Puros® Demineralized Bone Matrix (DBM)

Overview of *Puros* Demineralized Bone Matrix

Puros Demineralized Bone Matrix (DBM) represents advancement in bone graft substitute technology utilizing demineralized bone matrix (DBM) in a putty formulation. It is 100% demineralized human bone; in addition, putty with chips contains mineralized cortical cancellous chips. Many other allograft DBM based bone graft substitutes currently available contain a non-DBM-based carrier to facilitate handling and graft containment.[1] *Puros* DBM Putty differs from these products because its carrier is also DBM from the same donor, from a different stage of the proprietary processing methodology.

The mixture of human DBM powder with the DBM carrier from the same donor results in a *Puros* DBM product with a putty-like consistency exhibiting excellent handling properties and graft containment. There is also a *Puros* DBM Putty formulation that contains cortico-cancellous chips from the same donor as that of the DBM. The product is easy-to-use and convenient as it is ready for immediate use without extra preparation required in the OR. It should be stored at controlled room temperature (15°-25°C).

Both the active DBM powder and the carrier are 100% demineralized human bone from the same donor. *Puros* DBM consists of active human DBM mixed with osteoconductive DBM derived from the same donor. The only excipient in the product is sterile water. Because the product is 100% natural bone matrix, it is resorbable and remodels readily. Through proprietary processing procedures, *Puros* DBM Putty is generated such that it retains a high degree of osteoinductive potential* in addition to serving as an osteoconductive matrix. *Puros* DBM Putty is pre-mixed and delivered in open bore dispensers and jars to serve as a 'ready-to-use' product.

In addition to extensive donor screening, procedures for proper handling of the tissue, and RTI's proprietary processing methods, the final product is terminally sterilized using low-temperature, low-dose gamma irradiation to provide safety against disease transmission. This methodology, the *Cancelle SP*™ DBM Sterilization process, has been validated to inactivate or remove bacteria, viruses, fungi and spores, while preserving protein activity.

History of DBM

Bone consists of two major components: organic proteins and inorganic mineral. Collagen is the main constituent of the organic material that gives bone its toughness and resilience. In addition, there are many growth factor proteins that closely regulate bone formation and remodeling.[2, 3] Proteins constitute nearly 30-35% of bone by weight.

The mineral part of bone, which constitutes nearly 65-70% by weight, is essentially comprised of calcium phosphate salts that give bone its stiffness and strength. This component of bone dissolves readily in acid medium resulting in demineralized bone matrix rendering the growth factors more readily accessible[4, 5] to initiate the cascade of bone regeneration while simultaneously retaining the natural scaffold matrix.

The first reported clinical use of DBM dates back to 1889 when Senn[6] used DBM as a vehicle for the delivery of antiseptics in the treatment of bone cavities and demonstrated that demineralized bovine bone could successfully repair large osseous defects that would not completely heal by themselves. He demonstrated this using DBM in long bone and cranial defects in dogs, and tibial and femoral defects in humans. For decades this field lay dormant until Urist[7] (in 1965) published his seminal work demonstrating the osteoinductive properties of demineralized bone. He showed that DBM had the capacity to induce bone formation in a non-bony tissue by implantation in muscle pouches of rats. It is now known that only highly osteoinductive substances can induce bone formation at such heterotopic sites. Urist also demonstrated that demineralized bone could be implanted into human long bone defects and lumbar vertebrae to regenerate bone. Further, he demonstrated that the osteoinductive fraction could be extracted from demineralized bone. He identified this fraction as bone morphogenetic protein[8] (BMP) after establishing that the activity resided in a protein.[9] BMPs are known to signal precursor cells to regulate bone formation.

Since 1965, numerous animal and clinical studies have demonstrated the effectiveness of DBM in a variety of osseous defects. In 1981, Mulliken *et al.*[10] reported using DBM in craniomaxillofacial defects in 44 patients with 55 implants including demineralized bone powder and allograft cancellous chips and blocks. Radiographic healing was evident in 3-6 months, and biopsy sections revealed evidence of induced bone throughout the defects rather than bone formation at the edge of the defect as in creeping substitution.

* DBM induced bone formation when implanted in an athymic rat assay. Findings from an animal model are not necessarily predictive of human clinical results.

Tiedman *et al*.[11] studied the efficacy of DBM alone and as a composite with bone marrow for several indications including spinal fusion, augmentation of bone in total joint replacement, acute fractures, non-unions, defects, and joint arthrodesis. Union occurred in 77% of the cases. The author stated that "patients demonstrate the efficacy of DBM to graft osseous defects ranging from acute fractures with bone loss, to fracture non-unions, to bone defects resulting from tumor excision or total joint revision surgery."

More recently, Leriche and Policard[12], Levander[13], LaCroix[14], Huggins[15], and Reddi[16] established themselves as pioneers in the field of induced bone formation.

Osteoinductive Nature of DBM

Native bone is the largest reservoir of osteoinductive factors. These factors reside in a dormant fashion bound to the collagen matrix in bone. Following injury, the growth factors are mobilized and activated so they can stimulate migration, differentiation and proliferation of surrounding progenitor cells to initiate the cascade of bone repair[17]. The demineralization process not only retains these osteoinductive factors but also helps to expose them to surrounding cells for easy access. Thus, DBM can provide an optimal natural matrix for osteogenic progenitor cells to adhere, differentiate and form bone if the osteoinductive factors remain active. Because demineralization of bone results in exposing the growth factors that are bound to the collagenous matrix, it is critical to optimize the demineralization process to ensure that growth factors remain active through this process. The observed high variability in the osteoinductive potential of commercially available DBMs can, to a large extent, be attributed to the differences in the demineralization processes employed.[18]

Osteoinductive factors are critical to bone induction *in vivo*. Successful delivery of these factors requires their association with suitable matrices. These matrices, among other characteristics, should be biocompatible, biodegradable and capable of maintaining the proteins in their active state. They should serve as a reservoir for these proteins for extended periods of time. Demineralized bone provides all these features and is also the natural reservoir for the proteins *in vivo*. BMPs 1-16 have been identified in DBM.[19] These proteins start the osteoinduction cascade by initiating chemotaxis of progenitor cells which is the directed migration of cells in response to a chemical gradient of signals released from insoluble DBM.[20]

DBM: An Alternative to Autograft

Surgical management of a number of orthopedic problems necessitates the use of bone graft to ensure a clinically successful outcome.

Bone undergoes constant remodeling in a living organism. Small fractures of bone heal without the need for intervention because of its high potential to regenerate. However, larger defects created either by trauma or surgical procedures will not self-heal effectively. Fast healing with good quality bone occurs when the defect is filled with proper matrix (osteoconductive agent) that can serve as a scaffold for the osteogenic cells to infiltrate. Bone healing is further augmented in the presence of proper growth factors (osteoinductive agents). Autogenous bone is considered the gold standard for treating such large osseous defects because it provides all the components mentioned above that are required for bone regeneration. Additionally, it poses no issues such as disease transmission and immunogenicity. However, the greatest limitation it poses is the creation of a second surgical site for harvesting the tissue resulting in greater morbidity and increasing the risk for infection. Availability of sufficient autogenous bone may add to this limitation. These issues have been addressed by developing synthetic bone graft substitutes. Although synthetic bone graft substitutes provide the scaffold/matrix, they lack both the osteoinductive and osteogenic factors which provide great advantage for effective bone healing. The resorption of some of these synthetic matrices does not parallel bone regeneration, thus compromising the quality of tissue repair. Biological scaffolds will serve as more suitable substitutes for autogenous bone. Orthopedic surgeons are now using composite grafts consisting of osteoconductive scaffolds and some osteoinductive components to provide more of the required elements for bone regeneration. Allograft derived DBM is widely used as a composite graft because it provides the natural scaffold and the osteoinductive proteins that are found in bone.

Commercial DBM Preparations

Since the initial studies performed by Urist, the osteoinductivity of DBM has been well established.[21] DBM products have been on the market since 1991. The first DBM used glycerol as the carrier to improve the handling properties and ensure graft containment. Today, DBMs are used in various surgical applications including those of spine, reconstruction, maxillofacial, and trauma. Some of the surgical applications of DBMs include:

- Filling of voids following removal of a bone cyst or bone tumor
- Filling contained and uncontained defects
- Long bone fractures
- Tibial plateau and pilon fractures
- Talus and calcaneal fractures
- Condylar defects
- Distal radius and scaphoid fractures
- Supracondylar fractures
- Non-unions
- Segmental defects with fixation
- Impaction grafting

- Osteotomies: opening and closing wedge, translational
- Osteolytic defects
- Joint fusions
- Revisions
- Iliac crest harvest backfill
- Avascular necrosis
- Dental intraosseous, oral and cranio-/ maxillofacial defects
- Ridge augmentation
- Sinus lift
- Socket preservation
- With dental implant placement

Demineralization of bone powder results in a dry granular material which is difficult to handle and contain within the graft site. To improve handling characteristics, cohesiveness and prevention of dispersion from the graft site, the granular product is mixed with an inert carrier. Table 1 below depicts some of these carriers used in DBMs that are currently available in the market along with that of *Puros* DBM Putty.

***Puros* DBM Putty: What is it?**

Puros Demineralized Bone Matrix (DBM) is donated human allograft tissue intended for transplantation. Each lot of *Puros* DBM Putty is processed from a single donor and combines DBM processed using two slightly different methods to provide optimal handling characteristics while retaining the osteoconductivity and osteoinductive potential of the active DBM. The only excipient in the product is sterile water. *Puros* DBM Putty has excellent handling properties and can be molded to fit various defect shapes and sizes. It also provides good graft containment properties resisting irrigation. As it is supplied in open bore dispensers and jars and stored at controlled room temperature (15-25°C), the product is ready for immediate use without the need for any preparation. Like all other DBMs, it is restricted to homologous use for repair, replacement, or reconstruction of musculoskeletal defects without an intrinsic need for load bearing, by or on the order of a licensed practitioner.

DBM/Company	Carrier
Grafton®/OsteoTech, Inc., Biohorizons	Glycerol
DBX®/Synthes, Inc., Dentsply	Sodium Hyaluronate
Allomatrix® Putty/Wright Medical Technology, Inc.	Calcium Sulfate
DynaGraft®/DynaBlast™ Putty/Integra Life Sciences, Keystone	Reverse Phase Medium
Accell Connexus® DBM/ Integra Life Sciences	Reverse Phase Medium
Accell® DBM 100/Integra Life Sciences	Soluble DBM
Puros DBM Putty / RTI Biologics, Zimmer	Human DBM

Table 1. Commercially available DBM Putties* and the carrier utilized

* All trademarks are the property of their respective owners and all competitive information can be found on their respective web sites

Water is known to facilitate protein breakdown because it aids in keeping proteases in a hydrated state. This may result in progressive loss of osteoinductive potential of DBMs that are reconstituted with water. Han *et.al*.[22] demonstrated that hydrated DBM loses its osteoinductive potential when stored at or above room temperature for as little as 5 weeks. To address this issue, *Puros* DBM Putty has been tested in the *in vivo* rat ectopic assay and shown to maintain osteoinductive potential over the period of its shelf life when stored as indicated.

Patient safety is of paramount importance during all phases of DBM processing and handling. Each lot of DBM is obtained from a single human donor and is not mixed with other donors in any formulation. The tissue donors are rigorously screened in compliance with AATB standards and FDA regulations. In addition *Puros* DBM Putty is terminally sterilized via gamma irradiation as the final step in the proprietary *Cancelle SP* DBM Process.

Proprietary Processing

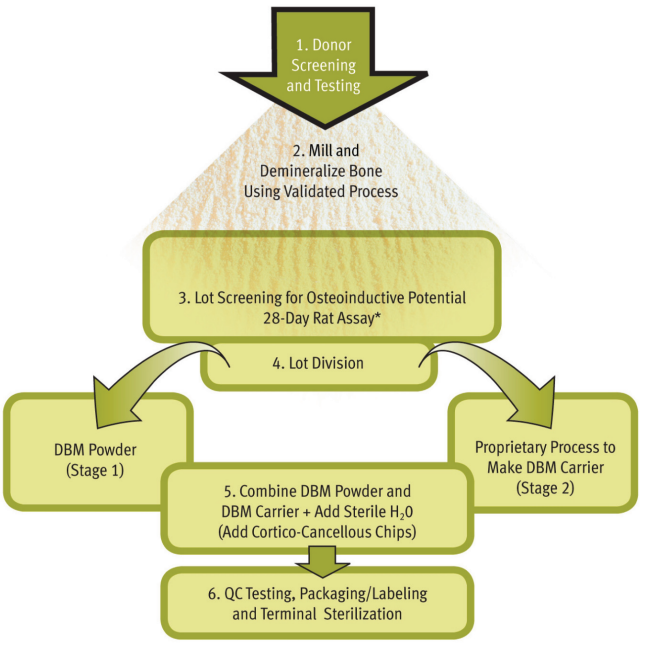

Figure 1. *Puros* DBM Process flowchart

* DBM induced bone formation when implanted in an athymic rat assay. Findings from an animal model are not necessarily predictive of human clinical results.

Step 1: Strict Donor Screening and Testing

Before processing any tissue, a risk assessment is performed on every potential donor, family members are interviewed, the donor's medical records are evaluated, and if necessary, the donor's physician is consulted. Blood samples from donors are tested for the presence of infectious diseases, including HIV and Hepatitis B & C.

RTI uses many different review processes and tests, including but not limited to:

- Medical / Social History Evaluation
  - Family/Next-of-Kin interview
  - Medical/Hospital record review
  - Behavioral/Lifestyle risk assessment
  - Medical Examiner/Coroner's report (autopsy report, when available)
  - Laboratory, pathology and radiology reports
- Serological Testing
  - HCV Antibody
  - HBV Surface Antigen
  - HIV 1 & 2 Antibody
  - HBV Total Core Antibody
  - HTLV I &II Antibody
  - RPR for Syphilis
  - HIV-1/NAT
  - HCV/NAT
- Microbiological Testing
  - Pre-processing culturing: Performed before processing begins; removes potentially unsuitable tissue from process
  - Environmental controls: Monitors cleanliness of processing environment

The final determination of donor eligibility is made by RTI's medical director-a licensed physician-utilizing all available, relevant information.

Step 2: Milling and Demineralization

a. Milling

Bone from donors that pass the above criteria is processed to make *Puros* DBM Putty. Tissue processing for each donor lot occurs in controlled, clean-room environments and the room is cleaned between donors. The first processing step involves milling the cleaned bone to optimal particle size so that it retains both osteoconductive and osteoinductive properties.

Donor bone tissue is milled such that *Puros* DBM Putty has DBM particles that fall within size ranges shown to produce bone growth (125-1000 μm), thus providing an osteoconductive matrix.[24]

b. Demineralization

Bone is a natural reservoir of growth factors that are required for its formation. In native bone, these factors are sequestered within the matrix and remain inaccessible unless the matrix is broken down. Demineralization is the first step during matrix breakdown, which improves accessibility of the growth factors. While several methods can be employed for demineralizing bone, the most common and effective way utilizes mineral acids as described by Urist.[7] Proper demineralization can result in high quality matrix which retains all or most of its osteoinductive potential. According to the AATB standards, DBMs should have a calcium content of 8% or less. A study further examining residual calcium levels within this range demonstrated that the optimal residual calcium content is less than 1%. *Puros* DBM Putty is therefore processed so as to fall within this optimal range.[25]

The *Cancelle SP* DBM Process is validated to inactivate the following model, relevant and challenge viruses providing an additional level of safety to the recipient of the *Puros* DBM Putty implant:

- Bovine Viral Diarrhea Virus (BVDV) Model
  - Human Immunodeficiency Virus (HIV)
  - Hepatitis C Virus Model (HCV)
  - Human T-lymphotropic Virus (HTLV)
- Pseudorabies Virus (PrV) Model
  - Hepatitis B Virus (HBV)
- Human Poliovirus (Polio-1) Challenge
- Porcine Parvovirus (PPV) Challenge

Step 3: Test for Osteoinductive Potential

The osteoinductive potential of DBM can be highly variable depending on the individual donor, processing methods used to demineralize bone and the carrier used to make DBM.[26] Because variability among donors exists, each lot of DBM must be assessed to assure osteoinductive potential.

Ectopic bone formation in an athymic rodent muscle pouch is a standard method of demonstrating the osteoinductive properties of DBM. Although a few *in vitro* assays have been validated against these animal models, *in vivo* testing in rodents is considered the gold standard because it evaluates the osteoinductive potential of the graft material through the entire cascade of steps leading to the formation of bone. In the presence of an osteoinductive graft in a non-bony site, a process similar to endochondral bone formation is observed where mesenchymal stem cells differentiate

into cartilage cells (chondrocytes) and bone-forming cells (osteoblasts), producing new bone. Bone grafts that do not trigger this process of endochondral ossification may be biocompatible but are not osteoinductive.

Each lot of DBM used in *Puros* DBM Putty is tested for osteoinductive potential after irradiation. Biological activity is assessed (qualitatively and quantitatively) using the Urist[7] athymic rat model. This *in vivo* model has been utilized by RTI Biologics to identify DBM with acceptable osteoinductivity for use in all *Puros* DBM Putty products. The implants are extracted after 28 days and analyzed histologically for osteoinductivity and inflammatory responses in accordance with the scoring system of Edwards *et al.* (1998).[27] In addition, bone maturity is also scored in accordance with Katz *et al.* (2006).[28] Only those lots of DBM that pass this in vivo rat assay with minimal inflammatory response are further processed into *Puros* DBM Putty.

Delivering patient safety

The primary goal is to ensure patient safety. To fulfill this goal, stringent tissue donor screening, laboratory testing and tissue preparation processes validated to address the potential for disease transmission are employed for *Puros* DBM Putty. As discussed earlier, the redundant safeguards include a) screening donor medical history by evaluating medical records and interviewing family and next-of-kin, b) conducting an extensive panel of serological and microbiological tests, c) demineralizing using validated process tested using model viruses in accordance with FDA guidance[23], and d) terminal sterilization of product using low-temperature, low-dose gamma irradiation to achieve a validated 10^{-6} sterility assurance level. These redundant safeguards provide a high level of confidence that patients will receive safe, high quality tissue.

General Information

For further information regarding *Puros* DBM products, please refer to the Instructions For Use.

RTI Biologics Inc. Credentials

- RTI Biologics is accredited by the American Association of Tissue Banks (AATB) for processing, storage and distribution of skin, pericardium and musculoskeletal tissue for transplantation and research
- RTI Biologics is registered as a Tissue Establishment with the U.S. Food and Drug Administration (FDA).
- RTI Biologics is registered as a Medical Device Manufacturer with the FDA.
- RTI Biologics' quality system is certified to ISO 13485:2003 (with CMDCAS for Canada) Medical Devices Quality Management Systems.
- RTI Biologics is registered with Health Canada for Human Cells, Tissue and Organs for Transplantation (CTO).
- RTI Biologics is licensed as a Tissue Bank by the following states:
    - Florida
    - Maryland
    - California
    - New York
    - Other state registrations as applicable: (Delaware, Illinois, and Oregon)
- RTI Biologics is licensed as a Medical Device Manufacturer with the State of Florida.
- RTI Biomedical Laboratory holds:
    - FDA Registration
    - Clinical Laboratory Improvement Amendments (CLIA) Certificate of Compliance (Federal)
    - State of Florida Clinical Laboratory License
    - New York State Department of Health Clinical Laboratory Permit

All lab tests are performed using kits approved by the FDA for donor screening and cadaveric specimens, if applicable. Laboratory procedures comply with the kit manufacturer's instructions for use regarding testing protocol, specimen type and specimen handling/storage requirements.

Zimmer, Inc. Credentials

Zimmer's tissue bank establishment, Zimmer Spine, is registered with the U.S. Food and Drug Administration, and is licensed as a tissue bank in the following states:

- Florida, Maryland, California, New York

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