Comparision of Osteoinductivity of DBM Powder to DBM Fiber – StaGraft® Fiber Test Report Summary

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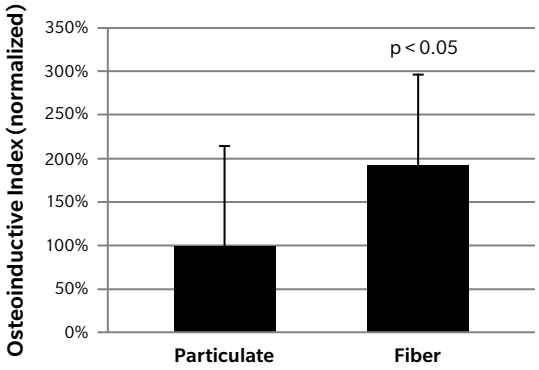
Traditionally, demineralized bone matrix (DBM) is processed from cortical bone. The cortical shafts of long bones are harvested, separated from the cancellous portion, and cleaned of soft tissue and marrow components. The cortical bone is ground into particulate, demineralized to a residual calcium content of less than 8%, and lyophilized. The resultant particle size distribution is typically around 125 – 850 microns.[1]

Zimmer Biomet has developed a method of processing cortical bone to make a new form of DBM. The cortical bone is machined in a precise manner, resulting in needle-like "fibers", or "ribbons". The machined cortical bone fibers can then be demineralized and lyophilized for storage, similar to ground particulate. The resulting human tissue allograft is easily rehydrated with fluids, and provides a moldable, putty-like graft that consists of 100% DBM without any added synthetic carrier.

Human cortical bone is known to contain a wide variety of growth factors, including BMP-2, -4, and -7. When cortical bone is demineralized, these growth factors are more readily bioavailable.[2] It is because of the presence of these active growth factors that DBM is said to be osteoinductive. Overexposure to the acid used to demineralize bone, as well as exposure to some of the caustic chemicals used to reduce bioburden and inactivate viruses may have a detrimental effect on the activity of these growth factors, and therefore the osteoinductivity level of the final DBM.[3,**] For this reason, it has become common practice to measure the osteoinductivity level of each lot of DBM processed. One commonly accepted assay used to quantify the osteoinductive potential of DBM is known as the C2C12 assay. This assay measures the alkaline phosphatase production of a myoblast cell line (C2C12) in the presence of DBM. When compared to a positive and negative control, an Osteoinductive Index (OI) is calculated. Results from this assay have been correlated with results from implantation of DBM into athymic rat muscle.[4]

The osteoinductive potential of DBM powder and DBM fiber as measured in the C2C12 assay were compared. All testing was performed by the Tissue Engineering lab of Dr. Bo Han at the University of Southern California, using the same test method.

As a whole, the Fiber DBM formulations demonstrated OI values approximately twice as high in the C2C12 in vitro assay as compared to the particulate formulations. It is theorized that this difference is due primarily to a difference in particle size and demineralization protocol between the two groups. DBM particulate has a wide range of particle size (typically 125-850 microns). In order to demineralize to a level of <8% residual calcium, the DBM particles must remain in acidic solution for a period of time long enough to demineralize the largest particle, which means the smaller particles are "over-demineralized". This could lead to a decrease in the OI scores. The Fiber DBM is machined to a more uniform thickness. This allows all fibers to demineralize at approximately the same rate, minimizing the time in acid, and therefore preserving a larger portion of the growth factors.



References

1. Zimmer Biomet Internal Specification C1-2063
2. Pietrzak, William S. *et al.* Assay of Bone Morphogenetic Protein -2, -4, and -7 in Human Demineralized Bone Matrix. *J Craniofac Surgery*;17(1):84-90, 2006.
3. Pietrzak, William S. *et al.* BMP Depletion Occurs During Prolonged Acid Demineralization of Bone: Characterization and Implications for Graft Preparation. *Cell Tissue Bank*. 12(2):81-8, 2011.
4. Han, B., *et al.* Quantitative and Sensitive in vitro Assay for Osteoinductive Activity of Demineralized Bone Matrix. *J Orthop Res.*21(4):648-54, 2003.

*Benchmark testing not indicative of clinical performance.
**Based upon bench testing performed with bovine bone.

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