GRAFTON™ DBF AND GRAFTON™ DBF INJECT:
PRECLINICAL EVIDENCE OF BONE FORMATION
DEMINERALIZED BONE MATRIX (DBM)

Deminerlized bone matrix (DBM) has been widely used as a bone graft extender in Spine, Trauma, Orthopedics and Dental surgeries.\(^1\) The ability of DBMs to form de novo bone comes from its osteoinductive and osteoconductive properties. When bone tissue is demineralized using gentle processes, such as aseptic techniques and sterilized without irradiation, the exposed Bone Morphogenetic Proteins (BMPs) are well preserved. These preserved BMPs are active and impart osteoinductivity to DBM.\(^2\) And when the DBM is milled into fibers as opposed to particulate, the interconnected fibers impart osteoconductivity to DBM resulting in higher bone formation capability.\(^3\)

GRAFTON™ DBF

Grafton™ DBF is a deminerlized bone allograft consisting of deminerlized cortical fibers. The graft does not have any carriers and must be hydrated with blood/BMA/saline prior to its use in spine, orthopedic or reconstructive bone grafting procedures. This product can also be used in bone grafting procedures in combination with autologous bone or other forms of allograft bone, or alone as a bone graft. The Grafton™ DBF employs Medtronic’s proprietary D-MIN™ process and fiber technology that has been perfected over 25+ years of experience. In addition, the processing is performed using aseptic techniques that results in a highly osteoinductive\(^2,4\) and osteoconductive product.

GRAFTON™ DBF INJECT

Grafton™ DBF Inject is an injectable version of Grafton™ DBF packaged within a delivery syringe that is well adapted for delivering the hydrated graft into deep surgical sites. The graft delivery syringe cannula can easily access the interbody space, making Grafton™ DBF Inject well suited for filling both into and around approved cages placed during both Open and MIS TLIF, PLIF and OLIF procedures. The product consists of deminerlized bone fibers pre-loaded inside a delivery syringe with a long cannula. For ease of use, the patent-pending fibers have been carefully manufactured to specific length and optimal shapes which can be quickly hydrated inside the syringe with blood/BMA/saline. The integrated cannula-clearing plunger and stylet ensures all the DBM can be seamlessly delivered to the site with minimal steps. Grafton™ DBF Inject offers a streamlined graft delivery method with procedural adaptability and includes:

- All-in-one Hydration & Delivery system
  - Cannulas enable MIS delivery
  - Ease of mix and use
  - Deliver directly into bone voids

- Flowable Fiber Technology
  - Injectable fibers
  - Enhanced Osteoconductivity\(^3,4\)
  - Cohesive compared to particulates\(^3,4\)

- 100% DBM
  - High OI based on preclinical testing\(^2,4\)
  - Hydratable with CBMA/BMA & Sterile water/saline
  - Aseptic processing with less harsh chemicals and no terminal irradiation

Potential Application Shown. If used in an interbody cage, DBF must be hydrated with BMA.
OSTEINDUCTIVITY

Osteoinduction is the capacity of extracellular signals (in the DBM) to attract, proliferate, and differentiate mesenchymal stem cells (MSCs) or immature bone cells into osteoblasts and form healthy bone tissue. The most direct and accurate way of measuring osteoinductivity is to evaluate the bone forming ability of a graft in a rat’s muscle pouch. Since the rat’s muscle pouch is devoid of any bone, only osteoinductive materials can induce bone formation. Animal studies should not be interpreted to predict clinical performance.

Osteoinductivity by Product

- Grafton™ DBF is osteoinductive.
- The cortical cancellous chips had a minimal osteoinductivity of 0.5.
- All the competitors that we tested scored lower than Grafton family DBMs.
2-LEVEL RAT POSTEROLATERAL SPINE FUSION\textsuperscript{2,4}

The rat two-level posterolateral spine fusion model is a recognized model designed to determine the fusion capabilities of bone grafts in a challenging pre-clinical environment. The results of fusion were analyzed by X-ray and confirmed by manual palpation. In addition, histology was conducted to determine the extent of new bone formation and incorporation of the bone graft. Assessment of space maintenance was performed to confirm the graft material retained its volume during remodeling. A control using cortical cancellous chips was included for comparison to Grafton™ DBF. Animal studies are not necessarily indicative of clinical performance.

Assessment of Fusion by X-ray and Manual Palpation

X-ray assessment and Manual palpation of the implant site allows assessment of bone formation and stability of the spinal segment immediately after the rat has been sacrificed. These data verify the extent of mineralization and lack of motion, and can detect any pseudarthrosis that may be present.

Radiographic Analysis

- Immediately post-op, Grafton™ DBF is invisible on X-ray as it is completely radiolucent due to being fully demineralized.
- At 8 weeks, the radiograph shows high radiodensity across the treated segments indicating fusion and evidence of robust new bone formation with trabecular structure and cortical shell.
- 100% of rats implanted with Grafton™ DBF showed fusion. However, residual mineralized implant material impeded radiographic fusion assessment in rats implanted with cortical cancellous chips.

Manual Palpation

- 100% of rats implanted with Grafton™ DBF showed fusion with manual palpation compared to 0% for those implanted with cortical cancellous chips.

Space Maintenance Assessment

Maintaining the graft volume during remodeling is critical to the effectiveness of the bone graft as it ensures strong bridging, good end plate contacts, and the entire void is filled.

- On a scale of 0 to 4, Grafton™ DBF scored 4 indicating excellent maintenance of volume while cortical cancellous chips scored 2.4 indicating construct collapse.
2-LEVEL RAT POSTEROLATERAL SPINE FUSION

Histological Assessment

- Histology verified *de novo* bone developed throughout the original implant area. Cortical shelling and trabecular structure indicative of bony maturation were observed.
- At week 8, new bone already accounted for 77% of the implant space for rats implanted with Grafton™ DBF compared to only 3% for rats implanted with Cortical Cancellous Chips.

- Very little residual Grafton™ DBF material and fibrous tissue were observed indicating high bioactivity and extensive remodeling of the product.

**Grafton™ DBF/Grafton™ DBF Inject**

**Cortical Cancellous Chips**

![Histology images](image)

**Figure 2.** 1X and 5X magnified histology images (H&E stain) of sagittal sections prepared from rat spine explants of rats implanted with Grafton™ DBF and cortical-cancellous chips shown in dorsal/ventral orientation.

- Red arrows indicate residual implant material.
- Green arrows indicate new bone.
- Yellow arrows indicate bone marrow development.
- Black arrows indicate fibrous tissue.
MATERIALS AND METHODS

Grafton™ DBF is a bone graft comprised entirely of human demineralized bone tissue fibers and is regulated as a tissue product (HCT/P) under PHS Act Section 3616. Grafton™ DBF Inject is Grafton™ DBF Fibers pre-loaded in a syringe for easy delivery in bone grafting applications.

OSTEOINDUCTIVITY TESTING

The athymic rat muscle pouch assay is used to evaluate the osteoinductive potential of human bone graft products using immune compromised athymic rats to avoid problems associated with cross-species compatibilities that would normally be encountered when implanting human tissue into an animal. Products were implanted into intermuscular sites in the rats’ hind limbs. After twenty-eight days, new bone formation was assessed using a previously validated model and a 0-4 scoring system described by Edwards et al. and shown in table 1.

Table 1. Scoring Quartiles Definitions

<table>
<thead>
<tr>
<th>Representative Images</th>
<th>Score</th>
<th>% of Explant Involved in New Bone Formation</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>1-25%</td>
</tr>
<tr>
<td></td>
<td>2</td>
<td>26-50%</td>
</tr>
<tr>
<td></td>
<td>3</td>
<td>51-75%</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>76-100%</td>
</tr>
</tbody>
</table>

Explant contains bone forming elements such as calcified bone, osteoid, osteoblasts, fatty and cellular bone marrow, cartilage, fibrocartilage and chondrocytes.

RAT POSTEROLATERAL SPINE FUSION MODEL

Products were implanted across the decorticated L3-L5 transverse processes of athymic rats (n=8 rats). After eight weeks, the animals were sacrificed, and the operated segment of their spinal columns were removed. Manual palpation of the operated segment was conducted. If no motion was detected at the operated level as compared to non-operated segments, then the site was considered fused. In addition, postoperative and eight-week radiographs were reviewed. Sites were considered radiographically fused if continuous bridging bone could be clearly seen between the transverse processes. If excessive radiopaque residual implant material was present, the radiographic assessment was scored indeterminate. Spines were placed in 10% formalin and processed for decalcified histopathology with H&E staining. The amount of new bone formation (consisting of woven bone, lamellar bone, cartilage, and marrow elements) was determined by a blinded pathologist. In addition, the ability of the graft material to maintain the implant space was measured. A space maintenance score of 0-4 was assigned, with a score of ‘0’ indicating complete collapse of implant, ‘1’ indicating 1-24% maintenance of original implant volume, ‘2’ indicating 25-49% maintenance of original implant volume, ‘3’ indicating 50-74% maintenance of original implant volume, and ‘4’ indicating 75-100% of the original implant was maintained.
KEY TAKEAWAYS

- **Results at a glance**

<table>
<thead>
<tr>
<th></th>
<th>Grafton™ DBF</th>
<th>Cortical Cancellous Chips</th>
</tr>
</thead>
<tbody>
<tr>
<td>Osteoinductivity Score&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>3.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Fusion by Manual Palpation</td>
<td>100%</td>
<td>0%</td>
</tr>
<tr>
<td>Radiographic Fusion</td>
<td>100%</td>
<td>indeterminate</td>
</tr>
<tr>
<td>New Bone Formation at 8 weeks</td>
<td>77%</td>
<td>3%</td>
</tr>
<tr>
<td>Space Maintenance Index</td>
<td>4.0</td>
<td>2.4</td>
</tr>
</tbody>
</table>

- Proprietary D-MIN™ processing, aseptic techniques without terminal irradiation, lack of carrier – all ensures Grafton™ DBF has the highest osteoinductivity among all the competitors tested.<sup>2,4</sup>

- The fiber technology imparts osteoconductivity and this helps rats implanted with Grafton™ DBF achieve 100% fusion both radiographically and by manual palpation. In contrast, rats implanted with cortical cancellous chips showed 0% fusion by manual palpation.

- Grafton™ DBF retains the volume at the implant site ensuring good endplate contact when injected in interbody space.

- The long cannula makes Grafton™ DBF ideally suited for MIS applications and the pre-loaded syringe allows for easy hydration with BMA /blood /saline.

- Radiolucency of Grafton™ DBF allows for clear visualization and monitor progress of bone formation.

REFERENCES


2. Data on File.


4. Animal studies are not necessarily indicative of clinical performance


**Grafton™ DBF/Grafton™ DBF Inject**

**INDICATIONS FOR USE**

Grafton™ DBF can be used in orthopedic or reconstructive bone grafting procedures. The product can also be used in bone grafting procedures in combination with autologous bone or other forms of allograft bone, or alone as a bone graft.

**CONTRAINDICATIONS**

The presence of infection at the implantation site is a contraindication for the use of this allograft.

**CAUTION**

This allograft may contain trace amounts of antibiotics (gentamicin), antiseptic (povidone-iodine) and alcohol solutions. Caution should be exercised if the patient is allergic to these antibiotics or chemicals.

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**Syringe Accessory Kit**

**INTENDED USE**

The Graft Preparation and Delivery Device is intended for the delivery of hydrated allograft, autograft or synthetic bone graft materials to an orthopaedic surgical site. In addition, it is designed to facilitate the premixing of bone graft materials with fluids such as I.V. fluids, blood, plasma concentrate, platelet rich plasma, bone marrow or other specified blood components as deemed necessary by the clinical use requirements.

**WARNINGS/PRECAUTIONS**

There are no specific warnings, precautions, or adverse effects associated with the use of this device.