony defects. Grafted test sites were compared with control sites treated with open-flap debridement.

Results: Significant improvement in all variables was found, including reduction of probing depth, and gain in clinical attachment level and bone fill for test and control sites at 3 months and 6 months postoperatively. For test sites, reduction of probing depth was 2.80 mm at 3 months and 4.05 mm at 6 months, and for control sites, 1.75 mm at 3 months and 2.65 mm at 6 months. Gain in the level of clinical attachment for test sites was 2.80 mm at 3 months and 4.00 mm at 6 months; for control sites, this gain was 1.75 mm at 3 months and 2.60 mm at 6 months. The mean amount of defect resolution was 2.02 mm and 3.27 mm for test sites and 0.82 mm and 1.17 mm for control sites, at 3 months and 6 months, respectively. The mean percentage of defect resolution was 37.1% and 56.5% for test sites compared with 20.5% and 28.6% for control sites, at 3 months and 6 months, respectively.

Conclusions: DMBM improves healing outcomes, namely, reduction of probing depth, resolution of osseous defects and gain in clinical attachment, compared with open flap debridement.

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Regeneration is the growth and differentiation of new cells and intercellular substances to form new tissues or parts. To achieve tissue regeneration, the cells in the wound must be the same type and be oriented in the same pattern as the original cells. In addition, the extracellular matrix produced by the new cells must be the same type and orientation, and must form the same structures as those originally present.[1] New attachment is the embedding of new periodontal ligament fibres into new cementum and the attachment of the gingival epithelium to a tooth surface previously denuded by the disease.

Periodontal regenerative therapy must produce a structural and functional periodontium constituted by several tissues, namely, cementum, periodontal ligament and the alveolar bone.[2] A variety of materials and regenerative procedures have been used for periodontal regeneration, including grafting of biomaterials and bone substitutes. In

Figure 1: Osseograft material used in the study.

Figure 2: Radiographic assessments were done with an x-ray mesh (a millimetre grid).

comparison with open-flap debridement alone, implantation of these materials produced a more favourable gain in clinical attachment level, reduction of pocket-probing depth and increased defect fill.[3]

A brief review of the terms associated with bone grafting should help readers more fully understand this clinical article. *Osteoconduction* is the formation of bone by osteoblasts from the margins of the defect on the bone-graft material, which acts as a scaffold for bone growth. *Osteoinduction* involves new bone formation by stimulating osteoprogenitors from the defect to differentiate into osteoblasts and begin forming new bone. *Osteogenesis* is the formation of mineralized bone by osteoblasts that is achieved only with autogenous grafts. A *xenograft* is a graft that is taken from a donor of a different species.[4] *Infrabony defects* are all angular or vertical periodontal bony defects that are below the crest of the bone.[5]

Many demineralized freeze-dried bone allografts have been used in combination with antibiotics, such as tetracycline, to treat periodontal osseous defects, but with little benefit.[6,7] Considerable effort has been made to develop osseous implant materials that are readily available, osteoinductive, biocompatible and similar in structure to human bone. Type I collagen fulfills some of these criteria for bone formation. This type of collagen stimulates osteoblast proliferation and differentiation of bone marrow cells. Since it is chemotactic for osteoblasts, fibroblasts and endothelial cells, it has been used for filling empty sockets, periodontal fenestrations and bone defects.[8]

In this context, demineralized xenograft (Osseograft, Chennai, India) was developed for bone regenerative procedures. Demineralized bone matrix (DMBM; Osseograft) is a bone inductive sterile bioresorbable xenograft composed of type I collagen. It is prepared from bovine cortical bone samples, resulting in nonimmunogenic flowable particles of approximately 250 µm that are completely replaced by host bone in 4–24 weeks[9] (**Fig. 1**).

The purpose of this study was to compare the effectiveness of DMBM bone grafts with that of open-flap debridement for the treatment of osseous defects, and to evaluate these grafts, both clinically and radiographically.

Materials and Method

A total of 40 sites, 20 test (grafted) and 20 control (open-flap debridement), were selected in 30 patients for this study from those attending the outpatient department of periodontics, D.A.V. Dental College, Yamunanagar, India. Complete medical and dental histories were taken. These patients met the inclusion criteria of Yukna and others[10]: a diagnosis of moderate-to-advanced periodontitis, radiographic evidence of periodontal osseous defects, age between 33 and 81 years, nonsmoker, and at least 1 intraosseous defect of ≥ 3 mm and pocket depth of ≥ 6 mm, as measured with a manual periodontal probe (UNC-15). The exclusion criteria of Scabbia and others[11] were used to eliminate patients from the study: having a history of severe acute or chronic systemic diseases, teeth with grade III mobility and inadequate endodontic lesions, and being pregnant or lactating.

Twenty of the 30 patients selected had at least 1 intraosseous defect; 10 patients had bilateral intraosseous defects. For patients with bilateral defects, 1 site was used as a test site and the other as a control site, and surgery was done on both test and control sites on the same day.

Presurgical Protocol

The presurgical protocol for this study was the same as that outlined by Mora and Ouhayoun.[12]

The initial preparation phase for treatment consisted of oral hygiene instructions, scaling and root planing. Occlusal therapy and re-evaluation was done 4 weeks after the completion of this first phase of therapy.

The variables investigated at baseline, 3 months and 6 months were the plaque index developed by Silness and Loe,[13] the gingival index of Loe and Silness,[14] pocket depth and clinical attachment level. The radiographic variables included the amount and percentage of resolution of defects.

The probing measurements were done with a customized acrylic stent that was used as a fixed reference point to minimize distortion. The stent was grooved in the occlusal apical direction with a tapered bur so that the UNC-15 probe was placed at the same position for each successive measurement. One site representing the same deepest point of the defect was included: the fixed reference point (FRP) to the base of the pocket (BP) and the

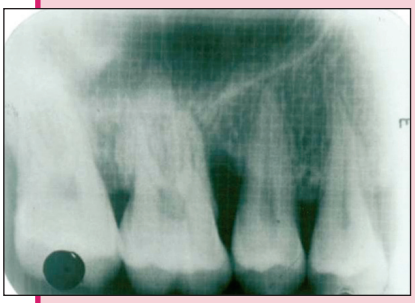

Figure 3a: Preoperative radiograph of the test site.

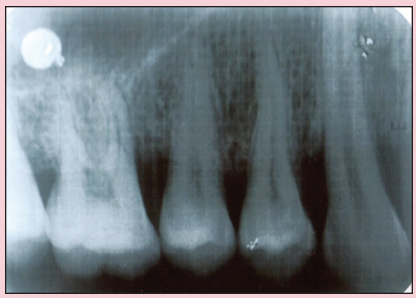

Figure 3b: Postoperative radiograph of the test site at 3 months.

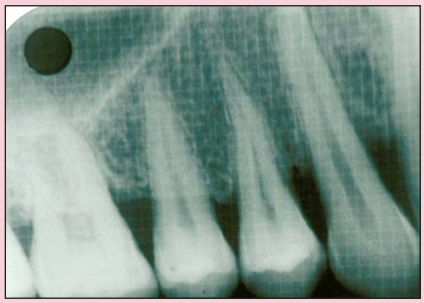

Figure 3c: Postoperative radiograph of the test site at 6 months.

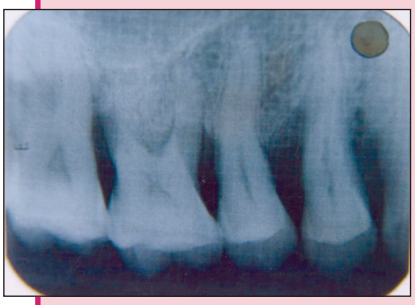

Figure 4a: Preoperative radiograph of the control defect.

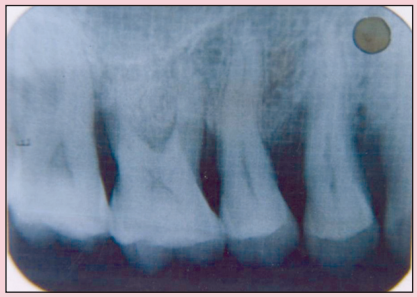

Figure 4b: Postoperative radiograph of the control defect at 3 months.

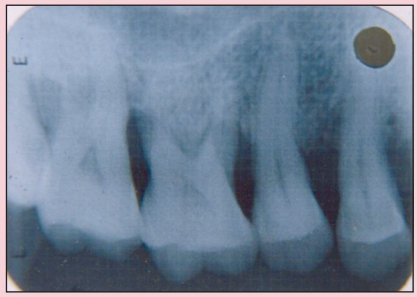

Figure 4c: Postoperative radiograph of the control defect at 6 months.

fixed reference point to the cementoenamel junction (CEJ). All the measurements were made by 1 examiner, using a periodontal probe, before and after surgery for test and control sites at baseline, 3 months and 6 months.

Pocket depth and clinical attachment level were calculated from the clinical measurements[15]:

pocket depth = (FRP to BP) – (FRP to gingival margin [GM])
clinical attachment level = (FRP to BP) – (FRP to CEJ)

Radiographic Assessment and Measurements

Radiographic evaluation of each defect was done at baseline, 3 months and 6 months for test sites and control sites. Intraoral periapical radiographs were taken with a millimetre grid (X-ray Mesh Meyer, Haake, Germany) (**Fig. 2**). Defects were measured as the distance from the alveolar crest to the base of the bone defect. The amount of defect resolution was calculated as the difference between the depth of the defect before and after surgery; the percentage of the resolution of the defect was then calculated (see **Figs. 3a–3c** and **4a–4c** for sample radiographs of each measurement).

Surgical Protocol

The surgical procedure was done under local anesthesia (2% lidocaine with epinephrine 1:100,000). Intrasulcular incisions with reflection of full thickness flaps were used to retain as much soft tissue as possible to obtain primary closure. Debridement and root planing were done with hand instruments (Gracey Curettes) (**Figs. 5** and **6**). After cleaning, the surgical area was irrigated with sterile saline. The surgical area was carefully inspected to ensure that the debridement procedure had been completed satisfactorily. The control sites were then sutured with interrupted sutures with 3-0 Mersilk suture (Ethicon Ltd., Aurangabad, India).

For the test sites, Osseograft was emptied into a sterile dappen dish and 4 to 6 drops of saline were added until the mixture was a paste-like consistency.[16] Increments of the graft material were added, starting from the bottom of the defect, and were condensed with an amalgam condenser to adapt the particles to the configuration of defect until it was completely filled (**Fig. 7**). The soft-tissue flap was then repositioned at the original level and closed with interrupted direct-loop sutures with 3-0 Mersilk sutures (Ethicon). Care was taken to achieve a tension-free primary closure of the flap during suturing. Silk sutures were used because of their outstanding handling and knot-tying characteristics.[17] However Nylon, Gore-Tex or Vicryl sutures are also good alternatives for regenerative procedures. The surgical site was protected with a periodontal dressing.

All subjects were given both oral and written instructions as a part of their postoperative regimen.[18] The patients were advised to rinse with 0.2% chlorhexidine (ICPA Health Products Ltd., Ankaleshwar, India) twice daily for 14 days to help control plaque. Patients were

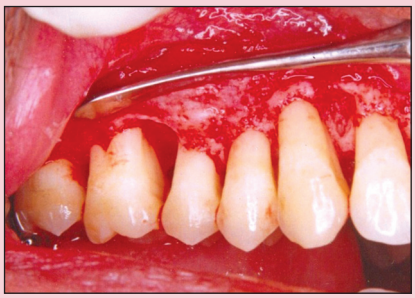

Figure 5: Osseous defect after debridement of the test site.

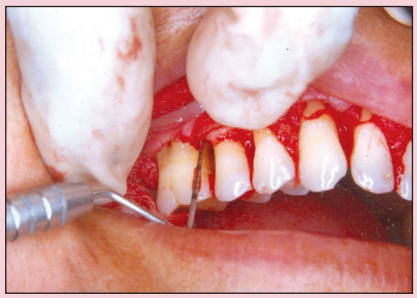

Figure 6: Osseous defect after debridement of the control site.

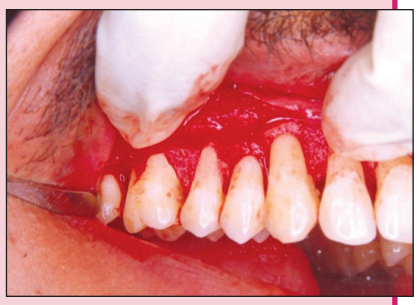

Figure 7: Osseograft placed and condensed into the test site.

advised to avoid chewing in area of the surgery for the same 2-week period and told not to brush at the surgical site or manipulate it for 10 days. Medications were prescribed: 400 mg of ibuprofen 3 times a day for postoperative discomfort, and 1-mg doxycycline hydrate, at a dose of 200 mg on the day of surgery and a single dose of 100 mg per day for the next 6 days to prevent infection.

After 7 to 10 days, the dressing, sutures and any plaque present in the area was removed. Recall appointments were scheduled at 3 and 6 months after the surgery for soft-tissue evaluation, plaque control, radiographic evaluation and recording clinical variables.

Data Analysis

Observational data were analyzed with the Student's *t*-test. Mean values and standard deviations were calculated for each variable and examination interval. The paired Student's *t*-test was used to evaluate and establish differences between baseline and postoperative measurements within a group. The unpaired Student's *t*-test was used to evaluate and establish differences between the test and control groups at baseline, and 3 and 6 months after surgery ($p < 0.05$).

Results

All 30 patients enrolled in the study reported for scheduled maintenance and postoperative evaluation visits.

Plaque Index

No statistically significant difference in the mean values for the plaque index was found between the test and control groups at baseline ($p = 0.065$), 3 months ($p = 0.279$) and 6 months ($p = 0.575$) (**Table 1**).

Gingival Index

No statistically significant difference in the mean values for the gingival index was found between the test and control groups at baseline ($p = 0.333$), at 3 months ($p = 0.093$) and 6 months ($p = 0.272$) (**Table 1**).

Pocket Depth

No statistically significant difference in the mean values for pocket depth was found between the test and control groups at baseline ($p = 0.370$). However, the mean values at 3 months ($p = 0.007$) and at 6 months ($p < 0.001$) were highly significant (**Table 1**).

Clinical Attachment Level

The difference between test and control groups in mean values for levels of clinical attachment at baseline ($p = 0.265$) was not significant. However, the differences in mean values of clinical attachment level at 3 months ($p = 0.025$) and 6 months ($p < 0.001$) were statistically significant (**Table 1**).

Amount of Defect Resolution

For test sites, the statistically significant mean difference in defect resolution at 3 months from baseline was 3.48 ± 1.51 mm and at 6 months 2.23 ± 0.99 mm ($p < 0.001$).

For control sites, the statistically significant mean difference in defect resolution at 3 months from baseline was 3.22 ± 0.83 mm and at 6 months, 2.87 ± 0.67 mm ($p < 0.001$) (**Table 1**).

The difference in the mean values of the amount of defect resolution at baseline ($p = 0.004$), 3 months ($p < 0.001$) and 6 months ($p < 0.001$) between test and control groups was statistically significant.

The mean amount of defect fill from baseline to 3 months and 6 months was 0.82 mm and 1.17 mm for control sites, which was also statistically significant ($p < 0.001$) (**Table 1**).

Percentage of Defect Resolution

For test sites, the mean defect resolution from baseline to 3 months after surgery was 37.1% ± 14.3%, and from baseline to 6 months, 56.5% ± 16.4%. For control sites, the mean defect resolution from baseline to 3 months after surgery was 20.5% ± 8.91%, and from

Table 1 Comparison of mean values of variables at baseline, 3 months and 6 months between test and control groups

	Time	Test	Control	*p*-value
Plaque index	Baseline	1.97 ± 0.29	1.78 ± 0.34	0.065
	3 months	0.97 ± 0.32	0.86 ± 0.30	0.279
	6 months	0.60 ± 0.30	0.55 ± 0.26	0.575
Gingival index	Baseline	2.03 ± 0.38	1.92 ± 0.29	0.333
	3 months	0.80 ± 0.30	0.65 ± 0.26	0.093
	6 months	0.53 ± 0.30	0.44 ± 0.20	0.272
Pocket depth	Baseline	6.85 ± 1.18	6.55 ± 0.89	0.370
	3 months	4.05 ± 0.76	4.80 ± 0.89	0.007[a]
	6 months	2.80 ± 0.70	3.90 ± 0.91	0.000[a]
Clinical attachment level	Baseline	7.05 ± 1.19	6.65 ± 1.04	0.265
	3 months	4.25 ± 0.72	4.90 ± 1.02	0.025[a]
	6 months	3.05 ± 0.51	4.05 ± 0.83	0.000[a]
Defect resolution	Baseline	5.50 ± 1.91	4.05 ± 0.89	0.004[a]
	3 months	2.02 ± 0.92	0.82 ± 0.37	0.000[a]
	6 months	3.27 ± 1.28	1.17 ± 0.47	0.000[a]
% of defect resolution	3 months	37.1 ± 14.3	20.5 ± 8.91	0.000[a]
	6 months	56.5 ± 16.4	28.6 ± 8.84	0.000[a]

[a]*Significant*

baseline to 6 months, 28.6% ± 8.84%. The difference in mean percentage of defect resolution was statistically significant at 3 months ($p < 0.001$) and 6 months ($p < 0.001$) for test and control sites (**Table 1**).

Discussion

Regenerative procedures have focused on the elimination of hard- and soft-tissue defects (i.e., probing depths and osseous defects) by regenerating new attachments. The main characteristic of these procedures is the minimal excision of gingival tissue, minimal extent of flap reflection, close adaptation of tissue and replacement of the flap close to the presurgical position, and open access to the root surface to facilitate root debridement. Many of these procedures include the use of bone grafts and bone-replacement materials to provide new attachments.[19]

The current study focused on the elimination of hard- and soft-tissue defects regenerating new tissue with the use of a xenogenic demineralized bone matrix (Osseograft) and to compare its effectiveness with that of open-flap debridement. Osseograft consists of type I collagen that is prepared from bovine cortical bone samples of 250 µm.

Sampath and Reddi[20] reported that subcutaneous implantation of coarse powders (74–420 µm) of DMBM results in local differentiation of bone. Once the Osseograft is placed in the osseous defect, a sequential differentiation of mesenchymal-type cell occurs to form cartilage and bone. There are 4 stages of cell differentiation and bone formation.[21] Stage 1 includes mesenchymal-cell migration into the vascular spaces of matrix within 2 days. In stage 2, mesenchymal cells differentiate into

giant cells and chondrocytes between day 2 and 18. In stage 3, the poorly vascularized areas of matrix show cartilage formation at day 8 and 20, and from day 10 to 20 woven bone develops in the vascularized areas of matrix. During stage 4, bone formation occurs between day 20 and 30.

The current study, which used Osseograft for 2- or 3-wall infrabony defects, showed bone gain of 56.5% at 6 months in all patients at all test sites. This study was conducted according to the methods of others.[22,23] Sonis and others[22] evaluated the efficacy of demineralized bone powder for the treatment of periodontal defects at 6 months. In their study, which included ½-wall, 1-wall and 2-wall defects, they found radiographic evidence of bone fill for only 61% of the patients studied. In their study, Blumenthal and Steinberg[23] evaluated the clinical efficacy of a combined graft of autolyzed antigen-extracted allogenic (AAA) bone and microfibrillar collagen (Zyderm, Collagen Corporation, Palo, Calif.) covered with a resorbable collagen membrane in human infrabony defects. Using membrane, AAA bone and collagen, they found 62.69% bone fill in defects, and with AAA bone and collagen bone fill, 48.53%. In the current study with Osseograft, bone gain was 56.5% at 6 months. More studies with Osseograft with membranes should be conducted to confirm these findings. The current study showed no statistically significant difference between test and control groups in the mean values of the plaque index and the gingival index at baseline, 3 months and 6 months (**Table 1**). These results concur with those of studies by Yukna and others[10] and Srikanth and others[24] who observed that patients undergoing periodontal therapy try to maintain optimal oral hygiene.

There was statistically significant reduction in mean values of pocket depth at baseline, 3 months and 6 months ($p < 0.001$) between test and control groups. There was statistically significant gain in mean values of clinical attachment level at baseline, 3 months ($p = 0.025$) and 6 months ($p < 0.001$) between test and control groups. The mean amount of defect resolution from baseline to 3 months and 6 months was 2.02 mm and 3.27 mm, respectively, for test sites, which was statistically significant ($p < 0.001$). The mean amount of defect fill from baseline to 3 months and 6 months was 0.82 mm and 1.17 mm for control sites, which was also statistically significant ($p < 0.001$).

In the current study, the mean percentage of defect resolution at 3 months and 6 months for both test and controls sites was statistically significant ($p < 0.001$) (**Table 1**). These results are similar to those for other studies.[10,22,23] In their study, Yukna and others[10] used the combination of anorganic bovine-derived hydroxy apatite matrix (ABM) and peptide (p-15) in periodontal osseous defects, which they evaluated at 6 months. They found that the mean defect fill was 72.9% with the combination of ABM and p-15, and 50.67% with ABM alone. This finding was due to the p-15 polypeptide with its sequence of 15 amino acids, which enhances the binding of fibroblasts and osteoblasts at the surgical site. Future studies with Osseograft in combination with p-15 should be carried out to see if results are similar.

Conclusion

DMBM (Osseograft) improves healing outcomes, compared with open-flap debridement, namely, reduction of probing depth, resolution of osseous defects and gain in clinical attachment. Better biocompatibility, excellent handling properties and the improved response of tissues to the material are definite benefits of using DMBM (Osseograft). Future studies with more patients and experimental animal studies should be conducted to analyze the maximum potential of xenografts for regenerative periodontal therapy.

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