ALLOGRAFT BONE

The Influence of Processing on Safety and Performance

Todd Boyce, PhD, Jean Edwards, PhD, and Nelson Scarborough, PhD

Bone grafts are often necessary to provide support, fill voids, and enhance biologic repair of skeletal defects. Autogenous bone recovered from the patient’s own iliac crest is generally considered the gold standard of bone grafts. There is a need for alternatives, however, because of limited autograft quantities, donor site morbidity, and other problems including limited biologic performance. It is estimated that 426,000 bone grafting procedures were performed in the United States in 1996; of these, 247,000 used autografts; 145,000, allografts; and 34,000, other materials. In comparison, it is estimated that only 5000 to 10,000 allograft procedures were performed in 1985.

Historically, allografts were primarily used for massive grafting procedures in which an autograft was not an alternative. In the 1980s, a notable shift in thinking occurred among surgeons, and they began substituting allografts, such as cancellous chips, Cloward dowels, and Smith-Robinson wedges for traditional autograft surgeries. Three factors that contributed to the increased demand for allografts were the National Organ Transplant Act, which paved the way for development of organ and tissue donor networks, dramatically increasing allograft availability; tissue donor screening and processing technologies that improved safety and quality thereby increasing surgeon and patient confidence; and newly developed allograft forms that expanded their utility into more procedures.

The sophistication of the tissue banking industry has increased significantly with much of the attention being focused on safety. In July 1997, the U.S. Food and Drug Administration (FDA) released industry requirements to establish rules for donor screening. These regulations help ensure that tissue donors are appropriately screened. The American Association of Tissue Banks also has requirements for donor screening, processing, labeling, and distribution procedures. Banks and tissue processors that meet these standards can be accredited.

Advances in tissue processing technology have been important for the success of allografts. From the work of early investigators, it has been shown that two major factors for a successful graft are sterility and the reduction of antigenicity by freezing or the removal of blood and cellular constituents. The challenge is to prepare allografts that are well cleaned, sterile, and free of virus, while still preserving the natural biologic and biomechanical properties of the tissue. It is these

From Allograft Research and Development, Osteotech, Inc., Eatontown, New Jersey
characteristics of allograft bone that make it such a useful biomaterial. This article discusses how processing techniques aimed to achieve the necessary safety and sterility can also affect the natural biologic properties that are vital for graft incorporation and healing.

**ALLOGRAFT SAFETY**

Transmission of human immunodeficiency virus (HIV), hepatitis B virus (HBV), hepatitis C virus (HCV), and other viral diseases is an issue for all biologically derived products. The risk of transmission of a disease depends largely on product type and preparation. Whole blood has a reported disease transmission rate ranging from 1 in 450,000 for HIV to 1 in 46,000 for HCV, with a cumulative risk of 1 in 36,000 for HIV, HBV, and HCV.\(^6^a\) Bone, in contrast, has a much lower incidence of disease transmission. The risk for HIV was estimated to be 1 in 1.6 million in properly screened allograft bone.\(^8\) Regulatory agencies in the United States and Europe have developed guidance documents for manufacturers of biologic products to follow to ensure that their products are safe.\(^3^9,80,92\) The basic principles are straightforward and can be applied to a broad range of biologically derived materials, including human tissue for transplant. Applying these principles provides safeguards at the supply, processing, and distribution phases:

1. Screen the source of the tissue for infectious agents.
2. Use processing techniques that have been validated to clear viruses.
3. Track the materials so that necessary follow-up actions are possible.

The value of this tiered approach to safety is illustrated by the following examples. The relatively high risk of disease transmission by blood transfusions is due largely to an approach that exclusively relies on donor screening for safety. The fragile nature of this product precludes the use of processing steps to address the potential viral burden. In contrast, factor VIII, a blood-derived product used to treat hemophilia, is highly processed. Advances in the methods used to prepare factor VIII have greatly increased its level of safety. Factor VIII is derived from plasma using various extraction procedures. Horowitz\(^33,35\) developed the solvent/detergent processing technique and demonstrated that combinations of Tri-n-butylphosphate and surfactants were effective in inactivating viruses. Rigorous studies have demonstrated that plasma infected with a broad range of viruses could be made noninfectious in animal models and in vitro studies used to quantify active viruses.\(^34\) The effectiveness of viral inactivation by this approach has been verified in clinical trials and patient usage. No incidents of HIV, HBV, HCV transmission have been reported since 1985, when factor VIII prepared by the solvent/detergent method became available. These examples demonstrate the value of tissue processing technologies to enhance product safety.

**VIRAL DISEASE TRANSMISSION BY ALLOGRAFTS**

The reported incidence of disease transmission by bone and tendon allografts is much lower than for other biologic products, such as whole blood. Because of incidents and increased allograft usage, however, this issue has received considerable attention. One result has been the implementation of donor screening requirements by the FDA in 1997.\(^6^9\) These regulations have established an algorithm for determining suitable medical history questionnaires for next-of-kin and serologic screening ensuring uniform practices across the tissue banking industry.

From reports that have documented transmission of viruses by musculoskeletal allografts,\(^12,16,73,83\) it is clear that donor screening can reduce, but not eliminate, transplantation of infectious tissue. Reports also suggest that thorough processing to remove blood elements further reduces the risk of disease transmission. Two reports of HIV transmission since 1980 have been documented through publications by the Centers for Disease Control (CDC).\(^12,73\) The first case involved a surgical femoral head removed during routine arthroplasty that was subsequently implanted during a spine arthrodesis procedure for idiopathic scoliosis. The recipient contracted HIV. If the donor patient had been screened according to current regulations, he could have been excluded based on serology, a history of intravenous drug use, and lymphadenopathy. This example demonstrates the value of proper donor selection.

The second case involved a donation of multiple organs and tissues that subsequently resulted in transmission of HIV to all four organ
recipients and three of four recipients of unprocessed musculoskeletal allografts (both femoral heads and one patellar bone–tendon–bone). The other 48 grafts that were more extensively processed did not transmit the virus. This case suggests that removal of blood and exposure to treatment solutions, such as ethanol, during processing can reduce the risk of transmission. These examples illustrate the value of the two-tiered approach to safety: screen the source of the material, and use processing techniques that can inactivate virus that may still be present. For allograft tissues, the screening step has been well standardized by the FDA guidelines, but this step alone is not adequate to eliminate infectious donors. Tissue processing is a central component for ensuring allograft safety.

Although specific guidelines for allograft tissue processing have not been developed, the guidance documents for other biologic products, including blood products and biopharmaceuticals, are relevant. These documents provide methodologies for quantitatively assessing the capability to clear viruses in a process. Viral clearance can be achieved by physical removal of the virus from the product or by inactivation of the virus (i.e., destroying the virus’ ability to replicate). Clearance studies are usually performed by spiking high titers of virus onto the product, executing a specific processing step, and then determining the amount of virus removed or inactivated. Results are expressed as log reduction in titer such that a 3 log reduction is equal to 1000-fold reduction.

To establish a claim on the capability of a processing technique to enhance the safety of allografts, the principles of validation and guidelines for viral clearance studies must be followed. A validation study on viral inactivation by a process used to produce demineralized bone has previously been reported. This study demonstrates how allograft safety can be enhanced by specific processing methods. It was performed following Good Laboratory Practice regulations and was rigorously controlled to model the process steps used in preparing demineralized bone matrix (DBM) for clinical use. Samples of bone were acquired from specific points in the manufacturing process, transferred to the laboratory, and spiked with one of the viruses listed in Table 1. After exposure to the virus, the sample was exposed to the next treatment step and assayed for remaining viable virus. The difference in the pretreatment and post-treatment titer of virus was expressed as the log viral inactivation value for that treatment step. Three treatment steps were assessed for each of the five viruses in the panel, which were selected to represent a range of biophysical characteristics of viruses and included those of primary clinical concern. Results from this study demonstrated high levels of viral inactivation by step 1 (acid treatment) and step 2 (ethanol treatment), but not by step 3 (lyophilization). For HIV, the total inactivation by these three steps was greater than 9.46 log indicating that the chance of an HIV virus surviving is less than 1.28 billion. The reported total inactivation levels provide a high level of safety assurance for DBM prepared using this process (Table 2).

Demineralized bone differs from mineralized bone in that once it is morcellized and decalcified, the blood elements are effectively removed, and treatment solutions are able to penetrate throughout the matrix. In contrast, mineralized allografts have a complex architecture that has potential reservoirs for viruses. The blood spaces of bone, such as the marrow, the spaces between trabeculae, and blood vessels including the Haversian and Volkmann canals of cortical bone, are the likely sites for contamination by the viruses of primary clinical concern (HBV, HCV, and HIV). Thus, an effective process must penetrate into these blood spaces and remove or inactivate the virus. It is a common practice of tissue banks to use high-pressure washes to surface clean bone. This practice is effective for surface cleaning but does not thoroughly penetrate the internal matrix of large segments. More sophisticated approaches to penetrate as well as clean blood and marrow from the internal spaces of bone are under development and have received U.S. patents. Rigorous studies are needed to demonstrate the ability of new technologies to provide allografts with improved safety and the optimal biologic performance characteristics.

**EFFECTS OF PROCESSING ON PERFORMANCE**

A variety of methods are used for processing allograft bone. Various treatment solutions have been used for washing, sanitizing, and demineralizing bone. Freezing and freeze-drying (lyophilization) are routinely used for product storage. Terminal sterilization by gamma irradiation, electron-beam radiation, or ethylene
Table 1. CHARACTERISTICS OF VIRUSES USED IN STUDY

<table>
<thead>
<tr>
<th>Virus</th>
<th>Model</th>
<th>Type</th>
<th>Enveloped</th>
<th>Comments</th>
<th>Assay System</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human immuno deficiency virus (HIV)</td>
<td>HIV-1,2</td>
<td>RNA-containing</td>
<td>Yes</td>
<td>AIDS virus</td>
<td>CEM-A cells sync-tium assay</td>
</tr>
<tr>
<td></td>
<td>HTLV-1,2</td>
<td>retrovirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Duck hepatitis B virus (DHBV)</td>
<td>CBV</td>
<td>DNA-containing</td>
<td>Yes</td>
<td>Similar infectious pattern in liver</td>
<td>Pekin duck in vivo assay</td>
</tr>
<tr>
<td></td>
<td></td>
<td>hepadnavirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bovine diarrheal virus (BVD)</td>
<td>Hepatitis C</td>
<td>RNA-containing</td>
<td>Yes</td>
<td>Same family as HCV flavivirdae</td>
<td>Bovine turbinate cells/plaque assay</td>
</tr>
<tr>
<td></td>
<td>virus (HCV)</td>
<td>pestivirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human cytomegalovirus (CMV)</td>
<td>CMV</td>
<td>DNA-containing</td>
<td>Yes</td>
<td>Model for herpes simplex and Epstein-Barr viruses</td>
<td>MRC-5 cells plaque assay</td>
</tr>
<tr>
<td></td>
<td></td>
<td>herpesvirus</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Human poliovirus type I</td>
<td>Polio, HAV</td>
<td>RNA-containing</td>
<td>No</td>
<td>Highly resistant to inactivation</td>
<td>VERO cells plaque assay</td>
</tr>
<tr>
<td></td>
<td>Parvovirus</td>
<td>picomavirus</td>
<td></td>
<td></td>
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</tr>
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</table>

oxide is often used. Many of these factors have been shown to affect significantly the performance characteristics of the allograft. Thus, apparently similar grafts may perform differently as a result of the processing treatments.

In the limited number of studies, the techniques used to assess the effects of bone processing have been biomechanical performance, biologic incorporation for mineralized allografts, and osteoinductivity for demineralized allografts. In these studies, a wide range of graft processing techniques and experimental methods are used. The results are also sometimes conflicting, making it difficult to draw strong conclusions. Nevertheless, it appears that some processing methods have deleterious effects on the tissue and should be minimized or avoided.

BIOMECHANICAL STRENGTH

A primary and essential characteristic of structural allograft bone is its ability to support mechanical loads and to resist failure. Collapse or breakage of the allograft before healing and initial incorporation can lead to clinical failure and the need for reoperation. The strength of the graft (maximal load that may be withstanded), elastic modulus (spring constant or stiffness), and work-to-fracture (a function of both the load and the deformation; the energy that is absorbed) before failure are all important measures of the allograft’s ability to support loads. Processing techniques can negatively affect each of these variables.

Fresh human bone is the appropriate comparison to relate the effects on material property in treated allograft tissues. Although fresh cadaveric tissue has been rarely tested, using rapidly obtained autopsy or surgically excised tissues, animal studies have established that freezing of bone tissue for storage at temperatures ranging from -20°C to -147°C does not adversely affect material properties. Well-established normal ranges (Table 3) for material properties for bone have been developed over the past 3 decades. Although clean, frozen allograft bone would be expected to perform similarly to the fresh-frozen bone standards, this has not been reported in the literature. Allografts are often freeze-dried, eliminating moisture from the tissue using low temperature and pressure, to make storage more convenient. The American Association of Tissue Banks states that lyophilized tissue should contain no more than 6% moisture, permitting storage of the packaged tissue at room temperature for up to 5 years after pro-

Table 2. VIRUCIDAL EFFECTS OF INDIVIDUAL STEPS IN THE BONE DEMINERALIZATION PROCESS (LOG_{10} REDUCTION)

<table>
<thead>
<tr>
<th>Process Step</th>
<th>HIV</th>
<th>DHBV (HBV)</th>
<th>BVD (HCV)</th>
<th>CMV</th>
<th>Pollo</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&gt;5.23</td>
<td>&gt;3.70</td>
<td>&gt;4.15</td>
<td>&gt;2.92</td>
<td>&gt;5.99</td>
</tr>
<tr>
<td>2</td>
<td>&gt;4.23</td>
<td>&gt;3.70</td>
<td>&gt;3.15</td>
<td>&gt;3.32</td>
<td>&gt;3.72</td>
</tr>
<tr>
<td>3</td>
<td>None</td>
<td>ND</td>
<td>1.77</td>
<td>None</td>
<td>2.30</td>
</tr>
<tr>
<td>Total inactivation</td>
<td>&gt;9.46</td>
<td>&gt;7.40</td>
<td>&gt;9.07</td>
<td>&gt;6.24</td>
<td>&gt;12.01</td>
</tr>
</tbody>
</table>

HIV = human immunodeficiency virus; DHBV = duck hepatitis B virus; HBV = hepatitis B virus; BVD = bovine diarrheal virus; HCV = hepatitis C virus; CMV = cytomegalovirus; ND = not determined.
cessing. Changes in mechanical properties of the tissue, however, are introduced as a result of the freeze-drying process. The mechanical changes appear to be associated with damage in the bone matrix, specifically microcracks along the collagen fibers. These effects appear to be magnified when freeze-drying and gamma irradiation are used together.

Rat bones have been used to model the effects of freeze-drying on the compressive properties of cancellous bone (compression strength of tail vertebrae) and the bending and torsional properties of the long bones. Results of these studies indicate that compressive strength is reduced by up to 30%, with little or no change in stiffness. Bending of freeze-dried long bones resulted in a 41% decrease in bending strength, whereas torsional strength was decreased by 60%. Bone becomes more brittle when the moisture is removed, but the material properties can be at least partially regained by rehydration before the grafting procedure.

**EFFECT OF TERMINAL STERILIZATION**

The issue of sterility is complex for biologic products because traditional terminal sterilization procedures may decrease their biologic performance. This problem has led to various strategies for processing allografts that can be categorized as either aseptic processing or terminal sterilization. Aseptic processing requires the tissue to be handled using sterile techniques in a controlled environment throughout processing. Key strategies employed to accomplish this include using sterile techniques during the recovery of the donor bone, processing the tissue in a clean room environment using aseptic techniques, thorough debride-ment and cleansing of grafts, judicious use of antibiotics and treatment solutions (e.g., surf-

![Figure 1. Treatment effects on the material behavior of allograft bone. Gamma-irradiated tissue, in particular, loses much of the plasticity of the frozen tissue.](image-url)
Table 3) that irradiation effects on the elastic properties are minimal at all irradiation levels.\(^ {18,30}\) For cortical bone, however, increasing dosages of radiation progressively embrittle the graft, reducing the energy that may be absorbed before breaking by more than 60% for 2.8 to 3.0 mrad-treated specimens.\(^ {30}\) Thus, although the graft may still be strong (ultimate strength reductions of only 10% to 20% have been reported for 3 mrad dose),\(^ {16}\) the tissue is now more brittle (little displacement may be allowed before breakage). Effects are greatest for grafts subjected to either torsional or bending loads,\(^ {18,27}\) and even low dosages appear to have some effect. Furthermore, the combination of gamma irradiation and freeze-drying appears to introduce more damage than either treatment alone.\(^ {54}\)

By contrast, cancellous bone grafts are reported to be much more resistant to damage from gamma irradiation. Iliac crest wedges subjected to dosages up to 2.0 to 2.5 mrad showed little effect in elastic modulus, compressive strength, or strain to failure.\(^ {97}\) Likewise, tibial cancellous blocks displayed no measurable effects in stress, strain, or modulus for treatments up to 5.1 mrad.\(^ {1}\) Effects of irradiation on bone fatigue cyclic loading properties are unknown.

It seems clear that cortical bone sterilized by terminal irradiation may have reduced biomechanical properties, particularly when loaded in bending or torsion. Cancellous bone seems to be less affected by sterilization. The significance of processing effects on graft performance in clinical settings has not been clearly determined.

**BIOLOGIC INCORPORATION**

In addition to mechanical performance, the mineralized allograft must also achieve several biologic functions, as follows: provide an osteoconductive surface for remodeling, become fully incorporated, be remodeled and replaced by host tissue, and be biologically acceptable to the host.\(^ {4}\) Many factors\(^ {75}\) are involved in successful graft incorporation, including combining the revascularizing and osteogenic capabilities of the host, skill of the surgeon in stabilizing the construct, and inherent strength and osteoconductive tissue matrix provided by the allograft. Among the factors relating to the graft itself, processing treatments are paramount, in the tissue's ability to incorporate.

Well-processed mineralized graft should contain little remaining cells or cellular debris. Minimally processed tissues in comparison have much more cellular debris, which can induce an immune response on transplant. This immune response in the host appears to be a significant factor in rejection and poor incorporation for unprocessed or minimally processed unmatched allografts.\(^ {76,77}\) In particular, marrow\(^ {6}\) and periosteal cells are highly antigenic. Thus, processes that remove (washing with water and mild solvents) or destroy (freezing, freeze-drying, irradiation) the cells of the donor tissue tend to reduce the antigenicity associated with the processed graft.\(^ {6,54}\)

Processed freeze-dried mineralized tissue with cellular components removed is well tolerated by the host.

Processes used for sterilization of the tissue can have unintended biologic consequences. Ethylene oxide has been used extensively for sterilization of allograft tissue, although studies have indicated that it can be detrimental to the incorporation of mineralized allografts. A bone chamber study by Thoren et al\(^ {81}\) has indicated that ethylene oxide–treated tissue containing residual concentrations below 20 ppm incorporates more poorly than fresh-frozen, hydrogen peroxide–treated, or irradiated tissue. Poor incorporation as well as relatively poor clinical outcomes associated with ethylene oxide–treated bone have led some to suggest that ethylene oxide–treated tissue is unsuited for weight-bearing fusion sites.\(^ {10}\)

**EFFECTS OF PROCESSING ON DEMINERALIZED BONE MATRIX PERFORMANCE**

When properly processed, DBM allograft materials have the advantage of two healing pathways or mechanisms. First, DBM can provide a suitable matrix for cells to infiltrate, populate, and produce new bone through osteoconductive healing. DBM can also aid the healing response through osteoinductive pathways, in which mesenchymal cells are stimulated by native bioactive molecules to differentiate into bone-forming cells. The osteoinductive property of bone originates from its extracellular matrix that contains noncollagenous bone inductive proteins. These factors can trigger the endochondral ossification cascade at the site of implantation. In contrast, mineralized bone allografts can support bone formation through osteoconduction only be-
cause the inductive proteins are within the mineralized matrix and not able to stimulate cellular differentiation. 26a

The ability of DBM to induce new bone formation can be demonstrated by evaluating implants in an animal model similar to that first described by Urist 86 DBM placed into subcutaneous or intramuscular sites in an appropriate host 2,62,63,86 is evaluated histologically for new bone formation. Bone forms through endochondral ossification, in which the presence of cartilage is seen around 14 days and converts to bone by 21 to 28 days (Fig. 2). 60 The use of an athymic (nude) rat to evaluate human DBM has been characterized and reported. 23

The current scientific literature does not provide a clear picture of the role that donor age and gender may have on osteoinductive potential. 40,50,66,79,95 Some reports are contradictory, often evaluating a limited number of donors. Others fail to assess human DBM, using animal analogue tissues instead. To address this issue, two of the authors (J.E. and N.S.) used a statistically relevant number of donors to investigate the effects of age and gender on the osteoinductivity of human DBM. A total of 200 donors (100 female, 100 male) age 65 years and younger were evaluated in five different age classes (20 donors for each gender in each age class). Samples of DBM prepared for clinical use from each donor were implanted into the athymic rat model and evaluated histologically for bone formation after 28 days. The results showed that for all donors, no statistical differences exist between genders or between any of the age classes (Fig. 3). This study provides a strong basis for the use of donor tissues of both genders from 15 to 65 years old for DBM products prepared by these methods.

The type of bone has also been suggested to affect osteoinductivity. In studies conducted in a nude rat model with cortical and cancellous bone of derived DBM from the same canine source, the cortical bone resulted in new bone formation, whereas the cancellous bone did not. 67,68

Processing methods for DBM also influence its osteoinductive performance. The choice of which demineralizing agent is used has been shown to be important. Hydrochloric acid (0.5 to 0.6 m) is the most commonly used agent. A 1:1 combination of formic acid and citric acid also yields osteoinductive DBM. Hydrochloric acid used in combination with alcohol (methanol, ethanol, or isopropyl) results in DBM that is not osteoinductive, however. 88 The use of acetic acid, nitrous acid, nitric acid, and lactic acid also fails to produce osteoinductive DBM. 86,90 The use of sonication (20,000 cycles/s) during demineralization in hydrochloric acid has also been reported to affect DBM osteoinductivity negatively. 88 Chelating agents, such as ethylenediaminetetra-acetic acid, have been shown not to complete demineralization and to be detrimental to DBM performance. 88

Demineralization time, which, in turn, affects final calcium content, is another variable to control in DBM processing. 26,29,37 Remaining mineral in the tissue affects the ability of the host cells to identify and respond to the osteoinductive proteins. It has been reported that mineral content must be reduced to at least 40% of the normal level before a strong osteoinductive response results. 29

Organic solvents used in fat removal have been shown to have no effect or may even enhance osteoinductivity. 31,90 Among the acceptable agents are alcohol, ether, acetone, hexachlorophene, and many detergents. Antibiotics are also frequently used in the process-

**Figure 2.** Bone formation events when demineralized bone matrix (DBM) is placed in a rat heterotopic site. Maximum bone volume is achieved at approximately 28 days.
ing of DBM. Oxytetracycline, erythromycin, streptomycin, chloramphenicol, and penicillin are among those agents that have been shown not to have any inhibitory effects on osteoinduction.\(^\text{90}\) Temperature extremes during processing have documented effects on inductivity. Excessive heat (70° to 100°C) and multiple freeze/thaw cycles (more than three) are both reported to be detrimental.\(^\text{31,86,90}\)

Among other factors that affect osteoinductivity is the size of the DBM particles. There is evidence that when DBM particulate is below about 75 μm, a chronic inflammatory response occurs that inhibits the osteoinductive response. Particle sizes from greater than 75 μm to 2 mm\(^2\) have been shown to be osteoinductive.\(^\text{64,67,81,89}\)

Lyophilization is commonly one of the last steps in DBM processing and is useful for extending product shelf life. Several studies have shown that freeze-drying has no effect on osteoinductivity.\(^\text{21,36,86,88,90}\) Freezing DBM at \(-4^\circ\) or \(-70^\circ\)C also allows for storage for extended periods without affecting osteoinductivity.\(^\text{45}\)

Terminal sterilization procedures may also have significant effects on the performance of DBM. In some studies, gamma irradiation at levels 2.0 to 2.5 mrad or greater has been shown to reduce greatly or eliminate the osteoinductive response.\(^\text{38,48,85,96}\) The data for ethylene oxide also suggest that osteoinduction can be significantly diminished depending on treatment temperature and duration\(^\text{5,22,38,46,96}\) and is destroyed at the conditions that are necessary to achieve sterilization of the tissue.\(^\text{3}\) In addition to these more commonly used methods for sterilization, glutaraldehyde solution and formaldehyde gas have been shown to eliminate the osteoinductivity of DBM.\(^\text{48}\)

Many factors must be controlled to produce DBM with good osteoinductive performance. The importance of osteoinduction to bone-graft healing has been demonstrated by Martin et al\(^\text{45}\) and Morone et al\(^\text{47}\) in nonclinical studies using a rabbit posterolateral spine fusion model. In a series of experiments, the performance of active DBM was compared with inactive DBM (guanidine-hydrochloride extracted). Table 4 shows the fusion results, with osteoinductive DBM being significantly better than noninductive DBM of the same form.

Table 4. RABBIT POSTEROLATERAL SPINE FUSION RATES WITH ACTIVE AND INACTIVE DEMINERALIZED BONE MATRIX IN VARIOUS FORMS (n = 9–12)

<table>
<thead>
<tr>
<th></th>
<th>Gel (%)</th>
<th>Putty (%)</th>
<th>Flex (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Active</td>
<td>58</td>
<td>83</td>
<td>100</td>
</tr>
<tr>
<td>Inactive</td>
<td>0</td>
<td>33</td>
<td>36</td>
</tr>
</tbody>
</table>

DBM = Demineralized bone matrix.
These data clearly demonstrate that superior bone formation can occur when two healing mechanisms, osteoconduction and osteoinduction, are both present.

CONCLUSIONS

Several factors have contributed to the increased demand for allografts over the last decade, particularly improved availability and safety. This success has attracted the attention of several major orthopedic companies. New efforts are now aimed at expanding the role of allografts by tailoring them for specific surgical procedures, such as dowels and wedges for spinal fusions and demineralized grafts with better handling and performance characteristics. The versatility of allograft bone offers broad market potential because it meets many of the requirements of an ideal graft, including sufficient strength for many load-bearing applications, the ability to incorporate and remodel, biocompatibility, osteoinductivity when demineralized, and a high level of safety. As new technologies, such as bone morphogenetic proteins, tissue engineering, cellular therapies, and gene therapies, become available, the role of allografts may change, but with the excellent biologic characteristics they offer, demand for allografts is likely to continue in the future.

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Address reprint requests to
Nelson Scarborough, PhD
Osteotech, Inc.
51 James Way
Eatontown, NJ 07724