

Osteoinductivity of Medtronic Grafton[®] Putty 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: Grafton Putty (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:

- **Grafton Putty was marginally*** osteoinductive in this model; only 43% of the samples were osteoinductive, with an average osteoinduction score (pooling data from 3 donors) of 0.52 ± 0.73 .

INTRODUCTION AND BACKGROUND

Demineralized bone matrix (DBM) is used for treating bone defects as an alternative to or in conjunction with bone grafts. The purpose of this study was to characterize the osteoinductivity of Grafton Putty, which is a commercially-available product containing DBM from Medtronic. The DBM tested in this study was processed from donors procured by Medtronic through two tissue banks. Each tissue bank determines the eligibility of the donor based on their respective donor eligibility requirements which are in compliance with FDA regulations.

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: **Medtronic Grafton Putty** (3 lots; *Table 2*). The test group comprised two lots (donors) procured from Tissue Bank A and one lot (donor) procured from Tissue Bank B. 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

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 industry³, the scoring system in *Table 1* was utilized.

| <i>Score</i> | <i>Criteria</i> |
|--------------|---|
| 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 \pm standard deviation.

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

RESULTS & CONCLUSIONS

Grafton Putty was marginally osteoinductive in this model; only 43% of the samples were osteoinductive, with an average osteoinduction score (pooling data from 3 donors) of 0.52 ± 0.73 (*Tables 2 & 3*).

The osteoinductivity scores for Grafton Putty 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

Figures 1 and 2 are representative histological responses to the Grafton Putty demonstrating the variable response of new bone formation. Figure 1 demonstrates a Grafton Putty implant with minimal osteoinductivity (< 25% new bone formation), while Figure 2 demonstrates a Grafton Putty implant which was non-osteoinductive in this model.

In conclusion, these results suggest that under the conditions of this study, and for the batches (donors) tested, the osteoinductivity for Medtronic Grafton Putty is significantly less than that of Enhance™ Demineralized Cortical Fibers.

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

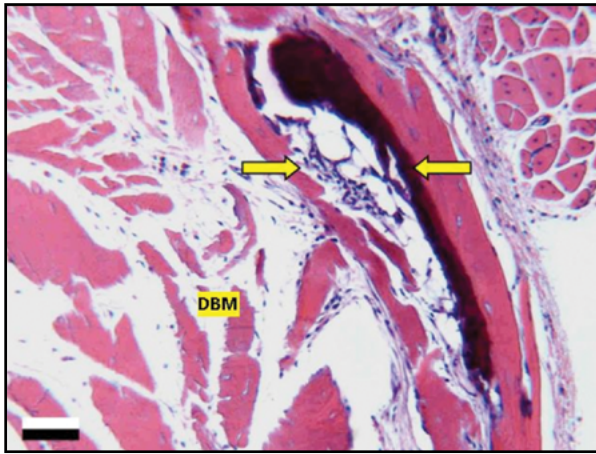


Figure 1: Grafton Putty. Osteoinductivity score of 1/4. H&E stain; 100X magnification; BAR = 250 MICRONS. There was <25% area of new bone formation (arrows) among the residual demineralized bone matrix (DBM) of the implant.

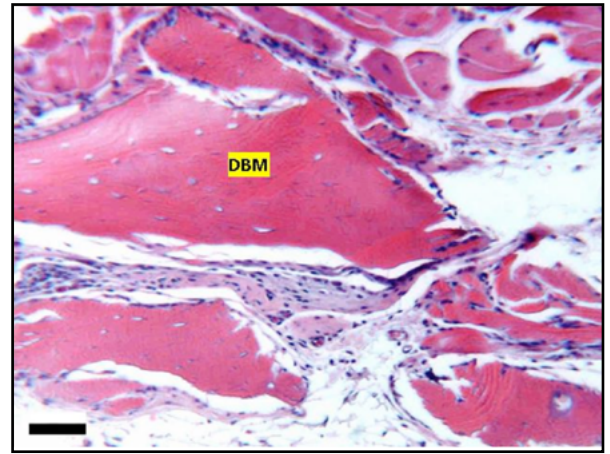


Figure 2: Grafton Putty. Osteoinductivity score of 0/4. H&E stain; 100X magnification; BAR = 100 MICRONS. No new cartilage or bone formation among the residual demineralized bone matrix (DBM) of the implant.

| Test Article | Lot | Average Osteoinductive Score | Group Std Dev |
|-----------------------|----------------------------------|------------------------------|---------------|
| Grafton Putty - TA IA | OTSCT0709144036 Tissue Bank A | 0.50 | 0.50 |
| Grafton Putty - TA IB | OTSCT0704164035 Tissue Bank A | 0.43 | 0.53 |
| Grafton Putty - TA IC | OTSLN0832304037 Tissue Bank B | 0.63 | 1.06 |

Table 2: Grafton Putty osteoinduction scores

| Summary Statistics | Osteoinduction Score (0-4 Scale) | | # Samples Ranked | Osteoinductive (Numbers & Percentages) Samples |
|--------------------|----------------------------------|---------|------------------|--|
| | Mean | Std Dev | | |
| Grafton Putty | 0.52 | 0.73 | 23/24 | 10/23 (43%) |

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. 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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