Osteoinductivity of Isotis Accell® DBM100 in the Athymic Mouse Model

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SUMMARY

The objective of this study was to characterize the osteoinductive properties of a commercially available product containing demineralized bone matrix: Accell DBM 100 (Isotis Orthobiologics). Osteoinductivity (OI), the ability to produce *de novo* heterotopic bone, was assessed histologically (OI ranked on a scale of 0-4) following intramuscular implantation of multiple samples for each test group in an athymic mouse model. Results of this study suggest that:

- **Accell DBM 100 was marginally osteoinductive** in this model; only 27% of the samples were osteoinductive, with an average osteoinduction score of 0.30 ± 0.55.

INTRODUCTION AND BACKGROUND

Demineralized bone matrix (DBM) is used for treating bony defects as a bone void filler. The purpose of this study was to characterize the osteoinductivity of Accell DBM 100, which is a commercially-available product containing DBM from Isotis Orthobiolgics.

When implanted into normal animals, human DBM is xenogeneic, and is expected to provoke an immune response that may compromise the analysis of osteoinduction. To avoid this, the athymic mouse model was used. The athymic mouse lacks a thymus gland and therefore cannot mount a humoral immune response to the human DBM implants. Precedence of the use of an athymic mouse (Nu/Nu) model for studying the osteoinductive potential of demineralized bone allograft was noted in Schwartz *et al.*[1]

Samples of the test groups were implanted bilaterally into the mouse

hamstring muscle. Intramuscular implantation of active DBM is expected to induce cartilage and then bone formation within the implants, a process termed osteoinduction. The hamstring muscle group (biceps femoris muscle) is a large, easily accessible muscle, which is commonly used as an implant site to evaluate heterotopic bone formation. Histological evaluation of the test articles was conducted 28 days after implantation to assess osteoinduction.

METHODS AND MATERIALS

This study utilized one test group: **Isotis Accell DBM 100** (4 lots; *Table 2*). For comparisons, this study references osteoinductivity data on Enhance™ Demineralized Cortical Fibers collected by the same investigator using techniques identical to those described in this study.[2] In some cases, the reference data was obtained contemporaneously with test samples in this study.

Eight samples (weighing 25 mg each) from each lot of material were prepared for implantation. The samples were randomized and implanted bilaterally in the hamstring muscles of athymic nude mice. Animals were sacrificed at 4 weeks post-implantation. Decalcified histology was then performed on the explanted samples; 5 histological slides with 2 sections per slide were prepared for each sample (10 sections total per sample). Slides were stained with hematoxylin and eosin, and samples were evaluated for osteoinductivity. A semi-quantitative scoring system was utilized to assess osteoinduction.

The relative amount of osteoinduction was evaluated semi-quantitatively by the study investigator using the scoring system described below; the observer was blinded to the identification of the implant. Osteoinductive scores were based

on the degree to which new bone, bone cells, osteoid, calcified cartilage remnants, and marrow elements were present. To be consistent with proposed standards in the industry[3], the scoring system in *Table 1* was utilized.

Score	*Criteria*
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

Table 1: *Osteoinductivity Scoring Scale and Criteria*

The overall score for each sample was obtained by averaging the highest 5 scores from the histological slides; scores for each experimental group were determined by pooling the overall scores of the individual samples. The results of semi-quantitative scoring are presented as a mean ± standard deviation.

Images of histological slides from each test group were also captured and stored using a digital camera and computer system (*Image-Pro Plus*™ imaging software).

RESULTS & CONCLUSIONS

Isotis Accell DBM 100 was marginally osteoinductive in this model; only 27% of the samples were osteoinductive, with an average osteoinduction score of 0.30 ± 0.55 *(Tables 2 & 3)*.

Figure 1 shows the representative histological response to Accell DBM 100, with a primarily fibrous tissue/inflammatory response with no new bone formation. This response was similar to that routinely found for the negative control heat-inactivated DBM.[2]

The osteoinductivity scores for Accell DBM100 are significantly lower than the osteoinductivity scores for Enhance™ Demineralized Cortical Fibers In all cases, 100% of Enhance™ Demineralized Cortical Fibers samples are osteoinductive when assessed using this model.[2]

In conclusion, these results suggest that under the conditions of this study, and for the batches (donors) tested, the osteoinductivity of Isotis Accell DBM 100 is significantly less than that of MTF Enhance™ Demineralized Cortical Fibers.

It is unknown how the osteoinductive potential, measured in the athymic mouse model, will correlate with clinical performance in humans.

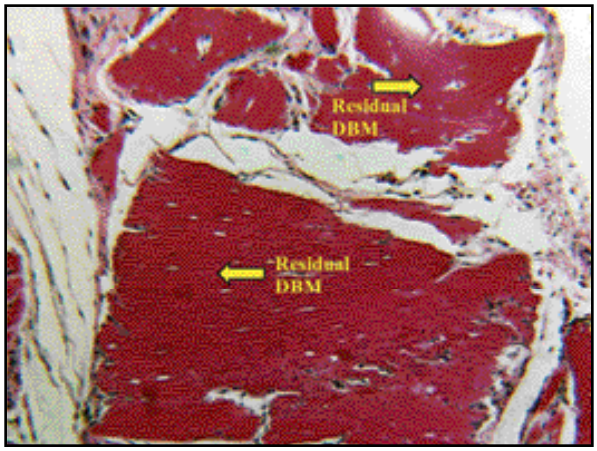

Figure 1: Accell DBM 100. *H&E stain; 100X magnification; BAR = 100 µm. No new bone formation. Fibrous and inflammatory tissue associated with residual DBM (arrows).*

Article	Lot	Average Osteoinductive Score	Group Std Dev
Accell DBM100	050836	0.40	0.50
Accell DBM100	051014	0.50	0.51
Accell DBM100	051023	0.37	0.73
Accell DBM100	410523	0.00	0.00

Table 2: *Accell DBM 100 osteoinduction scores*

Summary Statistics	Osteoinduction Score (0-4 Scale)		# Ranked Samples	Osteoinductive (Numbers & Percentages) Samples
	Mean	Std Dev		
Accell DBM100	0.30	0.55	22/32	6/22 (27%)

Table 3: *Summary statistics, number of samples that could be histologically evaluated, and number of osteoinductive samples for each group. Number of osteoinductive samples is divided by the number of evaluated samples to give the % of osteoinductive samples for each group.*

REFERENCES:

1. Schwartz, et al., J. Periodontol Surg. 69: 470 – 478, 1998.

2. Dunn, M.G. (2011). Osteoinductivity of MTF DBX Putty in the Athymic Mouse Model [White Paper]. Musculoskeletal Transplant Foundation (MKTG -810).

3. Draft Standard: **Standard Guide for the Assessment of Bone Inductive Materials**, ASTM F04.4 Division, Draft by Barbara Boyan, Univ. of Texas Health Science Center at San Antonio, downloaded from ASTM website 5-8-2000.