# Histomorphometric Evaluation of Extraction Sockets and Deficient Alveolar Ridges Treated with Allograft and Barrier Membrane: A Pilot Study

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The aim of the study was to determine the fate of demineralized freeze-dried bone allograft (DFDBA) used in conjunction with a barrier membrane in the management of extraction sockets and deficient alveolar ridges, and to compare the amount of bone formed with that found in untreated sites. Ten biopsies were obtained from 8 grafted patients. Five biopsies were harvested from untreated sites during routine implant placement and analyzed for comparison. In the socket management procedure, DFDBA was packed tightly into the socket and covered with an expanded polytetrafluoroethylene (e-PTFE) membrane. Primary closure was achieved in all cases. In the ridge regeneration procedure, cortical columns were placed in the ridge projecting outward approximately 3 mm to create and maintain space for DFDBA particles packed between them; the columns were then covered by an e-PTFE membrane. Healing time ranged from 8 to 23 months. At the time of implant placement, bone cores (7 mm × 2 mm) were harvested, fixed in 10% formalin solution, and prepared for histologic examination. At the light microscopic level, no inflammation or fibrous encapsulation was observed. New bone formation on and around DFDBA particles was widespread. Histomorphometric analysis of the grafted specimens and untreated sites was carried out using the trabecular bone volume (TBV) index. The TBV in the maxillary test specimens was 55.03%, as compared to 57.33% of control cores. Unaltered DFDBA made up 8.7% of the test specimens. In the mandibular biopsies, the TBV was 56.6%, while for the controls it was 40.9%. The volume of DFDBA still present was 2.45%. The results tended to indicate that treatment with DFDBA in conjunction with cell occlusive membranes will result in new bone formation, predominantly by the process of conduction, which appears to be similar in amount and nature to that found in cores harvested from healed nonfunctional edentulous areas.
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Key words: deficient ridges, demineralized freeze-dried bone allograft, extraction sockets, guided bone regeneration, histomorphometry, osteoconduction

A according to Amler et al,[1] uncomplicated healing of human extraction sockets takes place in approximately 40 days in an organized sequence of events, beginning with clot formation and culminating in a bone-filled socket with a connective tissue and epithelial tissue covering. However, disease of periodontic and endodontic origin or surgical trauma can adversely affect this normal pattern and result in extraction sites that have healed but have alveolar ridges that are quantitatively deficient. These deformed alveolar ridges do not permit appropriate pontic fabrication when conventional fixed prostheses are contemplated; nor do they permit the placement of endosseous implants when this form of tooth replacement is being considered. Defective ridge formation can be prevented by grafting the deficient or vulnerable sockets at the time of tooth loss to ensure the formation of alveolar bone within the sites,[2,3] and deficient alveolar ridges can be augmented or regenerated.[4–7]

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**Table 1** Patient Selection

| Patient | Age | Sex | Procedure | Location | Healing | Mo. |
|---|---|---|---|---|---|---|
| I.M. | 30 | F | Socket preservation | Maxillary left canine | Normal | 9 |
| D.M. | 60 | M | Socket preservation | Maxillary left central incisor | Normal | 14 |
| C.R. | 63 | M | Socket preservation | Maxillary right first premolar | Normal | 21 |
| F.A. | 44 | M | Socket preservation | Maxillary left lateral incisor | Normal | 23 |
| B.R. | 55 | F | Socket preservation | Maxillary left first premolar | Normal | 13 |
| S.M. | 38 | F | Socket preservation | Mandibular right first and second molars | Exposure | 10 |
| G.C. | 65 | M | Ridge augmentation | Mandibular left second premolar and first molar | Exposure | 8 |
| B.K. | 63 | F | Ridge augmentation | Maxillary right first premolar | Normal | 9 |
| L.T. | 60 | F | Control | Maxillary right first premolar | — | — |
| D.M. | 53 | M | Control | Maxillary right first premolar | — | — |
| I.T. | 51 | M | Control | Maxillary right first premolar | — | — |
| D.G. | 72 | M | Control | Mandibular right first molar | — | — |
| K.Z. | 56 | M | Control | Mandibular right first molar | — | — |

Requirements for successful alveolar regeneration were first presented by Melcher and Dreyer in 1962.[8] Later, Nyman and coworkers[9] proposed guidelines for guided tissue regeneration in the repair of defects associated with teeth and edentulous ridges.[10] These included creation and maintenance of space; protection of the blood clot formed; trephining of cortical plates to enhance the ingress of vascular, cellular, and molecular elements needed in the regenerative process; and the use of a cell occlusive barrier membrane to prevent invasion of the site by tissues that could impede regeneration. These criteria have since been successfully applied in endeavors to generate bone in extraction sockets[2,3] and to regenerate alveolar bone in defective ridges.[4,5,6,11] Human demineralized freeze-dried bone allografts (DFDBA) have been used in conjunction with the principles of guided bone regeneration to reconstitute and maintain bone during the placement of endosseous implants.[6,12–18] In these instances, DFDBA has been considered a space maintaining device and osteopromotive, and new bone formation takes place predominantly by a process of osteoconduction.[3,16,18] The role of DFDBA as an osteoinductive element has been both questioned[19–24] and affirmed.[2,25–27]

There is a paucity of human histologic and histomorphometric data pertaining to the amounts of new bone formed in extraction sockets and on deficient alveolar ridges augmented with DFDBA particles and barrier membranes. This study was undertaken to evaluate the potential of this type of treatment to produce new bone in these situations. An additional aim was to compare the amounts of newly formed bone with that found in untreated alveolar ridges.

## Materials and Methods

**Patient Selection**. Six patients, all requiring extraction of 1 or more teeth for traumatic or endodontic reasons (these were to be replaced by endosseous implants), were included in the socket treatment portion of the study. Two patients requiring ridge augmentation procedures prior to implant placement participated in this part of the study, while 5 patients requiring routine endosseous implant placement provided cores for the untreated areas (Table 1). The patients, 4 males and 4 females ranging in age from 30 to 65 years (mean 58.33), were all treated in Department of Periodontology and Oral Biology Implant Center

at Boston University School of Dental Medicine. All patients were in good health as determined by medical history screening, had no contraindications to treatment, exhibited good oral hygiene, and were informed of the nature of this investigation. All gave their informed consent according to the guidelines of the Internal Review Board, which approved this study.

**Surgical Technique.** *Socket Management.* Following administration of appropriate local anesthesia, intrasulcular incisions were made around the teeth to be extracted. Vertical releasing incisions were made both palatally and facially, either at the mesial and distal line angles of teeth adjacent to the tooth being removed or one tooth distal and mesial to it, and mucoperiosteal flaps were carefully elevated. The tooth in question was atraumatically extracted and the socket was thoroughly debrided (Fig 1). Intramarrow penetration with a fine, round bur promoted appropriate bleeding. Commercially obtained DFDBA (American Red Cross, St. Louis, MO) of 250 to 350 µm particle size was hydrated with sterile normal saline for 30 minutes prior to placement within the sockets. The DFDBA was placed in the socket and compressed using saline-saturated gauze, from which all excess saline had been expressed, and firm pressure from a hand instrument to eliminate dead spaces within the graft material. This was repeated until the socket was slightly overfilled (Fig 2).

A nonresorbable expanded polytetrafluoroethylene membrane (e-PTFE) (GTAM, WL Gore, Flagstaff, AZ) of appropriate dimension was trimmed so that it extended approximately 3 mm over the socket onto sound bone buccolingually but did not engage adjacent tooth surfaces. The buccal flaps were further released by periosteal separation to permit coronal positioning of the tissues and primary closure. Suturing was accomplished with nonresorbable e-PTFE, vertical mattress, and interrupted sutures. The patients were placed on doxycycline 100 mg/day for 2 weeks and nonsteroidal anti-inflammatory medication for pain control for 5 days, and chlorhexidine gluconate mouthwash (twice daily) was prescribed until mechanical plaque control could be recommenced after 1 week. Sutures were removed after 1 week and the patients were seen weekly to monitor for possible membrane exposure.

Membranes were kept in place for a minimum of 5 weeks; they were removed at the time of implant placement if they had not already become exposed and been removed. The membranes were removed following administration of local anesthesia by elevating mucoperiosteal flaps and separating the membranes from the soft tissue and underlying bone. Primary closure of the wounds was obtained using suturing methods previously described. Those membranes that were retained until implant placement were removed in the same manner at the time of stage 2 surgery (Fig 3).

*Ridge Regeneration.* Appropriate local anesthesia was administered, and a distal-to-mesial incision was made on the crest of the edentulous ridge between the teeth adjacent to the edentulous space, from the mesiolingual line angle of the distal tooth to the distolingual line angle of the mesial tooth. Vertical releasing incisions were made at the distal line angle of the distal and the mesial line angle of the mesial adjacent teeth, both buccally and lingually, and carried around the teeth to join the crestal incision. Mucoperiosteal flaps were elevated, and the buccal alveolar surface was penetrated in multiple areas to expose the endosseum. Wider holes, approximately $2 \times 2$ mm (Fig 4a), were drilled to receive the specially prepared DFDBA cortical columns (Northwest Tissue Center, Seattle, WA), which, after being firmly placed in these holes, projected out laterally approximately 3 mm like tent poles (Fig 4b) to support the e-PTFE membrane to be placed over the site.[7] Particulate DFDBA with the same properties as that used in the socket treatment part of the study was similarly reconstituted and compressed onto the surface of the alveolar bone around and between the cortical columns (Fig 4c). The membrane was then suitably trimmed, using the same precautions as previously described, and adapted well to sound bone surrounding the augmentation site. The buccal flaps were further released via periosteal separation to permit primary closure of the wounds without undue tension, and the flaps were sutured with e-PTFE vertical mattress alternating with interrupted sutures. Postoperative medications and membrane removal were the same as described for the socket study.

**Core Harvesting.** At the time of implant placement, which varied between 8 and 23 months after grafting, the patients received appropriate local infiltration anesthesia and mucoperiosteal flaps were elevated. Utilizing the photographic records made at each surgical stage as a frame of reference, the center of each augmented extraction site was compared to the original, in an attempt to ensure that the core would be taken from the treated site. The use of templates would have been more accurate, but since the study did not include measurement of the ridges, templates were not used. As the first step in osteotomy site preparation, a 2-mm surgical trephine was used to remove a 7-mm-long bone core from the center of the regenerated site.
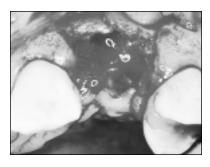
**Fig 1** Photograph illustrating extraction site. Note the fracture of thin, vulnerable labial plate.
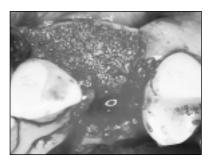


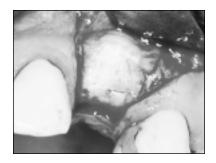**Fig 2** Demineralized freeze-dried allograft in socket.



**Fig 3** At stage 2 surgery, the e-PTFE membrane can be seen in situ over the extraction site.



**Fig 4a** Right mandibular knife-edged ridge, with holes trephined in the buccal plate. Some of the holes will have cortical columns placed in them.
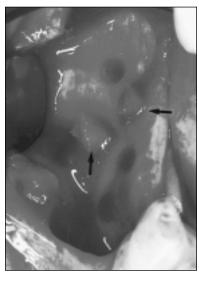


**Fig 4b** Two cortical columns projecting facially about 3 to 4 mm out of the buccal plate *(arrows)*.



**Fig 4c** Demineralized freeze-dried bone allograft has been packed around the cortical columns prior to coverage with an e-PTFE membrane.
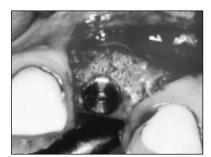


**Fig 5** Implant is placed in the healed extraction site. The width of the ridge has been maintained.

This was immediately fixed in 10% formaldehyde; site preparation was then completed, the selected implants were placed (Fig 5), and the wounds were closed with vertical and interrupted sutures. Sutures were removed in 7 days; healing generally progressed uneventfully. The same modus operandi was utilized when cores were obtained in the ridge augmentation part of the study. Similarly, the cores from healed, untreated sites were procured at the time of routine implant placement.

**Histologic Preparation.** Following fixation, the cores were decalcified in nitric acid for 2 weeks. The bone specimens were embedded in paraffin; cut serially, in a longitudinal plane, to a thickness of 6 to 8 μm; and stained with hematoxylin and eosin and toluidine blue in preparation for microscopic evaluation. Only the 6 central sections obtained from each core were used for comparative study. This selection was made in an attempt to minimize the inclusion of artifacts related to the harvesting process, which were more likely to appear on the surfaces of the cores. In addition, it was assumed that these sections would be more representative of the specimens.

**Histomorphometric Analysis.** The 2 most central sections of each core were evaluated histomorphometrically by the same person, who was blinded. The microscopic slides were viewed on a Nikon FXA microscope with a digital analytic interface (MicroVideo Instruments, Avon, MA) The microscope was attached to a video camera, which was linked to a computer, the software used being Image Pro+ (North Reading, MA). The amounts of bone, marrow, and DFDBA particles within a given field were measured and expressed in pixels. The average number of fields for each section analyzed was 5.53 (SD 2.67). The magnification used was 200 ×. The trabecular bone volume (TBV) index[28] was used to establish the ratio between trabecular bone and marrow spaces.

**Photographic Data Collection.** At strategic times during the treatment, 1-to-1 35-mm photographs were taken to permit evaluation of clinical results and to help ensure that cores were always taken from the center of a regenerated site (Figs 1, 4a, and 5). Reference to pretreatment photographs permitted verification of ridge width maintenance in the treated sites. Measurements were not made, as this was not part of the study protocol.
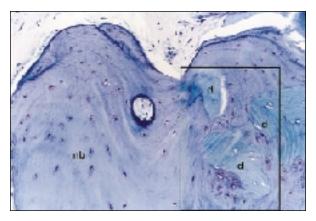
## Results

Primary closure was achieved in all surgical procedures, the postoperative period being generally uneventful. Two membranes became exposed pre-

maturely and were removed at 5 weeks. Socket treatment and ridge augmentation both resulted in ridges of adequate dimension for implant placement. Microscopic examination of the extraction site cores did not reveal any inflammatory response or fibrous encapsulation of particulate bone. Very little osteoclastic activity was noted around the remaining nonvital DFDBA particles; rather, those particles still present were surrounded by and appeared to be coalesced with newly formed bone (Fig 6a). Osteoblastic activity was observed to still be occurring on some newly formed bone surfaces, indicating a persisting remodeling process (Fig 6b). The newly formed bone was woven, woven undergoing lamellation, or lamellar with secondary osteons being present in the mineral phase (Fig 7a). In some sections, basophilic staining material was noted, sometimes eccentrically placed in lacunae, within the DFDBA particles near their peripheries and adjacent to newly formed bone (Fig 7b). This material appeared to resemble the eccentrically placed nuclei within newly formed vital bone.

The cores from the untreated, healed sites exhibited the trabecular bone pattern typically seen in alveolar bone, with large marrow spaces filled with adipose-type tissue usually seen at maturity (Fig 8). The experimental histologic sections seemed to reveal a more compact picture, with new bone formation on and about the enduring DFDBA particles. The marrow spaces appeared to be smaller and scarcer; some contained a mature adipose cellular arrangement, while others exhibited an apparently more active cellular pattern (Fig 9).

The cores from the augmented ridge sites revealed essentially the same picture. The cortical columns were still recognizable histologically. They appeared to be virtually unchanged, with only early signs of cellular activity seen in isolated areas. Newly formed bone was present and in intimate contact with the surfaces of the cortical columns (Fig 10).

Histomorphometrically, the TBV for the maxillary test cores was 55.03% (SD = 15.02), while that for maxillary untreated sites was 57.33% (SD = 11.37) (Fig 11, Table 2). In the mandible, the TBV for the experimental areas was 56.60% (SD = 32.77) and 40.95% (SD = 2.76) for the untreated areas (Fig 12, Table 3). The DFDBA still present in the specimens was 8.7% (SD = 7.58) for the maxillary and 2.45% (SD = 1.04) for the mandibular cores.

**Fig 6a** Photomicrograph demonstrating a large amount of new bone (nb) formation surrounding DFDBA particles (d) in a 21-month postoperative maxillary extraction site specimen (toluidine blue stain, original magnification ×200).
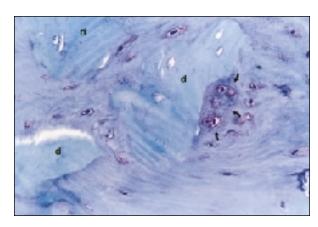


**Fig 6b** High-power view (×400) of outlined area in Fig 6a, rotated 90 degrees counterclockwise. Note DFDBA particles (d) surrounded and coalesced with new bone. Note cellular activity *(arrows)*.
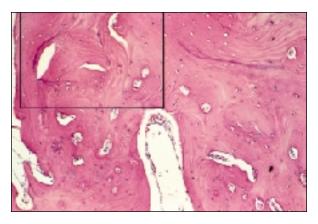


**Fig 7a** Photomicrograph representing newly formed bone of woven and lamellar nature in a mandibular ridge augmentation at 8 months (hematoxylin and eosin, original magnification ×100).
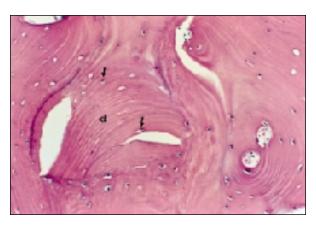


**Fig 7b** High-power view of area outlined in Fig 7a. Note woven and lamellar bone, primary osteons, and occurrence of what could be nuclei in DFDBA particle *(arrows)* (hematoxylin and eosin, original magnification ×400).
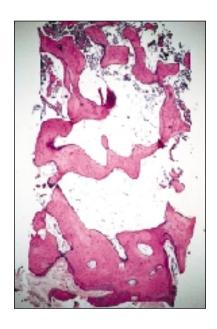


**Fig 8** *(Left)* Low-power view of a 7-mm core from a nonfunctional, untreated edentulous maxillary area. Compare the relative amounts of bone and marrow space sizes with those in Fig 9 (hematoxylin and eosin, original magnification ×40).

**Fig 9** *(Right)* Low-power view of a 7-mm core from a treated maxillary socket site after 9 months of healing. Note the denser appearance, scarcer and smaller marrow spaces, as compared to Fig 8 (hematoxylin and eosin, original magnification ×40).
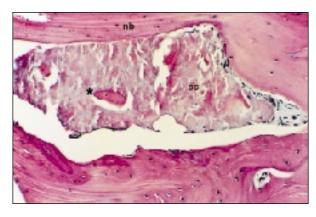
**Fig 10** Photomicrograph illustrating a cortical column (cc) surrounded by newly formed bone (nb) in an 8-month mandibular ridge augmentation. The asterisk (*) indicates what could be an osteocyte surrounded by bone in the otherwise unaltered cortical column (hematoxylin and eosin, original magnification ×400).
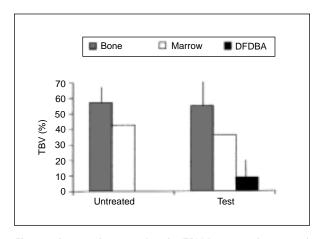


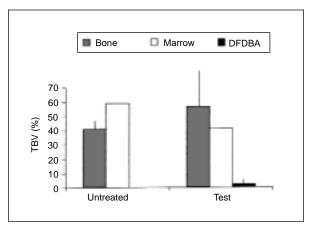**Fig 11** Bar graph comparing the TBV in test and untreated sites in the maxilla.



**Fig 12** Bar graph comparing the TBV in test and untreated sites in the mandible.

**Table 2** Maxillary Trabecular Bone Volume

| Patient | % bone | % DFDBA |
|---|---|---|
| Test sites | | |
| I.M. | 64.0 | 10.4 |
| B.K. | 38.5 | 3.1 |
| B.R. | 47.3 | 11.2 |
| D.M. | 57.6 | 21.5 |
| C.R. | 43.7 | 6.0 |
| F.A. | 79.1 | 0.0 |
| Mean | 55.03 | 8.7 |
| SD | 15.02 | 7.58 |
| Untreated sites | | |
| L.T. | 58.1 | — |
| D.M. | 45.6 | — |
| I.T. | 468.3 | — |
| Mean | 57.33 | — |
| SD | 11.37 | — |

**Table 3** Mandibular Trabecular Bone Volume

| Patient | % bone | % DFDBA |
|---|---|---|
| Test sites | | |
| S.M. | 48.4 | 0.9 |
| S.M. | 14.0 | 2.8 |
| G.C. | 77.0 | 3.0 |
| G.C. | 87.0 | 3.1 |
| Mean | 56.6 | 2.45 |
| SD | 32.77 | 1.041 |
| Untreated sites | | |
| D.G. | 39.0 | — |
| K.Z. | 42.9 | — |
| Mean | 40.95 | — |
| SD | 2.76 | — |

## Discussion

The present study appears to confirm that DFDBA can be used to successfully treat sockets prior to the placement of endosseous implants.[2,3,16,18] It also corroborates the findings that utilization of particulate DFDBA, in conjunction with the principles of guided tissue regeneration for the treatment of extraction sockets, will result in the gradual replacement of the allograft by newly formed bone.[3,18] The finding that bone formation takes place in an appositional manner on and around the allograft particles verifies the findings of others and tends to substantiate a conductive role for the DFDBA particles.[3,7,17,18] The fact that osteoblastic activity was still occurring on the surfaces of the newly formed lamellar bone indicates that active remodeling of the DFDBA particles and bone formation continued to take place for up to 23 months in this study, a phenomenon also noted by others.[3,17,18] It is noteworthy that Simion et al[18] were still able to identify, in human peri-implant tissue, apparently unaltered DFDBA particles some 4 years after placement. In more apical portions of the same specimen, DFDBA particles could be seen completely embedded in bone matrix and still showing signs of ongoing mineralization. This would appear to indicate that allograft reconstitution or replacement may take many years to complete.

It was not possible to corroborate the presence of mineralization nodules within the DFDBA particles.[16] However, the occasional appearance of deeply stained basophilic material in osteocyte lacunae, in the most peripheral portions of the demineralized particles nearest to adjacent new bone formation, which may be osteocyte nuclei, was noteworthy. This may permit the assumption that some sort of ongoing creeping substitution of the graft with new bone may be occurring.

How these events take place would be difficult to explain, but as Zhang et al[29] suggest, they may be related to the stimulating effect of residual calcium levels in allografts, or the degradation of organic matrix (collagen/proteoglycan), which may diffuse from the implant-stimulating cellular chemotaxis into the implant. Zhang et al further suggest that the degraded matrix could also act as a site to which cells attach and receive appropriate regulatory signals. Growth factor release may also be involved in cellular infiltration, differentiation, and establishment of a matrix that facilitates appropriate cell infiltration. However, it should be noted that in an investigation of chemical and "autodigestive" methods to remove "alloantigenic" tissues from "undemineralized" allogeneic bone implants, Urist et al[30] described some basophilic staining elements in some of the osteocyte lacunae. These elements may merely be a feature of nonvital bone implants. Urist et al[30] also stated that all the treated and untreated allogeneic or autogenous implants used in their study exhibited empty lacunae within the same period after implantation. In addition, Urist in 1980[31] again stated that lacunae of implanted decalcified allografts were predominantly empty and may remain so for extended periods of time. The findings of empty lacunae in the remaining allograft particles in the present study tend to agree with this.

The observation that the experimental cores presented a more compact picture than the cores from the untreated healed areas is probably merely an aggregation of the new bone formation on and around the remaining DFDBA particles and the sparser and smaller number of marrow spaces. Whether this phenomenon would persist as the grafts mature or whether the new bone would come to more closely resemble bone found in the untreated sites still needs to be determined.

The histomorphometric analysis revealed that the TBV for the maxillary cores was 55.03% in the experimental sites and 57.33% in the untreated sites. Similarly, the mean TBV for mandibular experimental cores and cores from untreated sites was 56.60% and 40.95%, respectively. It is apparent that the amount of bone present in grafted areas is similar to that found in nongrafted, nonfunctional edentulous ridge sites. The percentage of DFDBA particles still present in the maxillary test specimens was 8.70%, as opposed to 2.45% in mandibular specimens. This difference may be the result of a more rapid reconstitution of the DFDBA particles in the mandible, or there may also be individual biologic variations in response to allograft placement. The small sample size does not permit a more definitive statement.

The standard deviations for the maxillary test and untreated specimens were large (15.02 and 11.37, respectively), but in the mandible the even larger SD for the experimental core (32.77) was much greater than that of the untreated areas (SD = 1.25). It is noteworthy that among the 4 mandibular test specimens, 1 of the 2 sides with exposed membrane became infected, resulting in very poor bone formation (14.0%). If this site is excluded from the analysis, the TBV for the mandibular test cores would be 70.80% (SD = 20.03), suggesting an even more marked difference from the untreated sites. The large differences seen in both control and experimental data for most parameters examined

could well be a reflection of the small sample size or perhaps indicate a biologic variation that is common in nonfunctioning alveolar bone.

The value of DFDBA as an additive in bone regeneration procedures has also been questioned by Becker et al.[19,24,32] The same authors claim that DFDBA has no inductive effect in promoting bone formation in human extraction sockets, which they found healed, with nonvital bone particles being surrounded by connective tissue. Sockets treated with autologous bone grafts, on the other hand, exhibited healing with vital woven bone. Other studies[20–23] agree with these findings. Studies that investigated the properties of particulate human allografts have demonstrated, in ectopic sites or when gaps in bone are bridged, that the allografts do exhibit inductive properties.[26,27] Zhang et al,[33] in investigating the osteoconductivity of human demineralized bone matrix implanted into ectopic sites in athymic mice, more recently confirmed an inductive role for human particulate allograft. None of these studies used the principles of guided tissue regeneration, where cell occlusive membranes are used in conjunction with allograft placement as a basis for treatment. The effect of this approach on induction needs to be elucidated.

In addition, Schwartz and coworkers[34] have shown that human particulate allografts obtained from different bone banks vary in biologic activity when placed in ectopic sites. This variation has been attributed to the age of donors, the method of sterilization used, irradiation, and residual acid content. What effect, if any, these factors played in the studies cited is not known. In an in vitro investigation comparing the biologic activity of fresh bone and allografts, Shigeyama and coworkers[35] were able to show biologic activity for the allograft material, which was only slightly inferior to that of the fresh bone. It is thus obvious that more information is necessary to more definitively resolve the controversy over the inductive capacity of DFDBA. The present limited study did not shed light on the inductive capacity of DFDBA, but it did seem to indicate a conductive role for the allograft material, as evidenced by the appositional bone formation on and around the DFDBA particles. Therefore, it may be possible to conclude that, irrespective of the inductive potential of human allografts, the endogenous inductors, together with the conductive effect of DFDBA and utilization of principles of guided tissue regeneration, are sufficient to promote appositional bone growth in the treatment of extraction sockets and deficient alveolar ridges.

## Conclusions

Based on the findings and within the limits of this histologic and histomorphometric study, it may be concluded that:

1. In human extraction sockets, commercially available DFDBA, in conjunction with cell occlusive barrier membranes, appears to play a positive conductive role in new bone formation.
2. Histomorphometric analysis indicates similar trabecular bone volume in untreated sites and extraction sockets grafted with DFDBA, when guided bone regeneration principles are followed. The same applies to edentulous ridges treated in the same fashion.
3. The new bone growth appears to be appositional, and the DFDBA particles appear to undergo a creeping reconstitution that may take many months, if not years, to complete.
4. These results do not offer conclusive evidence regarding the osteoinductive capacity of commercially available particulate bone allografts.

## Acknowledgments

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