



Demineralized Bone Matrix:
Development of a Process for Optimal Performance

the better approach

MTF Musculoskeletal
Transplant
Foundation

Introduction

MTF is a non-profit organization founded in 1987 by academic orthopaedic surgeons dedicated to providing tissue of the highest quality and safety for transplantation. Everything we do at MTF begins with safety. MTF has distributed almost 4.2 million allografts since our inception, and we have never experienced a case of viral disease transmission. MTF's exemplary safety record is directly attributed to our commitment to the donor families and to the tissue recipients we serve. This tremendous commitment provides our customers with the assurance that this gift of human tissue is safe and that it comes from a trustworthy source.

We also think beyond safety. While safety governs every decision we make, we know that quality and efficacy also matter. Current techniques used by some tissue banks to clean, process and sterilize demineralized allografts have been shown to be detrimental to the quality of the tissue. These methods vary widely from bank to bank, because the industry donor criteria and processing standards are open to interpretation. Demineralized allograft tissue of less-than-optimal quality may yield a graft that does not perform its intended function, leading to a less-than-optimal clinical outcome.

Principles of Bone Healing and Graft Incorporation¹

Four components are necessary for bone healing and/or bone graft incorporation: the presence of host cells, a signal to trigger differentiation of the host cells to bone forming cells, a scaffold or matrix on which the new bone can form, and an adequate blood supply.

When bone fracture or injury occurs, there is loss of mechanical integrity of the bone and disruption to the blood supply. The healing cascade begins immediately. The three phases are: inflammation, repair and remodeling. Inflammation is the process by which host cells remove debris from the injured site, prepare the local matrix into a site which can support cell growth, and enable new bone to be formed. Revascularization, which is required for new bone to grow, begins in the inflammation phase. Repair includes the recruitment and differentiation of host cells into osteoblasts, which in turn produce new bone at the injured site. Lastly, remodeling is the resorption of immature or extraneous bone coupled with reorientation of bone along the direction of mechanical loading to provide adequate structural support. These phases (**Figure 1**) are regulated by the release of local cytokines.

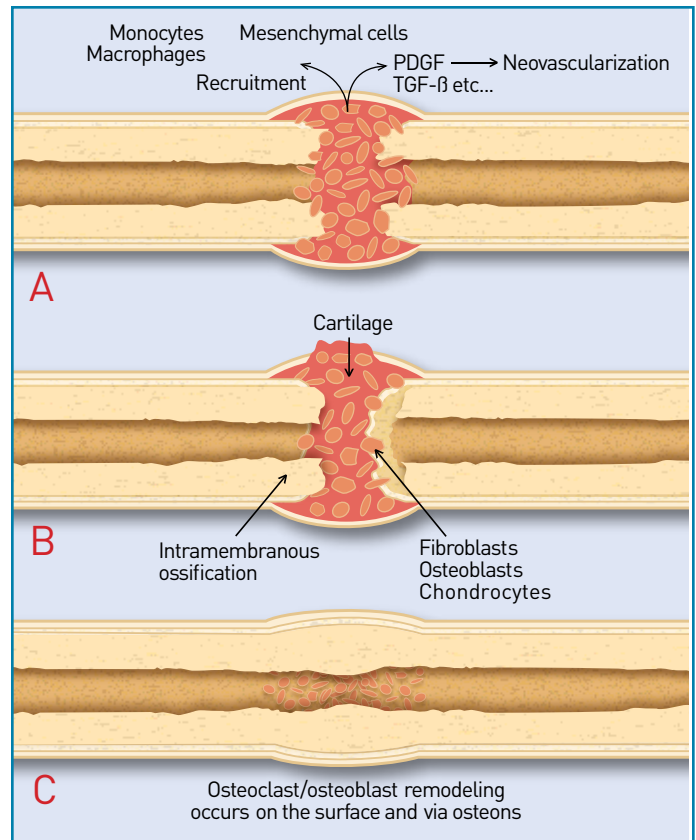


Figure 1: Example of the healing cascade in fracture repair. The three phases of fracture repair include A) the inflammatory phase, B) the reparative phase, and C) the remodeling phase.¹

New bone formation and bone healing are influenced by several factors in the bone graft material, some of which can be controlled, such as:

- Bone forming potential
- Porosity
- pH

Host factors are not as easily controlled:

- Age
- Systemic disease
- Vascularity
- Presence of infection
- Quantity and quality of host cells
- Use of anti-inflammatory drugs

Bone grafts are often used as bone void fillers to assist with bone healing. Grafts can provide support or cell signals. During the healing process, the bone grafts are incorporated into the host bone by remodeling and/or creeping substitution. Bone graft materials used as bone void fillers can be described as osteogenic, osteoinductive and/or osteoconductive.

Osteogenic tissues are capable of forming new bone from living cells. Osteoprogenitor cells proliferate and differentiate into osteoblasts (bone-building cells) and eventually into osteocytes (mature bone cells). These cells represent the osteogenic potential of the graft.¹

Osteoinductive tissues are ones which promote chemotaxis, mitogenesis and formation of osteoprogenitor cells that have osteogenic capacity (as described above).² Osteoinductive materials will form bone when implanted into tissues which would not otherwise form new bone.³

Osteoconductive tissues allow for fibrovascular tissue development and osteoprogenitor cell invasion of a porous structure. This material then acts as a temporary scaffold which will be replaced with newly formed bone.²

Rationale for Clinical Use of DBM

Autograft bone provides all three components necessary for bone healing. It has been widely used during bone grafting procedures due to availability of donor graft sites and good incorporation upon transplantation. However, the use of autograft bone requires a second surgical site (to procure bone) which has associated morbidity risks. Allogeneic demineralized bone matrix (DBM) eliminates the need for a second surgical site, and its demineralized state results in bone that is osteoconductive and has osteoinductive potential.

How DBM Works

The exact mechanism of the osteoinductive potential of DBM has not been very well defined. However, it is thought that the removal of the mineral component of bone exposes the active bone morphogenetic proteins (BMPs) present in the DBM while retaining the inherent osteoconductive properties of the bone. When implanted, the active BMPs are thought to signal the host mesenchymal cells thus causing the cells to proliferate and differentiate into chondroblasts, which in turn will form a cartilage matrix. Ultimately, the cartilage matrix is converted into a calcified extracellular matrix. This calcified matrix will become vascularized, and osteoprogenitor cells will form new bone on this matrix. This will be followed by the formation of bone marrow and marrow elements.³

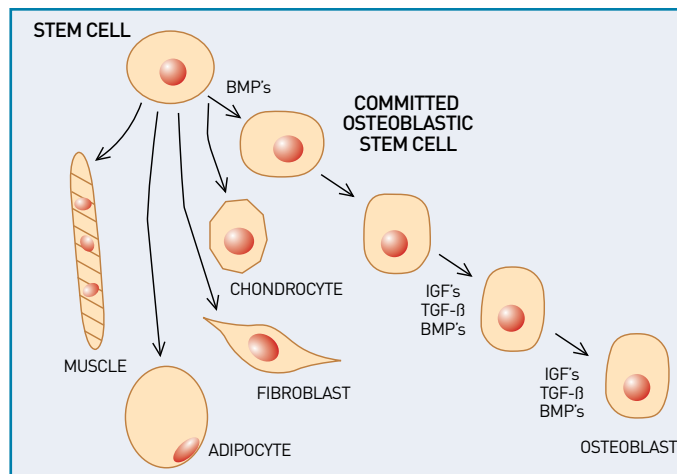


Figure 2: Schematic of Stem Cell Differentiation. Schematic showing differentiation of a stem cell into an active osteoblast, and demonstrating the influence of BMP-2.¹

What Constitutes Good DBM

MTF has developed and validated a demineralization procedure for processing bone into DBM. This procedure provides safe, high-quality allograft DBM and was developed through rigorous testing to ensure that the osteoinductive potential of the tissue was not compromised.

Many factors contribute to the high quality of MTF DBM, including:

- Quality tissue
- Careful processing
- A good carrier
- Quality control
- Scientific evidence

Quality Tissue

MTF's quality and safety standards consistently meet or exceed the requirements of the American Association of Tissue Banks (AATB) as well as the guidelines for screening and testing of tissue donors set forth by the Food and Drug Administration (FDA). The AATB and FDA set only minimal guidelines to ensure safety of tissue. Potential MTF donors must pass through an extensive quality assurance process.

Screening begins at the site of recovery with a comprehensive medical and social history that includes the cause of death. Tissue and blood samples are tested for infectious diseases, including hepatitis, HIV and syphilis. A team of medical/technical specialists from the infectious disease and tissue banking fields evaluates all information, including test results, before the donor is released for processing. **Figure 3** lists those areas in which MTF voluntarily defers donors for safety or quality reasons even when not required by the FDA or AATB.

SCREENING CRITERIA	MTF	AATB	FDA
Hepatitis B virus	X	X	X
Hepatitis C virus	X	X	X
HIV 1/2	X	X	X
Malaria	X	X	X
Sepsis	X	X	X
Syphilis	X	X	X
Transmissible spongiform encephalopathy (TSE)	X	X	X
Vaccinia	X	X	X
West Nile Virus (WNV)	X	X	X
Clinically significant metabolic bone disease	X	X	
Leprosy (Hansen's disease)	X	X	
Polyarteritis nodosa	X	X	
Rabies	X	X	
Rheumatoid arthritis	X	X	
Sarcoidosis	X	X	
Systemic lupus erythematosus	X	X	
Systemic mycosis	X	X	
Tuberculosis	X	X	
Active genital herpes	X		
Ankylosing spondylitis	X		
Antiphospholipid syndrome	X		
Autoimmune hemolytic anemia	X		
Autoimmune lymphoproliferative syndrome	X		
Autoimmune thrombocytopenic purpura	X		
Autoimmune vasculitis	X		
Cancer	X		
Chagas disease	X		
Clinically active Epstein Barr virus (mononucleosis)	X		
Clinically active gonorrhoea	X		
Clostridium difficile infection	X		
Cold agglutinin disease	X		
Encephalitis	X		
Endocarditis	X		
Guillain-Barre syndrome	X		
Illicit drug use	X		
Meningitis	X		
Methicilin resistant staphylococcus aureus (MRSA)	X		
Mixed connective tissue disease	X		
Multiple sclerosis	X		
Myasthenia gravis	X		
Peritonitis	X		
Poliomyelitis	X		
Pyelonephritis	X		
Reactive arthritis (Reiter's syndrome)	X		
Rheumatic fever	X		
Vancomycin resistant enterococcus (VRE)	X		
Varicella zoster	X		
Wegener's granulomatosis	X		
Any acute infectious/septic illness	X		

Figure 3: Tissue bank screening criteria. Note: MTF voluntarily exceeds both AATB and FDA screening criteria.

Careful Processing

To maintain biological integrity, MTF processes all tissue using aseptic techniques in ISO Class 4 (certified) clean rooms. MTF's use of these clean rooms is designed to prevent any environmental contamination of the tissue and thus eliminates the need for terminal sterilization by high-dose gamma radiation, which has been shown to compromise the biological and biomechanical integrity of allograft tissue (Figure 4).^{4,5}

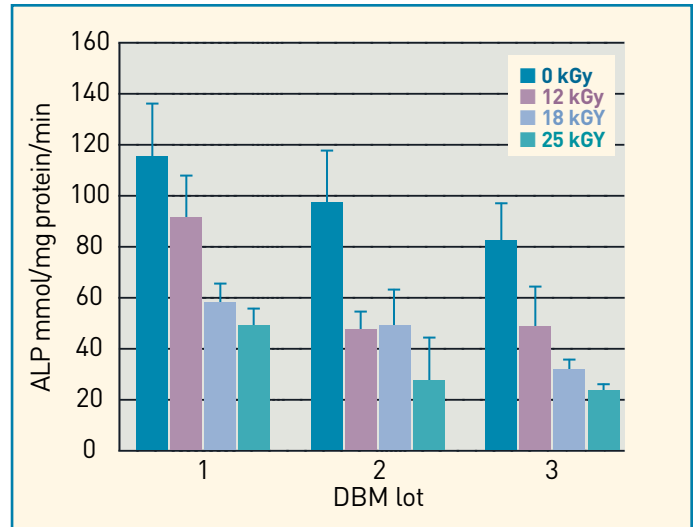


Figure 4: Gamma radiation decreases osteoinductive potential of DBM powder in a dose-dependent manner, as measured using an in vitro alkaline phosphatase (ALP) assay.⁵

For example, in an experimental study where DBX[®] Putty was exposed to a terminal gamma radiation dose of 17.2 kGy, the osteoinductive potential was reduced by approximately 50% (Figure 5).⁶

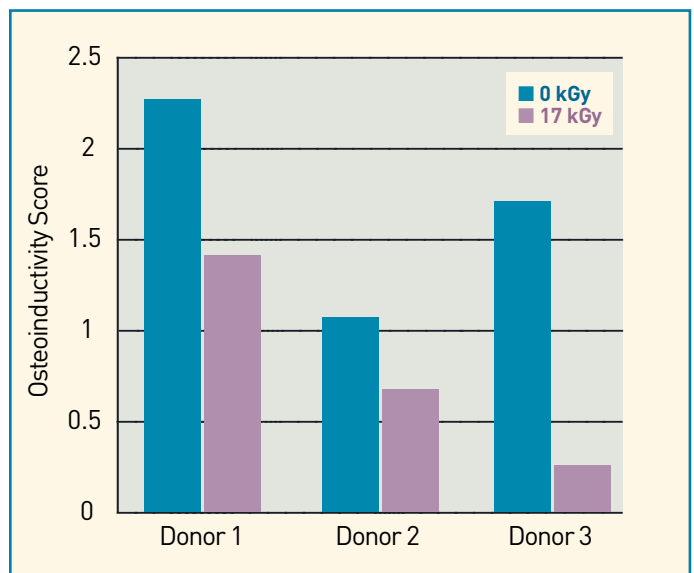


Figure 5: Gamma radiation decreases the osteoinductive potential of DBX Putty using the in vivo athymic mouse assay.⁶

A Good Carrier

DBM in its native form is a fine powder and as such is difficult to deliver to an operative site. DBM formulations should be designed to resist movement under irrigation while maintaining the physical integrity of the tissue form. Also, ideal DBM formulations should be provided ready

for use and not require any mixing or thawing prior to use. Therefore, MTF adds a carrier material to the demineralized bone to improve cohesion and provide an implant with good handling characteristics.

The carrier used in DBX is sodium hyaluronate which has been proven to be extremely safe. MTF uses high-quality, medical grade sodium hyaluronate, ISO 13485 certified, produced through fermentation processes using Good Manufacturing Practice (GMP) guidelines. It is designed to be isotonic and non-hemolytic. The sodium hyaluronate used in DBX tissue is not of animal origin.

Sodium hyaluronate was chosen because it is a polysaccharide formed by plasma membrane proteins. It is naturally occurring in the human body in the joints, eyes, extracellular matrix of skin and musculoskeletal tissue. Sodium hyaluronate plays an essential role in cell proliferation, migration and adhesion and has been correlated to angiogenesis.^{7,8} It also confers positional stability of the tissue.⁹

Formulations

DBX is demineralized bone matrix that has osteoinductive potential and is osteoconductive. It is composed of demineralized bone from human donors in a biocompatible carrier. The various DBX forms have been designed to meet surgical needs while maximizing the amount of bone delivered to the surgical site.

The demineralized bone powder is produced by first milling cortical bone to the appropriate size (212 – 850µm), followed by removal of the minerals from the cortical bone via an acid extraction procedure. The demineralized milled cortical bone is then rinsed and buffered to ensure neutral pH. Lastly, the demineralized bone is lyophilized (residual moisture content < 6%) to ensure stability during staging prior to mixing with appropriate carriers.

DBX Demineralized Bone Matrix is non-hemolytic, ensuring compatibility with the surrounding autogenous blood cells. The DBX formulations have been specifically designed to model the pH of human blood.

DBX Paste

DBX Paste provides a flowable consistency of granulated cortical bone in sodium hyaluronate. The bone content of DBX Paste is 26% bone (weight/weight).

DBX Putty

DBX Putty provides a moldable consistency of granulated cortical bone in sodium hyaluronate. The bone content of DBX Putty is 31% (weight/weight).

DBX Mix

DBX Mix provides a morselized cortical-cancellous bone texture in sodium hyaluronate, which eliminates the need to combine bone chips with a DBM. The bone content of DBX Mix is 35% (weight/weight).

DBX Pre-Formed Shapes

Certain carriers allow MTF to pre-shape the DBM tissue form into predetermined sizes and shapes. For example, DBX Strip provides a cohesive and flexible preformed DBM combined with sodium hyaluronate and gelatin. The bone content of DBX Strip is 45% (weight/weight).

DBX Strip differs slightly from other DBX forms since, in addition to sodium hyaluronate, its carrier includes porcine gelatin to give it unique handling characteristics. The combination of demineralized bone, gelatin, and sodium hyaluronate results in a pre-formed strip formulation.

Quality Control

Every lot of DBX undergoes strict release criteria testing. Sterility is tested per USP <71>. Each lot is also tested via an *in vitro* or *in vivo* assay for osteoinductive potential.

DBM Processing

MTF's demineralized bone matrices are produced from both cancellous and cortical bone which are subjected to controlled cleaning processes (including hydrogen peroxide and ethanol), followed by demineralization with hydrochloric acid. MTF's aseptic processes have been designed to preserve the biological integrity of the tissue. Each donor lot of DBM is verified for osteoinductive potential prior to distribution.

Osteoinductivity Testing

Osteoinductive materials have the ability to induce new bone formation even when implanted into non-bony tissue such as muscle. When bone is formed in this manner, the material is said to have osteoinductive potential. Osteoinductive potential can be measured using either *in vivo* or *in vitro* test methods.

MTF utilizes three methods to measure DBM osteoinductive potential prior to distribution: 1) athymic mouse muscle pouch assay, 2) alkaline phosphatase assay, or 3) BMP-2 content assay.

Athymic Mouse Assay

The athymic mouse assay for *in vivo* osteoinductive potential is based upon the Urist¹⁰ model and is designed to histologically confirm that DBX tissues have osteoinductive potential prior to distribution. In this model, implantation of demineralized bone in the hamstring muscle pouch of an athymic mouse will result in ectopic bone formation if the bone has osteoinductive potential. Athymic mice are used as they lack a thymus gland, and therefore are unable to mount an immune response against the human tissue. New bone formation is measured histologically after 28 days implantation (minimum) and scored using the scale in Table 1. This score is expressed as percent of new bone formation in the visualized area. **Figure 6** displays a representative histological section used for scoring osteoinductive potential.

Table 1: Osteoinductive Potential Scoring Scale	
OI Score	New Bone Formation
0	No evidence of new bone formation
1	1-25% of the section is covered by new bone
2	26-50% of the section is covered by new bone
3	51-75% of the section is covered by new bone
4	>75% of the section is covered by new bone

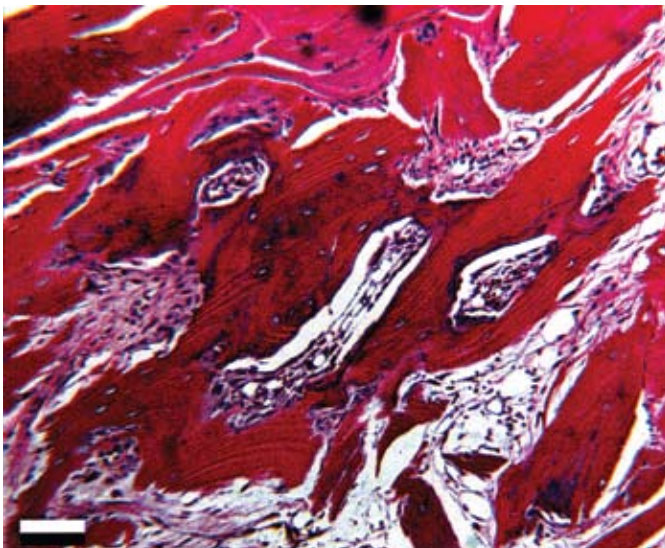


Figure 6: Example of histological section used to grade osteoinductivity in the athymic mouse Bar = 100 micron.

Alkaline Phosphatase Assay

Alkaline phosphatase (ALP) is an enzyme which is expressed by bone growing cells during new bone formation. Demineralized bone will activate these bone growing cells, thus resulting in increased levels of the ALP enzyme. *In vitro* test methods have been developed whereby bone growing cells are exposed to DBM and the resulting level of *in vitro* ALP enzyme is then measured. Correlations between the ALP levels and *in vivo* osteoinductivity scores from the athymic mouse model demonstrate that the ALP assay can be used as another means of measuring the osteoinductive potential of DBM tissue forms.¹¹

BMP-2 Content Assay

In vitro measurement via Enzyme-Linked ImmunoSorbent Assay (ELISA) of BMP-2 levels in DBM can be used as a surrogate measurement for the *in vivo* osteoinductive potential of DBX. Bone induction is a sequential, multi-step cascade which involves various growth factors. Bone morphogenetic proteins (and other intrinsic growth factors) in bone are exposed by the demineralization process. However, BMP-2 has been shown to be the best single predictor of osteoinductive potential based on statistical analysis of the correlation between *in vitro* levels of various growth factors and *in vivo* osteoinductivity.^{12,13}

ELISA is a quick and quantitative screening technique that can be used in place of methods with animal variability or a subjective, semi-quantitative scoring system. Therefore, measurement of BMP-2 level in DBM using ELISA techniques can be used as a surrogate *in vitro* test for evaluating osteoinductive potential of DBM.

There is a strong correlation ($R^2 = 0.72$) between *in vitro* ELISA results on DBM and *in vivo* osteoinductive potential of DBX Putty in the athymic mouse model.

BMP-2 quantified in the final formulation of DBX Putty was found to be of equivalent levels as in the respective DBM powder (from the same donor lots). The good correlation ($R^2 = 0.82$) between BMP-2 levels in DBX Putty and DBM powder (n=18, Figure 7), demonstrates that the addition of sodium hyaluronate to DBM powder does not alter intrinsic growth factor levels.¹⁴

Additionally, the strong correlation between BMP-2 levels and *in vivo* osteoinductive potential for DBM in sodium hyaluronate ($R^2 = 0.72$) and DBM in saline ($R^2 = 0.78$) demonstrates that there is no significant change in BMP-2 levels or *in vivo* osteoinductive potential of DBM after mixing DBM and the sodium hyaluronate carrier into the final DBX tissue form.¹⁴ Taken in its entirety, this data supports the validity of the BMP-2 ELISA assay as a batch release test method for OI.

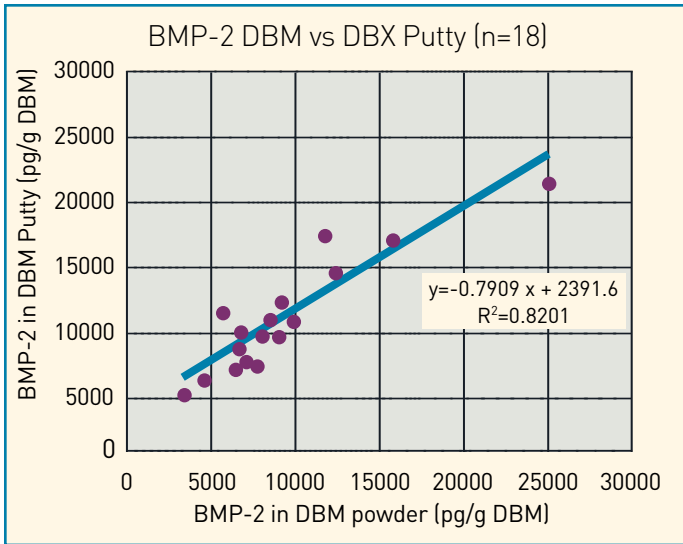


Figure 7: Correlation of BMP-2 levels in DBM powder and DBX Putty ($R^2 = 0.82$) from the same lots ($n=18$ donor lots).

In summary, each lot of DBX is validated using one or more of the following: the Urist athymic mouse model, the *in vitro* BMP-2 test, or the *in vitro* ALP assay.

Scientific Evidence

Indications

DBX is intended for use in voids or gaps that are not intrinsic to the stability of the bony structure. It is intended for treatment of surgically or traumatically created osseous defects of the posterolateral spine, pelvis, and extremities.

Additionally, DBX Putty and Paste are intended for the augmentation of deficient maxillary and mandibular alveolar ridges and the treatment of oral/maxillofacial and dental intra-osseous defects.

DBX is for single patient use only.

Clinical Studies

Numerous studies have demonstrated the clinical effectiveness of MTF's DBX Demineralized Bone Matrix Tissue forms.

Study 1: DBX Putty plus autograft has been shown to be as effective as autograft alone.¹⁵

A mix of DBX Putty and autograft was shown to be as effective as autograft alone in a multicenter, randomized, controlled clinical study of patients undergoing posterolateral spine fusion for the treatment for degenerative disc disease (DDD).

- Equivalent fusion rates were achieved between the two groups at 24 months.
 - DBX and autograft = 100% at 24 mo.
 - Autograft alone = 96% at 24 mo.
- Both groups exhibited significant decrease in VAS pain scores at 6 months relative to baseline that is sustained through 24 months.
- The authors conclude that DBX Putty is at least as effective as autograft alone when used as a bone graft extender in the treatment of DDD via posterolateral spine fusion.

Study 2: DBX Putty plus local bone and bone marrow aspirate has been shown to be as effective as autograft alone.¹⁶

Results indicate that DBX is as effective as autograft alone when used as a bone graft extender when combined with bone marrow aspirate from the iliac crest and local bone in the treatment of degenerative disc disease.

- Equivalent fusion rates were achieved at 12-months post-surgery:
 - Iliac crest autograft = 97.6%
 - DBX Putty + local bone + iliac crest bone marrow aspirate = 93.9%
- At 12 months, postoperative VAS pain scores improved 41.7% for the autograft control group and 53.1% in the DBX Putty + local bone and bone marrow aspirate group.
- Oswestry disability index rates improved 51.5% for the autograft control group and 53.5% in the DBX Putty + local bone and bone marrow aspirate group.
- Representative 12-month radiographs are shown in **Figure 8**.

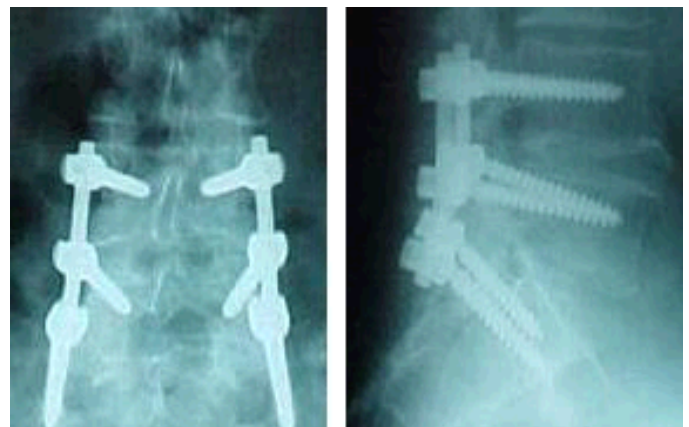


Figure 8: A-P and Lateral radiographs at 12-months demonstrated DBX Putty + local bone and bone marrow aspirate is as effective as autograft alone.¹⁶

Study 3: DBX Putty effectively treats periodontal intraosseous defects.¹⁷

Intraosseous periodontal defects in systemically healthy patients were treated with either DBX Putty or with demineralized freeze-dried bone allograft (DFDBA). Only sites with defects ≥ 3 mm in depth were considered. A statistical analysis was conducted at 6 months postoperative. Both groups of patients yielded significant improvements in percent bone fill, with $37\% \pm 18.5\%$ bone fill achieved in patients treated with DFDBA and $50\% \pm 25\%$ in cases of patients treated with DBX Putty.

Summary

At MTF, we are driven by our strong commitment to safety. It is because of this commitment that we continue to maintain an exemplary safety record, providing our customers with allograft tissue from a source they can trust. As part of our philosophy, we believe that providing safe tissue is not enough—we also must not compromise the biological properties of the demineralized bone. MTF's processes for bone demineralization and creation of DBM tissue forms have been designed and validated to ensure the safety of the allografts without adversely affecting their biological performance. The processes and studies described here demonstrate that MTF's demineralization processes and use of sodium hyaluronate in DBX have no harmful effects on the natural biological properties or *in vivo* performance of the allografts.

The demineralization process and additionally the use of sodium hyaluronate in DBX by MTF yields a safe, effective allograft designed and validated to maintain the natural healing function of allograft bone.

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