

# PROPORTIONAL OSTEOINDUCTION OF DEMINERALIZED BONE MATRIX GRAFT MATERIALS

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## Introduction

Autogenous bone remains the established standard bone grafting material because it contains the three essential elements for bone regeneration and maintenance. Bone morphogenetic proteins (BMPs) provide an osteoinductive trigger, autogenous osteogenic precursor cells give the potential for osteogenesis, and the bone structural form offers a means of osteoconduction.<sup>1</sup> However, limitations of autograft include significant donor-site morbidity, extended operating room time, as well as limited quantity and quality of bone available for harvest. In an attempt to create an optimal bone graft substitute in the absence of suitable quantities of autogenous bone, orthopaedic surgeons have begun to utilize composite graft formulations that provide two or more of the required elements for bone regeneration. Demineralized bone matrix (DBM) is widely used as the osteoinductive element within these composite grafts.

## DBM as an Osteoinductive Graft

DBM is derived from human cortical bone and is prepared by removing the minerals, allowing the organic and protein constituents to remain. It is well documented that DBM contains an array of naturally present growth factors, including a full complex of BMPs.<sup>2,3</sup> However, the DBM content in commercially available preparations

is highly variable. This paper documents the different DBM levels provided in commercially prepared formulations, and introduces the concept of *Proportional Osteoinduction*, so that surgeons can make an informed choice when selecting an osteoinductive element during assembly of a composite graft construct.

## Highly Variable Levels of DBM

The commercially prepared DBM-based osteoinductive products currently available contain a wide range of DBM content. The levels range from as low as 17% in first generation products, to as high as 100% DBM by weight in second generation implants (Figure 1, Table 1).<sup>4</sup> The non-DBM portion of many of these products is comprised of non-osteoinductive inert carriers, primarily to facilitate handling and graft containment. Only one

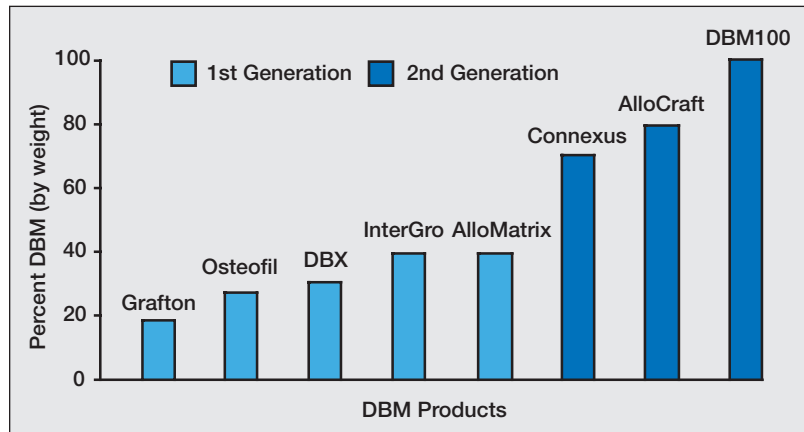


FIGURE 1. Percent DBM content of multiple DBM products.

Brand / Company	Carrier	% DBM
Accell® DBM100 ( <i>IsoTis OrthoBiologics, Inc.</i> )	DBM*	100%
AlloCraft™ DBM ( <i>Stryker Howmedica Osteonics</i> )	Acellular Matrix	80%
Accell Connexus™ ( <i>IsoTis OrthoBiologics, Inc.</i> )	DBM & Reverse Phase Medium	70%
AlloMatrix® Putty ( <i>Wright Medical Technology, Inc.</i> )	Calcium Sulfate	40%
InterGro™ Putty ( <i>Interpore Cross International, Inc.</i> )	Lecithin	40%
DBX® Putty ( <i>Synthes, Inc.</i> )	Sodium Hyaluronate	32%
Osteofil™ Paste ( <i>Regeneration Technologies, Inc.</i> )	Porcine Collagen	24%
Grafton® Putty ( <i>Osteotech, Inc.</i> )	Glycerol	17%

\* No inert carrier is added to the product. Putty consistency is achieved by softening the DBM.

TABLE 1. DBM brands, carrier utilized, and corresponding percentage of DBM (by weight).

commercially available DBM putty uses softened DBM as the carrier. Because the osteoinductive BMPs are contained within the DBM particles, it is intuitive that the greater the amount of DBM a product contains, the greater the bone formation potential. This concept is referred to as *Proportional Osteoinduction*.

### Studies Support Concept of Proportional Osteoinduction

A study published in 2003 in the Journal of Orthopaedic Research studied 20 DBM specimens from different bone banks<sup>5</sup>. An *in vitro* dose response assay was designed to assess alkaline phosphatase (ALP) effect on pluripotent C2C12 myoblasts in DBM lots with varied amounts of active DBM. To standardize the assay, inactive DBM was manufactured, and then mixed in varying amounts into five lots of active DBM from the same bone bank. A proportional osteoinductive response was observed (Figure 2).

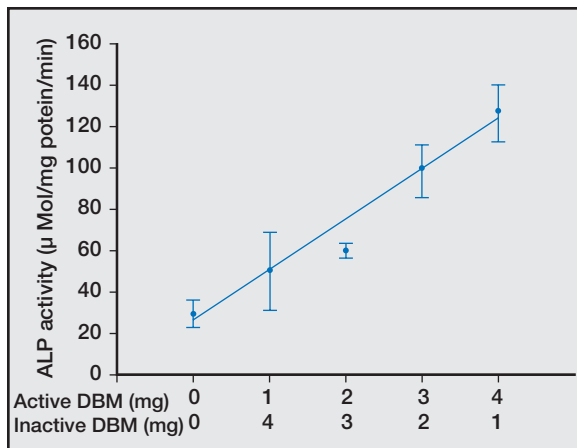


FIGURE 2. Dose response of ALP induction of active DBM *in vitro*.

A similar osteogenic dose response analysis of active DBM was performed in the athymic nude rat model. At 28 days post intramuscular implantation, the mineral density of explants was documented radiographically. In addition, ALP analysis was performed on the explants. Consistent with the results of the *in vitro* C2C12 cell response, a proportional osteoinductive response was observed radiographically; 100% active DBM produced a denser image of calcified deposits than the 100% inactive DBM. And not surprisingly, the ALP explant analysis showed low levels of induction in the implants with low relative percentages of active DBM, while the 100% DBM revealed high ALP activity (Figure 3).

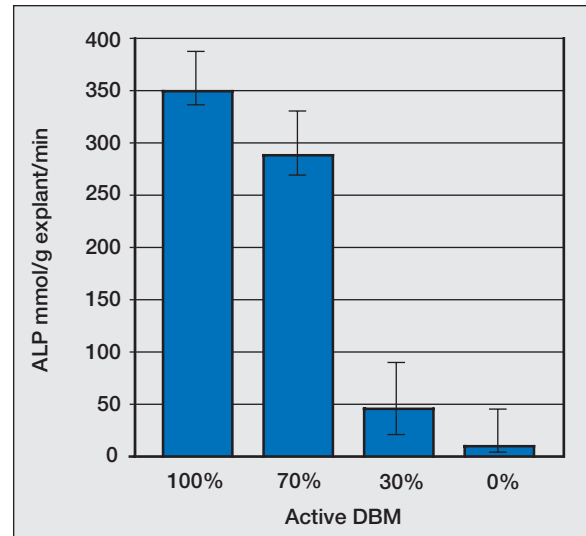


FIGURE 3. ALP of Explants

Additional research was conducted by Jung Yoo, MD, *et al.* (Case Western Reserve University) and supported by Musculoskeletal Transplant Foundation, to evaluate spinal arthrodesis in skeletally mature rabbits using DBM and bone marrow aspirate (BMA).<sup>6</sup> Two groups were implanted with varying amounts of DBM/Hyaluronan (HA) + BMA; one group with DBM/HA alone; and two control groups were used, one with autograft only and the other BMA only. Of the five groups studied, those with the most DBM (by weight), whether or not BMA was used, exhibited the highest bone volumes within the fusion mass, leading the authors to suggest that “the bone volume of the fusion mass appears to be dependent on the volume of bone or DBM/HA delivered to the surgical site” (Figure 4).

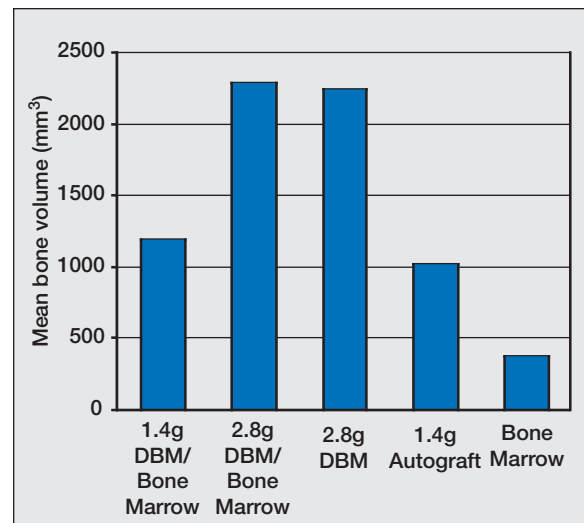


FIGURE 4. Bone volume of the fusion mass.<sup>6</sup>

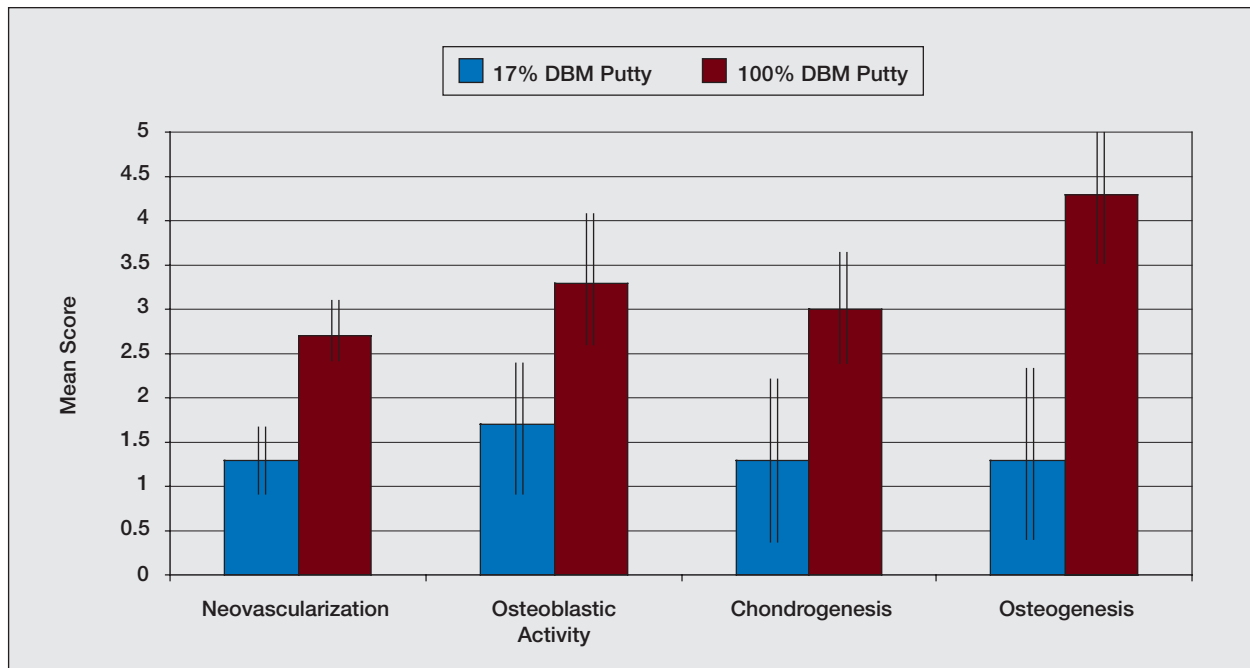


FIGURE 5. Mean graded scores of tissue responses to each implant at 28 days (n=3).<sup>7</sup>

Another study compared the osteoinductivity of two human DBM products in an established athymic mouse model.<sup>7</sup> One of the implant materials contained 17% DBM by weight and the other contained 100% DBM by weight. The 100% DBM material utilized a proprietary DBM softening process, allowing a portion of the DBM to serve as an osteoinductive carrier. The materials were implanted in an intramuscular pouch and nodules of ectopic bone were sectioned at 14 and 28 days post-implantation for quantitative histological analysis of bone formation. Enhanced bone formation was observed with the 100% DBM material as compared to the 17% DBM material at both time points (Figure 5). Evidence of good to excellent osteoinduction was noted for the 100% material, while the 17% material displayed lesser osteoinductive characteristics.

Finally, a study performed at the Hospital for Special Surgery in New York City assessed the osteoinductive potential of human DBM in an established athymic rat model.<sup>8</sup> Varying amounts of a DBM product (100 mg, 200 mg, and 300 mg) were implanted in an intramuscular pouch. The entire nodules of ectopic bone were excised at 28 days and analyzed by micro-computed tomography. Bone volume and density were calculated for the entire specimen. Results showed a direct correlation between the volume of DBM implanted and the volume of mineralized tissue (woven bone) formed (Figure 6). In other words, increasing the quantity of DBM resulted in increased osteoinductive activity (new bone formation).

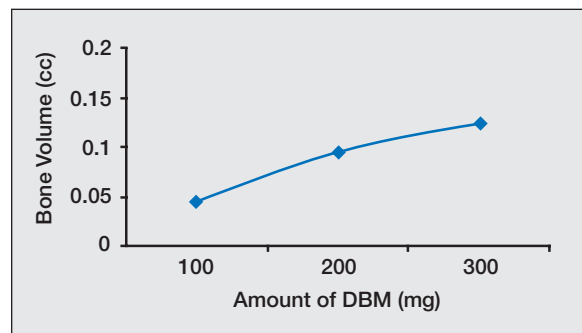


FIGURE 6. Volume of new bone formation per amount of DBM.

## Conclusion

The results of four recent studies support the concept of *Proportional Osteoinduction*: the higher the DBM concentration, the greater bone forming potential per unit volume of a given product. Furthermore, the Hospital for Special Surgery study confirms that utilizing more DBM results in greater bone volume at the graft site. Thus, to provide the strongest osteoinductive signal at a graft site with a finite volume, consideration should be given to choosing a product with the highest DBM concentration and proven osteoinductive potential.

## References

1. Lane JM, Yasko AW, Tomin E, et al. Bone marrow and recombinant human bone morphogenetic protein-2 in osseous repair. *Clin Orthop* 1999;(361):216-27.
2. Shigeyama Y, D'Errico JA, Stone R, Somerman MJ. Commercially prepared allograft material has biological activity *in vitro*. *J Periodontol* 1995;(66):478-487.
3. Reddi AH. Initiation of fracture repair by bone morphogenetic proteins. *Clin Orthop* 1998;(355S):S66-72.
4. Data on file. IsoTis OrthoBiologics.
5. Han B, Tang B, Nimni ME. Quantitative and sensitive *in vitro* assay for osteoinductive activity of demineralized bone matrix. *J Ortho Res* 2003;648:654
6. Yoo J, Birkedal J, Chang S, et al. Spinal arthrodesis using a demineralized bone/hyaluronan matrix and bone marrow. Proceedings of the NASS 18th Annual Meeting. *Spine J* 2003;3:143S.
7. Edwards JL. *In vivo* osteoinductivity of two human DBM materials: comparative histopathologic evaluation at 14 and 28 days. IsoTis OrthoBiologics; April 2003:AW-0166.0.
8. Atti E, Abjornson C, Diegmann M, et al. High resolution X-ray computed tomography as a technique to study osteoinductivity of demineralized bone matrix. Proceedings of the NASS 18<sup>th</sup> Annual Meeting. *Spine J* 2003;3:120S.