Osteoinductivity of Medtronic Progenix® in the Athymic Mouse Model

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SUMMARY

The objective of this study was to characterize the osteoinductive properties of two commercially available products containing demineralized bone matrix: Progenix DBM Putty and Progenix Plus (both Medtronic). 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:

- **Progenix DBM Putty was marginally osteoinductive** in this model; only 21% of the samples were osteoinductive, with an average osteoinduction score of 0.21 ± 0.41.
- **Progenix Plus was marginally osteoinductive** in this model: only 29% of the samples were osteoinductive, with an average osteoinduction score of 0.29 ± 0.46.

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 Progenix DBM Putty and Progenix Plus, which are commercially-available products containing DBM, both from Medtronic.

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 two test groups: **Medtronic Progenix DBM Putty** (3 lots; *Table 2*) and **Medtronic Progenix Plus** (1 lot; *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 3 sections per slide were prepared for each sample (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[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

Progenix DBM Putty was marginally osteoinductive in this model; only 21% of the samples were osteoinductive, with an average osteoinduction score of 0.21 ± 0.41 *(Tables 2 & 3)*.

Progenix Plus was marginally osteoinductive in this model as well: 29% of the samples were osteoinductive, with an average osteoinduction score of 0.29 ± 0.46 *(Tables 2 & 3)*.

Figures 1 and 2 show the most representative histological response to the Progenix Putty and Plus implants, respectively, with primarily a fibrous tissue/ inflammatory response, and no new bone formation. There were a few small regions with calcified cartilage formation.

The osteoinductivity scores for Progenix (both Putty and Plus compositions) 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 for both the Putty and Plus compositions of Medtronic Progenix DBM 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.

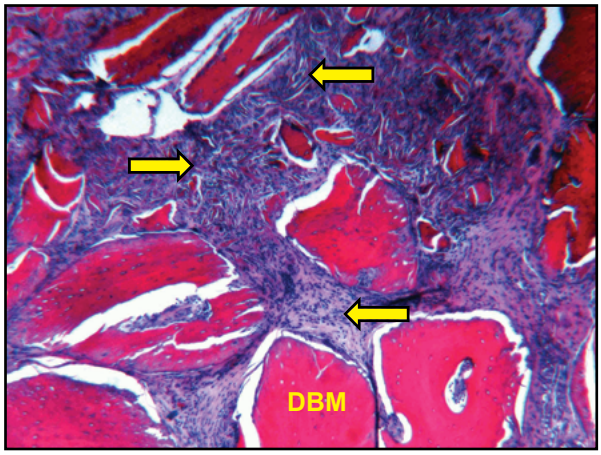

Figure 1: Progenix DBM Putty. *H&E stain; 40X magnification; BAR = 250 µm. No new bone formation. Fibrous and inflammatory tissue (arrows) associated with residual DBM*

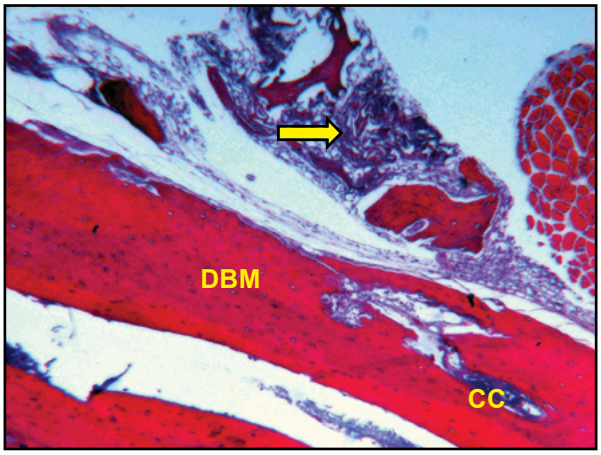

Figure 2: Progenix Plus. *H&E stain; 40X magnification; BAR = 250 µm. No new bone formation; just a very small area with calcified cartilage (CC). Primarily fibrous and inflammatory tissue (arrow) associated with residual DBM.*

Article	Lot	Average Osteoinductive Score	Group Std Dev
Progenix DBM Putty	1193280035	0.13	0.33
Progenix DBM Putty	1340040014	0.00	0.00
Progenix DBM Putty	1347670067	0.50	0.51
Progenix Plus	1413980100	0.29	0.46

Table 2: *Progenix osteoinduction scores*

Summary Statistics	Osteoinduction Score (0-4 Scale)		# Ranked Samples	Osteoinductive (Numbers & Percentages) Samples
	Mean	Std Dev		
Progenix DBM Putty	0.21	0.41	24/24	5/24 (21%)
Progenix Plus	0.29	0.46	7/8	2/7 (29%)

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.

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