Clinical Utility of Demineralized Bone Matrix for Osseous Defects, Arthrodesis, and Reconstruction: Impact of Processing Techniques and Study Methodology

James L. Russell, PhD
Jon E. Block, PhD

Demineralized bone matrix (DBM) in a variety of forms is a much-used type of bone graft because it has both osteoconductive and osteoinductive characteristics. Indeed, the majority of studies have shown that DBM directly induces new bone formation when implanted subcutaneously or intramuscularly.2,5

The addition of autologous marrow to DBM furnishes an immediate source of viable osteogenic precursor cells at the graft implantation site and may additionally provide a direct biochemical contribution to osteogenesis.6–12 Consequently, allogeneic DBM often in combination with autologous marrow has, with few exceptions,13 performed similarly to autologous bone in healing long-bone defects created in both lower- and higher-order animals.14–18 It should be noted that the unflattering findings of Schwarz et al19 may be partially explained by the use of a form of DBM that had been sterilized with cobalt irradiation prior to implantation, a practice with a documented effect of attenuating the osteoinductive potential.19

Demineralized bone matrix also has shown promise as a bone-graft substitute for spinal fusion. Among spinal fusions in rabbits grafted with either autologous bone or allogeneic DBM, Oikarinen20 found similar gross histologic evidence of new bone formation and remodeling within the fusion mass 6 months postoperatively. Undermineralized grafts were markedly inferior in terms of histologic evidence of new bone formation. Several studies by Ragni et al21–23 confirmed similar histologic evidence of new bone formation and radiographic evidence of solid fusion when comparing DBM with autologous bone in rabbit spinal fusions. The standard demineralization process as originally promulgated by Urist et al24 is easily reproducible, consisting of several basic steps. Briefly, long-bone segments are placed in 0.6 N HCl for 24 hours using 1 g of bone per 100 mL acid solution; the acid is removed by thorough washing in sterile water, and the samples are lyophilized. Reddi and Huggins25 provided a slight modification of this procedure by initially pulverizing the bone samples to a consistent particle size (i.e., 74–420 μm) followed by demineralization in 0.5 N HCl, 25 mEq/g for 3 hours; residual acid is eliminated by rinsing in sterile water, absolute ethanol, and ethyl ether.

While hydrochloric acid is the industry-recognized demineralization agent of choice, processing methods vary widely and include: the concentration of the demineralization agent4,26–28; the temperature and duration of the demineralization step(s)2,27–29; the
inclusion or exclusion at various points in the demineralization process of solvents or solvent combinations such as ethanol, methanol, ether, chloroform, chloroform:methanol, and chloroform:ether;24,30,31 the extent to which the matrix is washed following the demineralization step;26,27 and whether the resulting DBM is stored frozen or is lyophilized and stored at room temperature.2 In some reports, the initial bone tissue is irradiated to reduce the bioburden, while in others, the DBM is reported to be aseptically processed or is subjected to terminal sterilization with either ethylene oxide or irradiation.2,26,30

A number of these processing steps have been shown to affect the biological properties or bone-forming capacity of DBM. Table 1 lists several commonly used processing methods and describes their potential effects on osteoinduction. While some of these ancillary processing methods may enhance the osteoinductive potential of the matrix (e.g., lipid and lipoprotein removal using some detergents),6 others may be deleterious (e.g., terminal sterilization).33,34 For example, pulverization that yields particle sizes <125 µm has resulted in an attenuated osteoinductive response.37,38 Likewise, certain chelating agents, various acid-alcohol preparations, and alternative decalcifying solutions as well as selected physical agents and tissue fixatives inactivate the inductive substrate to varying degrees.5,24 In contrast, DBM that is either lyophilized or frozen (<70°C) has been as effective in new bone formation as freshly prepared demineralized bone powder.39 Irradiation (e.g., >2 megarads), however, generally has been found to have deleterious effects on the osteoinductive potential of DBM whereas lower levels of radiation have produced mixed results.19,39,40 Irradiation-induced adverse effects likewise have been reported to be most pronounced when applied to lyophilized, undemineralized bone.41

Ethylene oxide frequency is used to sterilize allograft and DBM. However, clinical failures or deleterious tissue effects have been associated with ethylene oxide sterilization.36,42,43 Doherty et al34 found that exposure of ethylene oxide sufficient to definitively eradicate a common bacterium (i.e., 1 hour) had the untoward result of markedly attenuating the osteoinductive potential of DBM. On the other hand, Hallfeldt et al45 reported that DBM terminally sterilized with ethylene oxide for 4 hours continued to provide a viable matrix for the formation of new bone when implanted into a bony defect in sheep, a result similar to that observed for DBM terminally disinfected with ethanol or for autologous bone graft. Thus, uncertainty remains as to whether graft performance, in some cases, will be compromised by the sterilization of DBM with ethylene oxide.

Ethanol is effective in reducing bacterial load46 and does not affect the bone-forming potential of the graft.3,24,45,47 Antibiotic treatments also are used and do not adversely affect biomechanical or bone-forming properties of bone.5,35 Importantly, a study by Scarborough et al48 provides strong evidence that important pathogens such as human immunodeficiency virus and hepatitis are effectively inactivated by
one particular demineralization process that does not include ethylene oxide sterilization. Consequently, variations in DBM processing methods and techniques can markedly affect the underlying biological potential and performance characteristics of the graft.

This article reviews the clinical literature, with processing variables in mind, as a means of interpreting the clinical utility of DBM.

**MATERIALS AND METHODS**

A literature review of published clinical studies of the use of demineralized bone matrix for various skeletal applications was conducted. The review was organized according to clinical indications. Four broad indications were identified:

- bone tumors and cysts,
- long-bone defects,
- spinal fusion, and
- craniofacial reconstruction.

Dental applications of DBM are not included in this article.

Articles were identified and selected for review using a number of methods. A MEDLINE search was conducted using various combinations of "demineralized," "decalcified," "bone," "matrix," "powder," "gel," and "allograft" (eg, "demineralized bone matrix") as primary search terms alone and also coupled with various limiting descriptors such as fracture(s), tumor(s), fusion, etc. On-line searches were restricted to display only clinical reports in English published from 1966 to present; preclinical and laboratory investigations were not included other than for background supporting information.

All articles were retrieved and examined for suitability by one of the authors (J.E.B.) prior to review. Articles also were culled from the personal libraries of both authors. Additionally, the bibliography lists of all articles were scanned to identify relevant articles published prior to 1966 as well as any other clinical reports of potential interest.

**RESULTS**

*Bone Tumors and Cysts.* Two studies reported the use of DBM for surgical reconstruction of bony defects of the hand.530 Upton and Glowacki49 examined a mix of 12 patients (primarily children and adolescents) with 26 hand defects of various types: 5 had one or more enchondroma defects (ie, benign bone tumor), 5 had congenital defects, 1 had a large phalangeal defect after radial excision of an osteochondroma, and 1 patient had a significant area of bone loss secondary to surgery to correct amputated digits. Surgical treatment consisted primarily of thorough curettage and defect packing with either rehydrated demineralized powder or cancellous chips; missing cortical surfaces were replaced with demineralized corticocancellous blocks.

Defect healing was evaluated radiographically at 3-month intervals (mean follow-up: 50 months [range: 14-92 months]) and rated on a four-part scale (1=lowest grade and 4=highest grade); a rating of 4 was given when the defect had been filled with trabecular bone similar to adjacent unaffected digits, with no residual areas of radiolucency.

Results were qualitatively compared with other defects in 5 of the same patients who received autologous bone grafting at a previous surgical session. By 6 months postoperatively, 23 of 26 defects were 50% to 75% filled with viable bone; by 12 months, complete defect filling (ie, a rating of 4) had occurred in 24 of 26 DBM-treated cases. These healing results were similar to findings observed among a small number of other defects managed with autologous bone.

Whiteman et al50 similarly reported on 20 consecutive cases (18 enchondromas and 2 intraosseous cysts) of bone defects of the hand treated with curettage and DBM powder. A matched control group consisted of 16 patients managed with curettage and autologous bone and 4 patients managed with curettage alone. Again, a four-part rating system (poor, fair, good, and excellent) was used to blindly judge healing radiographically at regular intervals through 12 weeks postoperatively.

Eighty percent (16/20) of the defects managed with DBM achieved complete (ie, excellent) healing, and all of the defects healed without recurrence, resorption, or refracture. Additionally, 19 of 20 DBM subjects regained their preoperative range of motion within 1 month of surgery. These findings contrast with results achieved with conventional treatment where a 25% overall failure rate necessitated reoperation, a finding significantly (P<.05) worse than the 0% rate in DBM subjects. Patients undergoing bone grafts from the iliac crest required, on average, 2 days of hospitalization due to hip-related symptoms. This complication was avoided entirely among patients managed with DBM.

Demineralized bone matrix likewise has been used to manage bony tumors and cysts in other areas of the skeleton.651 Kakuchi et al62 reported encouraging findings using DBM in 86 cases with a variety of tumorous bone conditions. Seventy-three (85%) of these cases were available for long-term radiographic and clinical follow-up (mean follow-up: 32 months). After packing the excised tumor site with DBM, >90% of cases showed complete defect repair; the remaining cases (3 of 15 giant cell tumors and 4 of 26 solitary bone cysts) showed recurrence. This success rate is strikingly similar to the 100% rate reported by Tiedeman et al6 and the 88% rate reported by Iwata et al63 among smaller samples of subjects with similar cavity defects (n=7 and n=24, respectively).

Xiaobo et al64 also reported encouraging results in a small subset of seven patients with varying-sized tumors and cysts that were grafted with DBM: the defects were located at various appendicular sites. Patients underwent follow-up for an average 14 months (range: 9-25 months). Using a four-part radiographic rating system, all of the patients achieved a rating of 4, representing complete defect healing by study completion.

Pals and Wilkins55 reported the novel use of DBM to facilitate the
reestablishment of the cortical shell in large, giant cell tumors initially managed with extensive curettage and filled with methylmethacrylate cement. Demineralized bone matrix was placed on the surface of the cement as a final surgical maneuver to fill the residual cortical defect. All 10 of their patients showed a reconstituted cortical shell after an average of 36 months of follow-up (range: 19-65 months) and 80% had an excellent functional result using the Musculoskeletal Tumor Society rating system (ie, a four-part rating system).

**Long-Bone Defects.** Four studies were identified that evaluated the clinical utility of DBM in the surgical management of long-bone defects, including fractures, delayed unions, or frank nonunions.\(^7\)\(^{53}\)^\(^{54}\)\(^{56}\) Xiaobo et al\(^4\) assessed defect healing using a four-part rating system among a subgroup of subjects with various long-bone defects: fracture (n=3), delayed union (n=7), and nonunion (n=7). All patients were treated with DBM, and postoperative follow-up ranged from 9 to 26 months (mean: 14 months). Bony union and healing was achieved in all patients by 2 to 6 months postimplantation, and the highest radiographic score (ie, 4) was reported among 88% (15/17) of DBM patients at final follow-up.

Kakiuchi et al\(^52\) also observed clinical success with the use of DBM among 8 traumatic defects requiring bone grafting. All eight of these traumatic bone injuries showed complete repair. Demineralized bone matrix was less successful among three challenging cases of delayed union, with only 1 (33%) of 3 cases showing complete healing. Similarly, Tiedeman et al\(^7\) reported that 11 (61%) of 18 subjects with frank nonunions refractory to previous interventions achieved union after grafting with a composite of DBM and autologous marrow.

Additionally, Johnson et al\(^56\) reported on their experience with a composite graft of DBM and human bone morphogenetic protein in healing resistant nonunions of major long bones (12 femurs, 7 tibias, and 6 humeri). All nonunions had been refractory to conservative management for an average of 30 months (range: 5-83 months). In 10 patients, bone grafting with the DBM composite was augmented with internal fixation, and cancellous autologous bone further augmented the composite implant in 7. Patients underwent radiographic and functional follow-up for an average of 21 months (range: 5-82 months).

Initially, 80% (20/25) of patients achieved healing; average healing time among these subjects was 6 months (range: 3-14 months). Complete bony healing was eventually achieved in all 1 patient following reoperation with repeat composite implantation in the 5 patients who failed initial surgical intervention. Excellent postoperative function was reported in 14/25 (56%) patients; 5 patients achieved either good or fair functional levels. The sole patient whose injury was unresponsive to this intervention reported poor postoperative function.

**Spinal Fusion.** Case reports of the use of DBM to facilitate spinal arthrodesis suggest similar effectiveness to autologous bone.\(^2\)\(^{55}\)\(^{56}\) Urist and Dawson,\(^57\) using a highly extracted form of DBM, reported similar performance to autologous bone among 40 subjects managed with posterolateral lumbar spinal fusions. Tiedeman et al\(^7\) reported an 87.5% solid fusion rate in 8 subjects undergoing spinal fusion procedures.

However, results achieved with DBM in spinal fusion in other studies have been equivocal, especially when directly compared with the performance of autologous bone. For example, Jorgenson et al\(^58\) reported significantly worse radiographically determined bone mineralization and solid fusion ratings in posterolateral fusion sites grafted with ethylene oxide-sterilized DBM (or DBM/autologous bone composites) than among sites grafted with autologous bone alone. In their study, 144 subjects undergoing posterolateral lumbar spinal fusion had different grafting materials randomly allocated to separate sides of the fusion construct within the same subject. Regardless of the type of DBM material or composite used, autologous bone was consistently found to offer statistically superior performance from a fusion integrity standpoint. The overall fusion rate among autologous bone-treated sites was approximately 80%. This contrasts with an overall fusion rate of approximately 40% among sites treated with various forms of DBM (ie, with or without autologous bone in the composite). It should be noted that the analytical methods used to evaluate the findings of this study have been questioned,\(^59\) therefore, it is unknown whether the overall conclusions of this study would be altered substantially.

An et al\(^60\) found mixed results among 39 patients treated for anterior cervical fusion with a composite of DBM and freeze-dried allograft compared with 38 subjects managed with autologous bone. Although the pseudarthrosis rate among those treated with DBM (46%) was higher than the rate among autologous bone subjects (26%), this difference was not statistically significant (P=1.1). Similarly, the proportion of subjects with graft collapse >3 mm was also higher among DBM subjects compared with the autologous bone comparison group (19% versus 11%). Again, this difference was not significant (P=3.2). On the other hand, the proportion of subjects judged as "clinical success" was similar between study groups (85% for DBM versus 84% for autologous bone), and the rate of revision surgery qualitatively favored subjects managed with DBM (8% for DBM versus 13% for autologous bone; P=68).

In a well-matched retrospective case series analysis of 90 subjects, Lowery et al\(^61\) compared patients treated with posterolateral fusion grafted with autologous bone (n=54) or a composite of DBM and autologous bone (n=36). For both study groups, autologous bone was obtained from decortication at the primary surgical site or was harvested from the iliac crest. This study reported
a similar graft-site bone mineralization pattern between study groups through 12 months of postoperative follow-up.

Using a four-point rating system, bone mineralization ratings were not significantly different between study groups at any of several postoperative follow-up intervals. Importantly, the requirement to harvest autologous bone tissue from the iliac crest to manage the fusion was significantly lower among DBM subjects (64% for DBM versus 87% for autologous bone; P<.02). The similarity in bone mineralization ratings at the fusion site between these two grafting interventions has been maintained through 24 months of postoperative follow-up, suggesting similar graft performance in the long-term (G. Lowery, personal communication).

Craniofacial Reconstruction. Bone grafts are a commonly used adjunct in craniofacial reconstruction and cosmetic applications. Much of the pioneering work involving the use of DBM in this setting was undertaken in a series of studies by Glowacki et al. Among 34 patients being treated for congenital (n=28) or acquired (n=6) craniofacial anomalies, solid healing of defects treated with DBM implants was observed by 2-3 months, postoperatively. Of this sample, only 6% (2/34) required implant removal due to wound infection; all other DBM implants healed without incident.

These encouraging results were corroborated by the same group in a subsequent report of 44 patients treated with DBM. Solidity of the implant was observed in 100% of cases by 3 months postoperatively, where the graft site could be manually palpated (n=31). Radiographic healing was shown in >70% of DBM implants after 6 months of follow-up, and implant resorption and graft site infection again occurred in <10% of cases (4/44).

In another study, DBM was used to successfully treat 50 jaw defects among 36 patients. Only 8% (4/50) of these interventions were judged as unsuccessful at study completion.

Neigel and Ruzicka reported successful osseous healing in 21 patients (31 implants) with craniofacial congenital deformities, orbital fractures, and tumors treated with DBM implants. In the defects that could be palpated, solid healing was observed as early as 2-3 weeks postoperatively. In radiographic evaluations, osseous healing was judged complete within 6 weeks after surgery. No reoperations were necessary, and none of the DBM implants required removal.

Moss et al. reported on a “perforated” form of DBM in 42 patients; 33 of these operations involved primary skull reconstruction for multiple suture synostosis. Demineralized bone matrix grafting procedures resulted in complete closure of the skull defects in all but 2 (5%) patients. These authors noted that this particular form of DBM exhibits a protracted remodeling process of up to 3 years in these challenging defects.

The findings of two studies appear at odds with the promising results reported above for DBM in craniofacial applications. Both studies used DBM procured from the same source where terminal ethylene oxide sterilization was the standard method of processing. Ousterhout reported mixed success with the use of DBM implants for facial augmentation procedures that were mostly cosmetic in nature (e.g., nasal dorsum, chin, and malar region) in 25 patients. Similarly, Toriumi et al. found that approximately 50% of DBM implants markedly resorbed among 41 patients who underwent dorsal nasal augmentation with demineralized cortical implants.

Discussion

Surgical management of a number of orthopedic problems necessitates the use of bone graft to ensure a clinically successful outcome. The preferred graft material for most surgeons remains autologous bone, usually harvested from the iliac crest. In addition to issues related to tissue availability, harvesting autograft from a secondary operative site is associated with significant postoperative morbidity. In fact, in a literature review of 47 studies of lumbar spinal fusion, the highest frequency of complications occurred not at the primary surgical site but at the autograft donor site. Consequently, a concerted research effort is under way to develop and validate alternative bone grafting materials that either diminish or eliminate the need to harvest autologous tissue.

This review evaluated the clinical findings from 21 studies in which allogenic DBM was used to manage orthopedic problems that typically require adjunctive bone grafting. Most of the authors (81%) reported that generally positive results were achieved with the use of DBM, even in particularly challenging cases (e.g., management of resistant nonunions). However, widespread recognition of the clinical utility of DBM has been hampered by less than rigorous experimental testing and processing techniques that in some cases may adversely affect graft performance.

The majority of studies aimed at evaluating the clinical effectiveness of DBM have been retrospective case reviews (Table 2); however, two of these reviews included matched autologous bone comparison subjects, and the radiographic determination of bony healing for both study groups was evaluated blindly and independently. Although both studies reported excellent results with DBM, they should be considered preliminary until the major study findings can be tested and validated using more rigorous prospective study designs with concurrent autologous bone controls (ie, randomized controlled trials).

Two studies, both in the area of spinal fusion, used prospective methodologies and only the study by Jorgenson et al. included a random allocation of DBM and autologous bone grafts. In the prospective, nonrandomized cervical fusion study by An et al., the authors present seemingly counterintuitive findings. Their results imply that subjects grafted with autologous...
bone had better results in regard to fusion integrity and graft collapse, while DBM subjects may require fewer revision surgeries. Subjects in both study groups had similar clinical success.

The findings reported by Jorgenson et al. suggesting superior performance in lumbar spinal fusions managed with autologous bone compared with DBM are likewise difficult to interpret. First, the DBM grafts used in that study had undergone terminal ethylene oxide sterilization prior to implantation without additional augmentation with viable osteogenic cells such as can be contributed by marrow. Thus, it is conceivable that the inductive characteristics of this matrix preparation were attenuated or even eliminated, resulting in an essentially osteoconductive graft. The demanding nature of spine fusion necessitates the use of bone grafts that can facilitate ectopic bone formation with solid bridging of contiguous vertebrae, a result unlikely to be achieved with an osteoconductive graft alone. Second, the analytical and statistical methods used by Jorgenson et al. to interpret their data have been criticized, raising some legitimate concerns about the overall study conclusions.

Allogeneic DBM is appealing as a bone grafting material, in part because when it is properly processed, it preserves the inherent osteoinductive and osteoconductive characteristics. Thus, DBM theoretically represents a viable extender or alternative to autologous bone, particularly in challenging applications in which bone must be formed ectopically (e.g., spinal fusion) or in which the defect is extensive (e.g., severely comminuted appendicular fractures). It is known that some supplementary processing methods attenuate the osteoinductive potential of the matrix, and there has been particular concern about the widespread use of ethylene oxide as a terminal sterilization procedure. Indeed, the deleterious effects of ionizing radiation on osteoinduction were documented several decades ago and more recently, ethylene oxide sterilization has been evaluated and found to attenuate osteoinduction in a dose-dependent manner.

Unfortunately, however, terminal graft sterilization with ethylene oxide is common, and many of the studies reviewed in this article used some form of sterilization, either ethylene oxide or radiation. Paradoxically, in most cases, these terminally sterilized grafts performed adequately. For example, Whiteman et al. reported similar graft performance between DBM and autologous bone for treating osseous defects in the hand irrespective of the fact that the DBM grafts had been irradiated with 2.5 megareads.

It has been postulated that the sterilization-induced attenuation of the osteoinductive potential of DBM renders the osteoinductive aspect of the matrix intact and in some clinical applications (eg, small osseous defects resulting from tumor excision), this compromised graft may be adequate to support bone formation albeit at a slower rate. However, in more challenging cases, a solely osteoconductive graft is unsatisfactory.

This may explain the less satisfactory results reported by some authors when ethylene oxide-sterilized DBM was used to create a fusion mass in the posterolateral lumbar spine to facilitate ectopic bone formation for cosmetic purposes in facial reconstruction. Alternatively, Tiedeman et al. reported encouraging results among a variety of difficult-to-manage cases, including resistant nonunions and joint arthrodeses; however, it was the consistent practice of these authors to combine autologous bone marrow with the ethylene oxide-sterilized DBM to form a composite graft prior to implantation.
SUMMARY

The findings of studies on DBM in the surgical management of osseous defects, arthrodeses, and reconstructive procedures have been promising. In general, DBM grafts have supported healing in a timely fashion without complication and with a diminished need to harvest bone from a secondary operative site. Nonetheless, controlled prospective trials are needed to confirm the comparative effectiveness of DBM and to quantitate the benefits of avoiding secondary site autologous bone harvesting.

Notwithstanding the known deleterious effects of certain processing steps, current commercial demineralization processes vary widely and use ancillary procedures aimed at attenuating potential residual antigens and pathogens. While some of these procedures may improve or facilitate graft performance (eg, lipid and lipoprotein removal with detergents), others may be deleterious (eg, sterilization with radiation or ethylene oxide) (Table 1). Therefore, it is important that DBM be processed using methods that consistently establish conditions known to preserve DBM's documented osteoinductive potential and that authors appropriately identify processing methods known to have effects on graft performance.

REFERENCES


