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Safe and Effective Osteobiologic Options

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Ground human bone matrix grafts have been used in clinical applications such as oral and maxillofacial procedures and spine surgery for decades. The bone scaffold formed by ground cortical bone and cancellous chips creates the favorable environment required for bone-forming cells to be able to generate new bone. The demineralization of bone matrix exposes bone morphogenetic protein (BMP) and other bone growth promoting factors. Because of this, demineralized bone matrix (DBM) not only provides a scaffold for bone formation, it also promotes differentiation of osteoprogenitor cells into viable bone-forming cells, a process called osteoinductivity.

In spite of their widespread use, ground human bone matrix grafts, and most specifically particulate demineralized bone matrix (DBM), can be difficult to handle in the operating room. To overcome issues with handling, many companies offer DBM in combination with a natural or synthetic carrier. The term “osteobiologics” has been introduced to refer to these manufactured bone graft substitutes.^{1,2}

This article investigates the principles of bone formation and healing as well as the role that osteobiologic products can play in promoting the healing of fractures and bone defects. The effectiveness of sterilization by gamma irradiation on the osteoinductivity of bone matrix using LifeNet Health’s patented Allowash XG[®] process is also discussed.

BONE REMODELING AND HEALING

Healthy adult human bone is made up of two components: cortical bone (also called lamellar or compact bone) and cancellous (or trabecular) bone. Cortical bone forms the dense outer layer of shaft and flat bones and makes up 75 to 80% of the body’s bone mass. In contrast, cancellous bone fills the

central cavity (medullary cavity) of the bone and has a structure that resembles a honeycomb. This network of spaces called interstices contains the hematopoietic, or blood-building, elements.

Bone tissue continuously undergoes remodeling that is tightly regulated by biochemical, biomechanical, cellular, and hormonal mechanisms as part of a naturally occurring cycle of bone renewal. These same steps of bone resorption and deposition are also part of the bone healing process and are made use of when bone is transplanted in the form of a bone graft.³

Three physiological properties play a role in the remodeling and healing of bone; these are osteogenesis, osteoconduction, and osteoinduction. Osteogenesis, which is dependent on the presence of viable cells with the ability to form new bone, is a property found only in fresh autograft bone and in bone marrow cells. Osteoconduction is the property of bone to serve as a favorable environment for bone-forming cells at the recipient site to infiltrate, proliferate, and form new bone. A number of scaffold materials can provide this favorable environment including autografts, ground cortical bone and cancellous chip allografts, and synthetic matrices such as hydroxyapatite and calcium phosphate. Osteoinduction is the ability of bone material to promote osteoprogenitor cells to differentiate into bone-forming cells upon stimulation by a matrix and associated protein factors such as bone morphogenetic proteins (BMPs). During the demineralization of bone matrix, BMP isoforms and other bone growth promoting factors are exposed, enabling them to facilitate the process of osteoinductive new bone formation.^{3,4} Demineralized bone matrix (DBM), also known as demineralized freeze-dried bone allograft or DFDBA, is the most frequently used osteoinductive allograft tissue available on the market.

WHY DEMINERALIZATION MATTERS

Ever since Urist et al. recognized that new bone formation is not caused by a single factor, but is rather the result of a large number of molecules interacting in a multi-step cascade^{5,6,7}, DBM has been accepted as the most optimal natural solution for clinical purposes. Residual mineral levels of DBM are considered a measure of the reproducibility of the demineralization process as well as a marker for the availability of active growth and differentiation factors. Although a detailed discussion of the impact of demineralization on new bone formation is beyond the scope of this article, studies have shown that DBM of a particle size of 250 to 710 micron (μ) with a residual calcium content of 2% displayed the highest osteoinductivity.^{8,9}

In the early 1990s, LifeNet Health introduced a patented demineralization process that involves solubilization of the mineral phase of the ground bone in a closed continuous-flow system to minimize the risk of contamination. This process was further enhanced through the development of LifeNet

Health's patented Pulsatile Acid Demineralization (PAD[®]) technology. Rapid "pulsing" of the ground bone with acid improves the precision and speed of the demineralization process. The PAD[®] process permits optimum residual calcium levels.¹⁰

OPTIMIZING THE SAFETY OF ALLOGRAFT BONE BIO-IMPLANTS

Allograft bio-implants should ideally contain a minimum of antigenic factors and be free of any microbial contaminants. It should be remembered that human bone tissue for grafting is procured from deceased donors, and despite stringent procurement and processing methods, human tissue might harbor microbial contaminants that are present in the tissue at the time of recovery. As there is no completely infallible way to exclude such donors, conscientious tissue banks have adopted cleaning and sterilization procedures that do not adversely affect the performance of implanted allograft tissue.

LifeNet Health has been a pioneer in allograft cleaning and disinfection since the introduction of

Table 1. Allowash XG[®]—six steps to sterile tissue

Steps to Sterilization	Description Summary
1. Bioburden Control	Meticulous and rigorous screening routine based on FDA and AATB guidelines with strict donor exclusion criteria
2. Bioburden Assessment	Extensive serologic testing for microbiological contamination that includes bacteria, fungi, and infectious diseases
3. Minimizing Contamination	State-of-the-art processing facilities to maintain cleanliness levels designed to eliminate the possibility of cross-contamination by exceeding regulatory standards
4. Rigorous Cleaning, Blood and Marrow Removal	Flushing, centrifugation, hypotonic processes, and ultrasonication to solubilize and remove blood elements, including marrow and lipids
5. Disinfection and Rinsing Regimen	Intensive decontamination, disinfection, and scrubbing regimen designed to remove and eliminate viruses and bacteria, followed by centrifugation and/or microabsorption to remove residual water
6. Terminal Sterilization	Low-level gamma irradiation at low temperatures resulting in sterile allograft tissue with an SAL of 10 ⁻⁶

An overview of the steps that make up the Allowash XG[®] process are shown in Table 1 and relevant steps in the processing of LifeNet Health's allograft bone tissue are described on the next page.

the proprietary and patented Allowash® technology in 1995. In a comprehensive and validated process, the vast majority of bone marrow and blood elements from the internal bone matrix are removed, and subsequent chemical disinfectant treatment lead to a significant log kill for bacteria and viruses. In 2005, LifeNet Health extended this process through the addition of a terminal sterilization step with the introduction of Allowash XG®. Allowash XG® results in sterile allograft tissue with a Sterility Assurance Level (SAL) of 10^{-6} without compromising the biochemical properties of the tissue needed for surgical applications.

Aseptic Recovery and Processing

Following consent of the donor family, donor tissue is procured using aseptic technique, then wrapped in sterile drapes and transported to the LifeNet Health on ice. The purpose of this practice is to retrieve and transport tissue in a manner that minimizes or prevents contamination or decomposition of the tissue.

All recovered tissue is tested for microbiological contamination before the donor tissue is processed into grafts. Standard microbiological assays under aerobic and anaerobic conditions are used to culture and identify bacteria and fungi. Blood samples taken from each donor are tested for viral infectious agents and must meet or exceed Food and Drug Administration (FDA) regulations for donor tissue testing. LifeNet Health also complies with the standards established by the American Association of Tissue Banks (AATB).

Additional Cleaning and Disinfection Processes

To maximize allograft safety and osteoinductivity, numerous tissue banks in the United States have developed processes to clean and disinfect human allograft tissue with the objective of reducing the potential of disease transmission, in particular emerging infectious diseases that we are not yet aware of. It is critical that the methods used to disinfect the bone tissue will not affect the osteoinductivity or osteoconductivity of the allograft and will not cause an inflammatory response at the implantation site. At the same time, a controlled

process should be used to remove infective agents, so the bone is not stripped of all of the protein elements that protect the organic bone matrix and contribute to the material's osteoinductive potential.

LifeNet Health's comprehensive validated and patented Allowash XG® technology includes an intensive decontamination, disinfection, and cleaning regimen that uses chemical and biomechanical steps to remove virtually all of the biological material that can harbor pathogens. During this process, the vast majority of bone marrow and blood elements that might harbor infectious agents are removed from the internal bone matrix. This step, along with a subsequent chemical sterilant treatment, have been shown to substantially reduce the bacterial and fungal bioburden and inactivate viruses.

Sterilization of Bone Allograft

As a final step in ensuring the utmost safety of allograft bone tissue, many tissue banks have adopted sterilization procedures that do not negatively affect the performance of implanted allograft tissue. LifeNet Health's Allowash XG® process concludes with a controlled-dose terminal gamma irradiation sterilization step administered at low temperatures that results in a Sterility Assurance Level (SAL) of 10^{-6} without compromising the biomechanical or biochemical properties of the tissue as needed for its intended surgical application.

OSTEOINDUCTIVITY OF DBM AND THE IMPACT OF GAMMA IRRADIATION

Gamma irradiation's bactericidal characteristic is due to its direct and indirect effects on nucleic acids which leads to genome dysfunction and destruction.¹¹ There is general agreement that a gamma radiation dose of approximately 20 kGy completely eliminates bacterial contamination from musculoskeletal tissue, however, it should be emphasized that the safety of musculoskeletal allografts depends on the incoming bioburden of the tissue and the testing and processing methods used by each tissue bank.¹²

The effect of numerous sterilization methods on the osteoinductivity of demineralized bone matrix has been extensively studied in a variety of animal

models implanted at both heterotopic and orthotopic sites.^{13,14,15,16,17} These methods included treatment with glutaraldehyde solution, formaldehyde gas, and ethylene oxide, as well as autoclaving and gamma irradiation. Of all sterilization methods examined, gamma irradiation demonstrated the most consistent results and appeared to be the most appropriate sterilizing method for demineralized bone in clinical use.

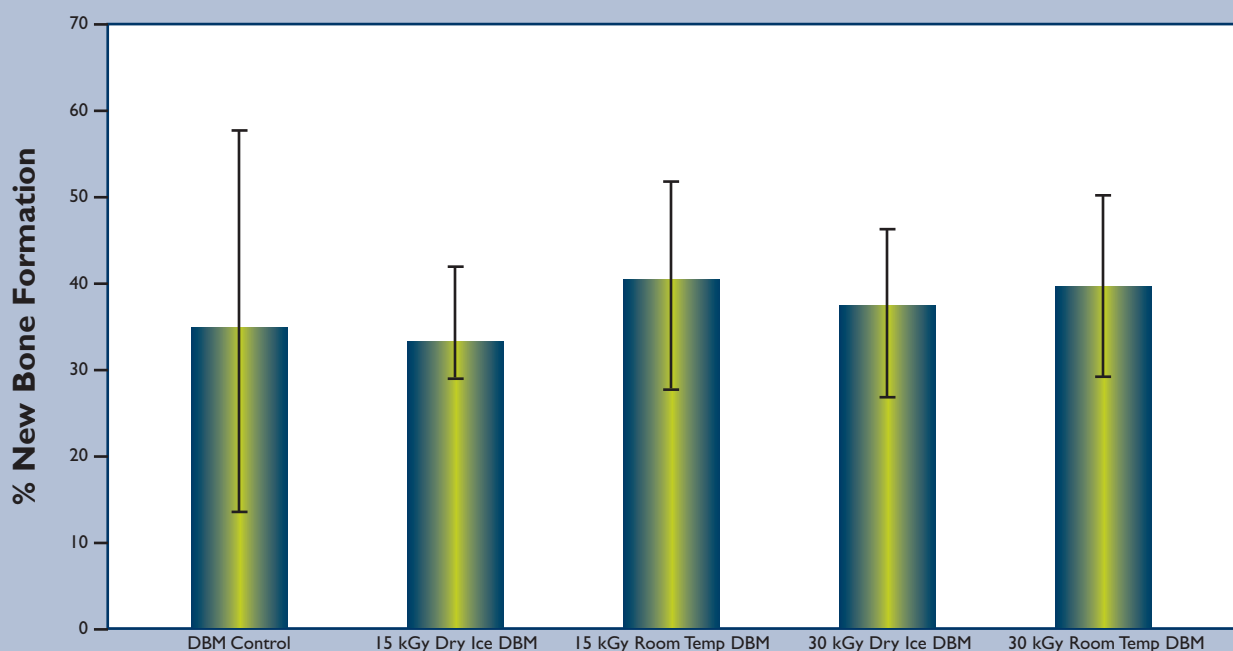
In further studies, the effect of gamma radiation at various temperatures on bone formation and remodeling were explored. In the experiments performed by Weintraub and Reddi¹⁴, samples were maintained in ice water during irradiation. Preparations that had been irradiated with doses up to 25 kGy showed inductive properties that were similar to the non-irradiated control. Dziejcz-Goclawska and colleagues¹⁵ irradiated DBM at room temperature or on dry ice (-72°C). While samples that were irradiated at room temperature had been completely resorbed five weeks after implantation into the muscle pouch of a rat, DBM irradiated on dry ice were osteoinductive and were resorbed more slowly. DBM that had been treated with a dose of 35 kGy at -72°C demonstrated new bone formation that was comparable to non-irradiated

control samples.¹⁵ These results lead both groups to hypothesize that temperature plays a critical role in the protection of osteoinductive properties of DBM against radiation damage.

DEPENDABLE IRRADIATION PRACTICES

To address the need to provide sterile tissue, LifeNet Health performed a series of studies to determine the threshold for safely irradiating bone tissue while maintaining its original osteoinductivity. In one experimental series demineralized bone particles were irradiated at 15 and 30 kGy (absorbed dose) on dry ice (-20 to -50°C) or at ambient temperatures and compared to non-irradiated control samples. The preparations were implanted heterotopically into athymic mice for 28 days and were then assessed for percentage calcium deposition and new bone formation in the implanted samples. No statistically significant differences in calcium deposition and percent new bone formation were found between the experimental samples irradiated at 15 and 30 kGy and the non-irradiated control (dose independence). Similarly, the results showed no significant difference between the experimental samples irradiated on dry ice or at room temperature relative to the non-irradiated control (temperature independence).¹⁸

Figure 1. Percent new bone formation determined by histomorphometric analysis of explanted DBM.¹⁸



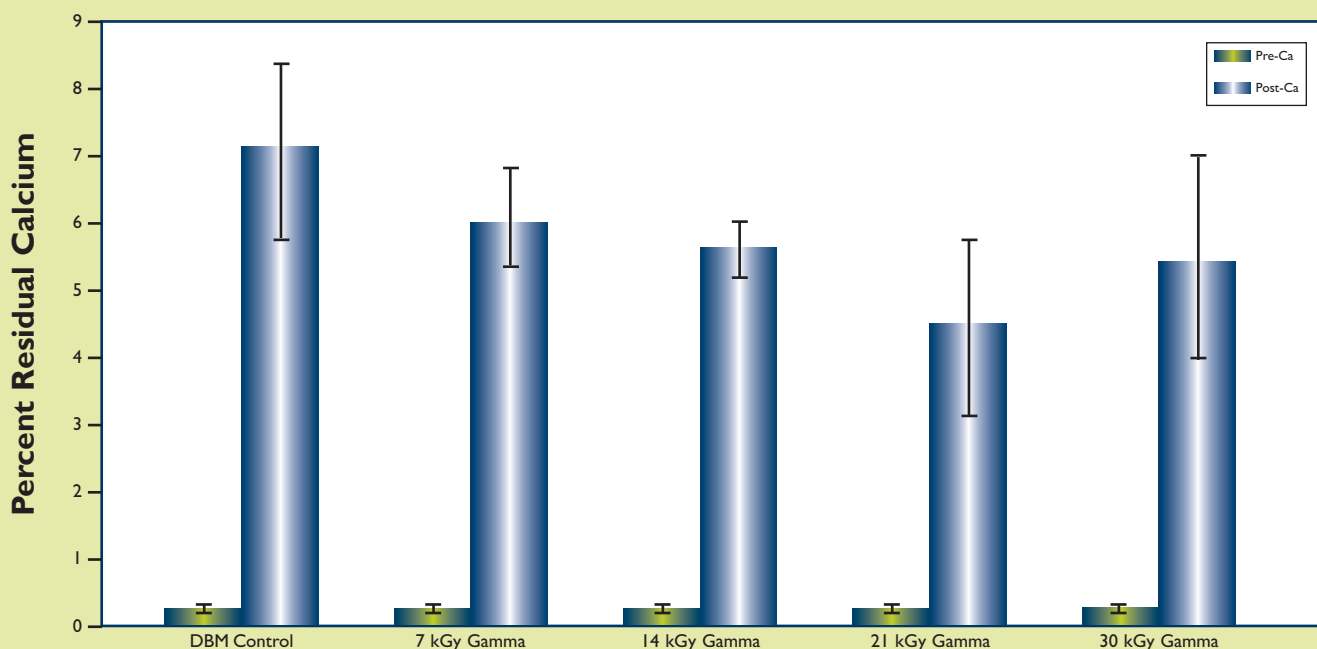
In light of the finding that demineralized bone particles that were irradiated at low temperatures were less susceptible to radiation damage^{14,15}, LifeNet Health also undertook a study to examine the results of a range of absorbed gamma radiation doses on demineralized bone particles.^{18,19} In this assay, bone particles 250 to 710 micron (μ) in size were irradiated at 7 kGy, 14 kGy, 21 kGy, and 30 kGy on dry ice. Experimental samples were implanted in muscle pouches of athymic mice for 28 days and were then compared to non-irradiated control samples in regard to percentage calcium deposition. The data demonstrated that gamma irradiation decreased the remineralization of the implanted DBM preparation to a point at which it allowed for greater resorption and replacement with new bone. This observation corroborates the results of experiments performed by Weintraub and Reddi, which suggested that irradiation of 30 to 50 kGy enhanced bone induction, leading to a higher level of mineralization than non-irradiated control samples in a heterotopic rat model.¹⁴ The experimental samples irradiated at each radiation dose and evaluated in the study did display significant osteoinductivity, however, regardless of the dose administered.²⁰

GAMMA IRRADIATION AND THE OSTEOINDUCTIVE POTENTIAL OF DBM

Two of LifeNet Health's osteobiologic products, I/C Graft Chamber[®] and Collect DBM[®] were evaluated using the athymic rat posterolateral lumbar spinal fusion (PLF) model.²⁰ Groups of 12 rats each were implanted bilaterally with I/C Graft Chamber[®] and Collect DBM[®] grafts that were either non-irradiated or irradiated at 15kGy absorbed dose. An additional six animals acted as the "empty" control group. The graft materials were combined with the rats' autologous blood that was taken from an ocular blood draw.

After eight weeks, the rats were euthanized and the explanted spines evaluated for bony fusion. Radiographs showed that fusion occurred in all implanted animals, but the animals in the irradiated groups demonstrated an increased bilateral fusion rate when compared to the non-irradiated groups. Taking micro computed tomography (μ CT) and histology (H&E staining) together with radiography into consideration, the assessments showed no overall significant difference between irradiated and non-irradiated products in terms of fusion. The

Figure 2. Residual weight changes in percent residual calcium of DBM irradiated on dry ice.^{18,19}



results of this study provide evidence that gamma irradiation of DBM at an absorbed dose of 15 kGy in a controlled low-temperature environment does not have a negative effect on the osteoinductivity of I/C Graft Chamber® and Collect DBM® graft materials.²⁰

This experiment was later repeated using a target absorbed dose of 22 kGy. All groups demonstrated either unilateral or bilateral fusion when assessed by radiography and mCT after eight weeks. Even the higher gamma irradiation dose of 22kGy did not result in a negative effect on the I/C Graft Chamber® and Collect DBM® graft materials in the athymic rat PLF model.²¹

SAFETY THROUGH STERILITY

With the increased concern about allograft safety in the medical community, allograft suppliers are focusing heavily on providing sterile grafts. Although there have been no reports of disease transmission through implanted demineralized bone products to date, medical professionals should be knowledgeable about the practices of individual tissue banks when making the decision to purchase and use allograft tissue.²² Physicians also have the responsibility to inform their patients about the risks and benefits of using allograft tissue.

LifeNet Health has been a pioneer in allograft cleaning and disinfection since the introduction of the proprietary and patented Allowash® technology in 1995. In 2005, LifeNet Health extended the Allowash® process with a terminal sterilization step, thus introducing Allowash XG®. Data derived from animal models and clinical results indicate that the Allowash XG® process has no detrimental effect on the characteristics of allograft tissue.^{12,23} Accordingly, LifeNet Health's validated Allowash XG® technology results in sterile allograft tissue at a 10⁻⁶ SAL without compromising the biomechanical and biochemical integrity of the tissue.

By terminally sterilizing our Osteobiologic bone allograft products, LifeNet Health demonstrates our commitment to the highest level of safety and most effective clinical outcomes for medical practitioners and their patients.

OSTEOBIOLOGIC PRODUCTS – A VARIETY OF CONFIGURATIONS FOR YOUR SURGICAL NEEDS

Although demineralized bone has been used in clinical applications for decades and has been shown in both *in vitro* and *in vivo* assays to be osteoinductive and to cause the formation of bone, it does have limitations. DBM, particularly DBM powder, can be difficult to handle. The small particles are flaky, do not adhere to each other, and can migrate from the implant site. In addition, DBM is generally provided in small quantities of 0.5 cc to 5 cc to fill small defect sites for which DBM is typically used, for example, during maxillofacial surgery. These small graft volumes are less effective for large bony defects. A further problem with packaging DBM in volume quantities is that the volume of a specific unit of weight of DBM may vary, depending on how the bone particles settle in the package over time. This variability in the volume due to settling may make it seem as though the package contains an insufficient amount of product.

To overcome the issues of handling limitations, the variability in fill volumes, and the need to extend the graft to fill larger voids, many companies offer DBM in combination with a carrier. Often, extender scaffolds such as non-demineralized cancellous bone chips are used to increase the volume of the implant material and are added to DBM either by the manufacturer or by the physician at the time of surgery.

Carriers and extenders for DBM can be synthetic or biologic in origin. Glycerol, synthetic polymers, hyaluronic acid, collagen, and autologous blood or blood components are examples of carriers used in commercially available osteobiologic products.^{24,25} Handling characteristics such as malleability, resistance to graft migration, and viscosity are often manipulated by varying the ratio of carrier to DBM. The medical professional should be cautious when comparing the osteoinductive potential of osteobiologic products with pure DBM, however. For example, in a bioassay that tests the osteoinductivity of a product, 20 mg of DBM and 20 mg of an

osteobiologic product should not necessarily be expected to perform equally well. In most products, demineralized bone is the bioactive component of the product, so variability in the DBM content may have a significant effect on the product's osteoinductive potential. Therefore, when comparing equal amounts of DBM and osteobiologic product in either a bench testing or a clinical evaluation, there may be significantly less osteoinductive material in the osteobiologic product. Nonetheless, this should not be interpreted to mean that osteobiologic products are less capable of stimulating new bone formation in every case.

Some osteobiologic products may use carriers that act synergistically with the DBM in improving osteoconductivity by helping to maintain porosity within the packed DBM matrix, which may enhance cellular infiltration or promote cellular activity in the synthesis of new matrix. Other carriers may lend better handling characteristics to DBM and will quickly dissipate from the implant site, leaving only DBM to provide osteoinductive and osteoconductive characteristics. In this regard, DBM and osteobiologic products with added growth and differentiation factors may have an advantage over DBM alone. These added growth and differentiation factors may also quickly dissipate from the implant site, however, so that the overall and time-dependent bioavailability of the factors is reduced. If DBM with its natural complement of growth and differentiation factors is not present at the surgical site, osteobiologic products, even with added growth and differentiation factors, may only be able to act as a scaffold in which infiltrating cells proliferate and grow without the capacity to stimulate these cells to differentiate and form bone.

Since autografts contain viable cells with the ability to form new bone and are therefore osteogenic, many surgeons have historically relied on autografts as the gold standard for bone grafting, usually using bone and bone marrow from the iliac crest. The main disadvantage of this technique is the potential for morbidity and the pain of a second surgery site generally associated with recovering autograft tissue. LifeNet Health offers three osteobiologic products

to avoid the issue of a second surgery site that take advantage of DBM's osteoinductive potential, provide the clinician with a variety of alternatives suitable for a wide range of surgical applications, and have a proven track record of clinical safety and efficacy. These osteobiologic products are I/C Graft Chamber[®], Collect DBM[®], and Optium DBM Matrix[®]. Both I/C Graft Chamber[®] and Collect DBM[®] can be combined with a patient's autologous tissue to create a grafting material that provides all three physiological properties of osteogenesis, osteoconduction, and osteoinduction for optimized bone healing and patient outcome.

I/C Graft Chamber[®]

LifeNet Health's I/C Graft Chamber[®] leverages the osteogenic advantages of the patient's autograft in combination with demineralized bone matrix and cancellous bone chips contained in a chamber. At the time of surgery, the platelet-rich plasma (PRP) component from the patient's own blood can be prepared and mixed with the contents of the I/C Graft Chamber. Once the mixture clots, a semi-solid graft is formed that contains the patient's own osteoconductive growth factors together with the osteoinductive DBM. Using the I/C Graft Chamber in conjunction with the Symphony[™] Platelet Concentrate System (PCS; DePuy, Inc.) results in an approximately five-fold increase in the platelet concentration per volume of blood.²⁶ In addition, recombinant platelet-derived growth factor (PDGF) can be added to the bone matrix and has been shown to enhance cartilage and bone formation. This may have particular therapeutic functionality in treatment of impaired bone formation in aged patients.²⁷

Similarly, bone marrow from the patient's iliac crest can be aspirated and combined with the bone in the chamber to make use of the marrow-derived osteoprogenitor stem cells with their growth and differentiation potential. Of course, whole blood can simply be added to the I/C Graft Chamber in combination with an activating agent to produce a fibrin clot that will improve the handling characteristics of the allograft bone.



Collect DBM®

Collect DBM's proprietary combination of allograft demineralized bone fibers and cancellous chips is designed for the selective retention of osteoprogenitor cells from a patient's own bone marrow. The bone marrow is aspirated from the patient's iliac crest and is conducted through the autograft bone contained in a chamber using the Collect® Graft Preparation Device (Depuy, Inc.). Retention of attachment-based cells in the bone matrix leads to a three- to four-fold enrichment of osteoprogenitor cells, which have osteogenic capacity and augment the osteoinductive and osteoconductive properties of the allograft tissue.

Two recently published studies investigated the effectiveness of Selective Cell Retention (SCR) technology using Collect DBM® in both an animal model and in clinical application.^{28,29} The animal model used was the canine critical-sized femoral segmental defect model, a methodology that is well established and published.³⁰ Four different bone graft materials were evaluated:

1. Autograft bone taken from the iliac crest
2. Collect DBM® enriched with osteoprogenitor cells using SCR technology
3. Collect DBM® saturated with whole bone marrow
4. Collect DBM® alone

Radiographs were taken to assess bony union post-operatively and every four weeks thereafter through 16 weeks. At 16 weeks the dogs were euthanized and the femoral bones with the graft materials were explanted. At four weeks, the Collect DBM® implants enriched with osteoprogenitor cells and the autograft bone implants showed early evidence of cortical bridging. This was confirmed at 16 weeks; at completion of the study, the Collect DBM® implants enriched with osteoprogenitor cells (Group 2) and the autograft bone (Group 1) implants demonstrated complete cortical bridging. In contrast, the

experimental groups with Collect DBM® alone or saturated with whole bone marrow consistently lagged in the healing process. Only 50% of the defects grafted with Collect DBM® alone (Group 4) and 67% of Collect DBM® saturated with whole bone marrow (Group 4) reached complete bone healing by week 16.²⁸

The results found in the animal model have been confirmed in clinical applications. In the spine, for example, autologous bone marrow combined with either demineralized bone matrix (DBM) or osteoconductive substrates has resulted in improved fusion rates. In a prospective lumbar spine fusion pilot study conducted at five centers, 51 patients underwent one- or two-level posterolateral fusion.²⁹ All patients with one exception received an interbody implant and Collect DBM® enriched with the patient's bone marrow aspirate was placed in the posterolateral gutters. Patients went through clinical and radiographic follow-up at six weeks as well as three, six, 12, and 24 months after surgery. In addition, samples of the aspirated bone marrow were taken before and after the Selective Cell Retention (SCR) Collect DBM® enrichment process and were analyzed in an independent laboratory.

The fusion rates at 12 and 24 months were comparable to fusion rates for posterolateral fusion procedures with autologous bone harvested from the iliac crest. Patients also reported improved function and reduced pain by over 50%. The analysis of cellular retention during the SCR process indicated that the number of osteoprogenitor cells retained in the Collect DBM® matrix is approximately 3.3 fold the level in native bone marrow with over 85% of all osteoprogenitor cells retained in the allograft matrix. At the same time, nucleated cells such as lymphocytes are reduced by about 45% and red blood cells are largely removed. Collect DBM® enriched with osteoprogenitor cells using SCR technology offers a viable alternative to iliac crest autografts that is minimally invasive and clinically effective.²⁹

Optium DBM[®] Matrix

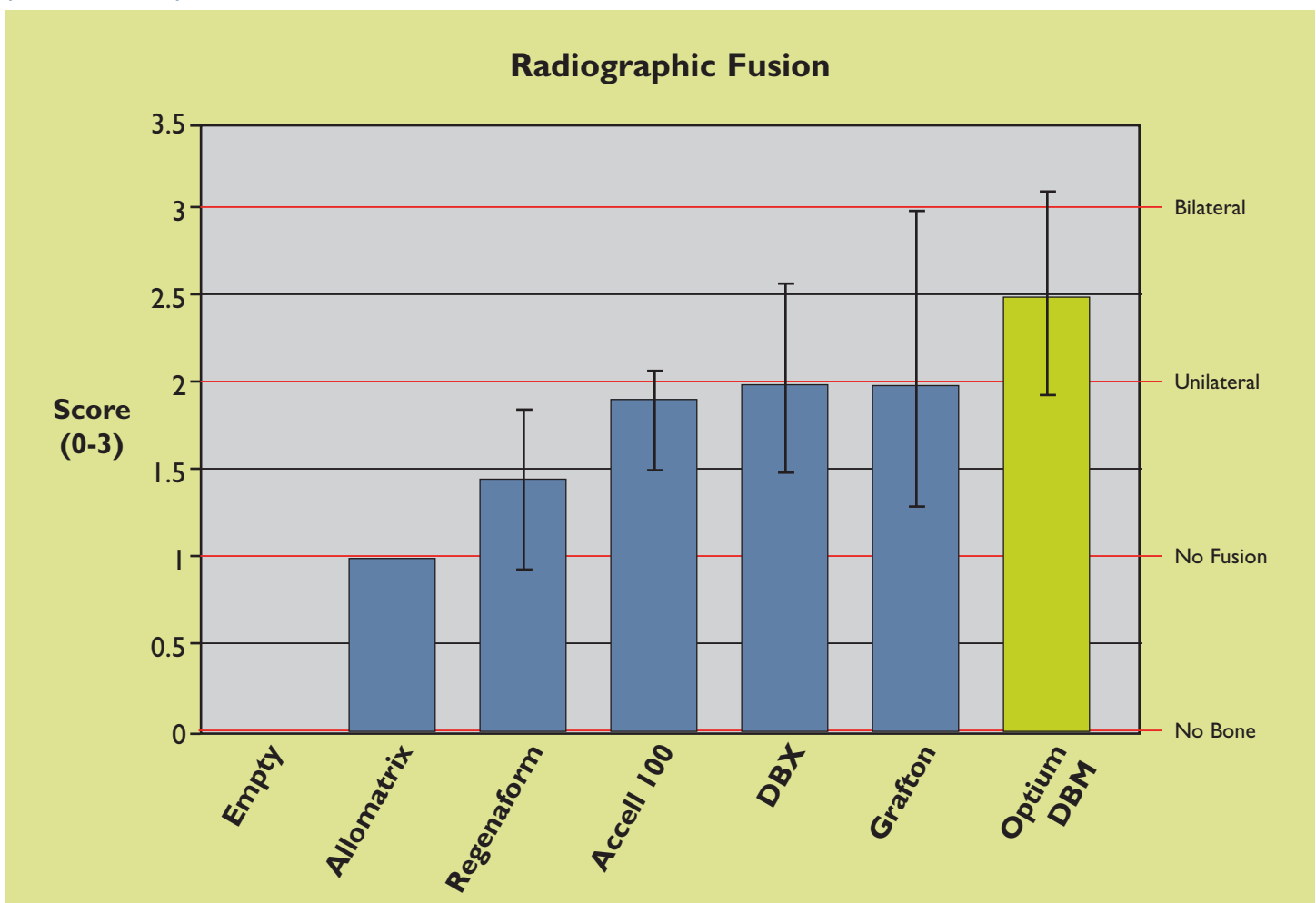
For those medical practitioners who prefer a ready-to-use osteoinductive allograft product, LifeNet Health offers Optium DBM[®] Matrix available in both gel and putty configurations. Optium DBM Matrix[®] is composed of demineralized bone matrix (DBM) and processed using a patented and proprietary glycerol-based carrier technology to enhance the product's handling characteristics.

Of critical importance when comparing the many DBM options available in the market today is to take the residual mineral content of each product into account. When DBM has been demineralized to an optimal residual mineral level, the bone morphogenetic proteins (BMPs) found in the bone

matrix are ideally exposed, allowing each individual BMP isoform to play its critical role in the multi-step sequence that leads to new bone formation.

The athymic rat spinal fusion model has been developed to evaluate the efficacy of various DBM products. In one comparative study³¹, six different commercially available DBM products were compared regarding their potential to induce spinal fusion in athymic rats. Sixty rats were included in the study with a group of six rats acting as an "empty" control. Each animal was implanted bilaterally with the same test material. After eight weeks, the rats were euthanized and the explanted spines evaluated for bony fusion. Of all DBM materials tested, Optium DBM[®] putty demonstrated highest total fusion rate at over 90% (Figure 3.).

Figure 3. Osteoinductive potential of commercially available demineralized bone matrices in an athymic rat posterolateral spinal fusion model.³¹



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