Osteoinductivity of MTF DBX® Putty in the Athymic Mouse Model

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July 15, 2011

SUMMARY

The objective of this study was to characterize the osteoinductive properties of a commercially available product containing demineralized bone matrix: DBX® Putty (MTF). 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 the test group, as well as negative controls, in an athymic mouse model. Results of this study suggest:

- **MTF DBX Putty was consistently osteoinductive** in this model; 100% of the samples were osteoinductive, with an average osteoinduction score of 1.65 ± 0.81.

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 DBX Putty, which is a commercially-available product containing DBM, from MTF.

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 and negative controls 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 two test groups: **MTF DBX Putty** (7 lots; *Table 2*) and **Heat-Inactivated MTF DBX Putty** (1 lot; *Table 2*) used as a negative control.

Samples (weighing 25 mg each) from each lot of material (N= 5-8 per lot) 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 2-3 sections per slide were prepared for each sample (10-15 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[2], 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

MTF DBX Putty was consistently osteoinductive in this model; 100% of the samples were osteoinductive, with an average osteoinduction score of 1.65 ± 0.81 *(Tables 2 & 3)*. Figure 1 shows the representative histological response to DBX Putty, with robust new bone formation including bone marrow.

Heat-inactivated negative control DBX Putty was not osteoinductive, as expected, with a primarily fibrous tissue/ inflammatory response and no new bone formation *(Figure 2)*. This is an important result that validates the utility and sensitivity of the animal model.

DISCUSSION & CONCLUSIONS

The semi-quantitative scoring system of the in vivo athymic mouse model does result in some inherent variability in the data. Therefore, when characterizing demineralized bone matrices it is imperative to assess both the average OI score and the variability in osteoinductive response reflected in the number of osteoinductive samples relative to the total number of samples.

There is minimal variability in the osteoinductive response of DBX Putty. Additionally, when periodically re-tested in this model DBX Putty is always osteoinductive.[3]

In conclusion, these results suggest that under the conditions of this study, and for the batches (donors) tested, that MTF DBX Putty is consistently osteoinductive with every sample of DBX Putty demonstrating osteoinductivity.

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

Figure 1: DBX Putty *demonstrating the presence of bone marrow and new bone formation. H&E stain; 100X magnification; BAR = 100 µm.*

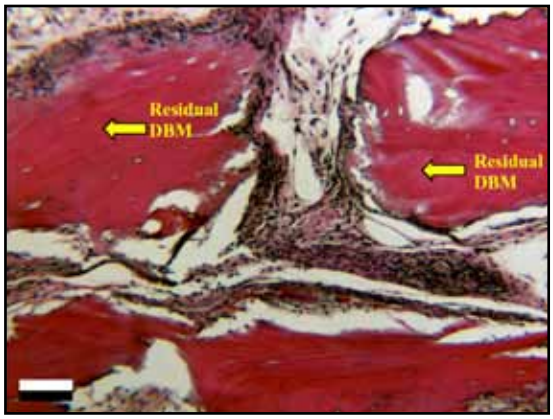

Figure 2: Heat-Inactivated Negative Control DBX Putty *does not demonstrate any new bone formation (as expected). H&E stain; 100X magnification; BAR = 100 µm.*

Article	Lot	Average Osteoinductive Score	Group Std Dev
DBX Putty	03347096	1.40	0.71
DBX Putty	00825797	2.55	0.51
DBX Putty	04418413	2.30	0.92
DBX Putty	02318567	2.15	0.67
DBX Putty	0650910907	1.00	0.00
DBX Putty	0080910057	1.00	0.00
DBX Putty	0571001878	2.00	0.72
Negative Control Heat-Inactivated DBX Putty	00825797	0.00	0.00

Table 2: *Osteoinductive scores for MTF DBX Putty and Negative Controls*

Summary Statistics	Osteoinduction Score (0-4 Scale)		# Ranked Samples	Osteoinductive (Numbers & Percentages) Samples
	Mean	Std Dev		
DBX Putty	1.65	0.81	40 /44	40 / 40 (100%)
Negative Control Heat-Inactivated DBX Putty	0.00	0.00	5/5	0/5 (0%)

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. 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.
3. Data on file, MTF