

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 Grafton® Putty (Medtronic). Osteoinductivity (OI), the ability to produce *de novo* heterotopic bone, was assessed histologically (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 matrices (DBMs) have been 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, 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 donors based on their respective donor eligibility requirements in compliance with FDA regulations.

When implanted into normal animals, human DBM is xenogenic and expected to provoke an immune response that may compromise the analysis of osteoinduction. The athymic mouse model was used, as the athymic mouse lacks a thymus gland and therefore cannot mount a humoral immune response to the human demineralized bone 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 (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 groups was conducted 28 days post-implantation to assess osteoinduction.

METHODS AND MATERIALS

This study utilized one test group: **Grafton Putty** (3 lots; *Table 2*). The test group comprised two lots (donors) procured from Tissue Bank A and one lot (donor) from Tissue Bank B. For comparisons, this study references osteoinductivity data on ENHANCE® demineralized cortical fibers collected by MTF.²

Eight samples (weighing 25 mg each) from each group 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 evaluated for osteoinductivity.

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.

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 group were determined by pooling the overall scores of the individual samples. The results 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™* 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 $.52 \pm 0.73$. (*Tables 2 and 3*)

The osteoinductivity scores of Grafton Putty are significantly lower than the osteoinductivity scores for the ENHANCE demineralized cortical fibers. In all cases, 100% of ENHANCE demineralized cortical fiber samples are osteoinductive when assessed using this model.² (*Table 4*)

Grafton Putty (Medtronic)			
Test Article	Lot #	Average OI Score	Group Std Dev
Grafton Putty: TA IA	OTSCT0709144036 Tissue Bank A	0.5	0.5
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 Osteoinductivity Scores.

Summary Statistics - Grafton Putty			
# of Samples Ranked	Osteoinductive Samples	Mean OI Score (0 to 4)	Std Dev
23/24	10/23 (43%)	0.52	0.73

Table 3: Summary Statistics for Grafton Putty Osteoinductivity Scores including number of samples, 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.

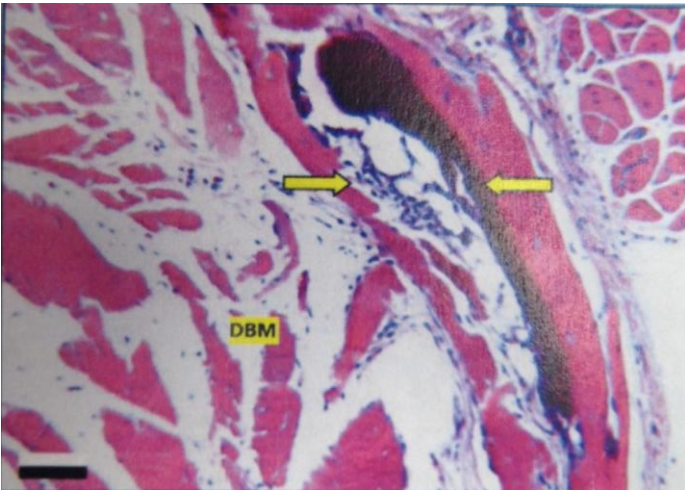


Figure 1: Grafton Putty Osteoinductivity score of 1. H&E stain; 100X magnification; BAR = 100 MICRONS. There was <25% area of new bone formation (arrows) among the residual DBM of the implant

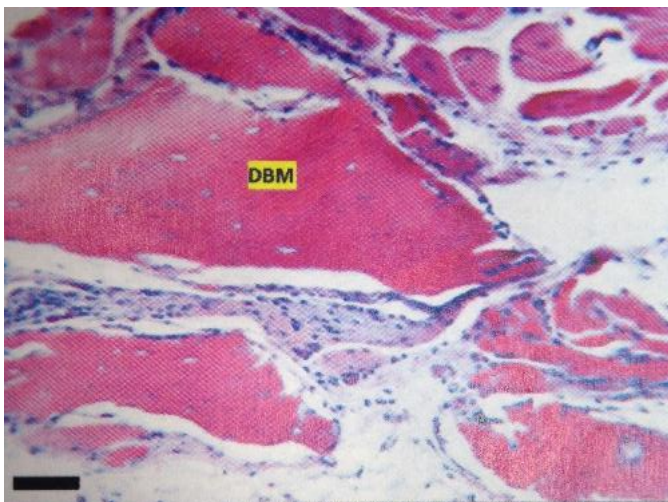


Figure 2: Grafton Putty Osteoinductivity score of 0. H&E stain; 100X magnification; BAR = 100 MICRONS. No new cartilage or bone formation among residual DBM of the implant.

ENHANCE Demineralized Cortical Fibers (MTF)		
Summary Statistics	Mean OI Score	Std Dev
Cortical Fibers	2.5	1.07
Cortical Fibers + PRP	3.25	0.96

Table 4: Osteoinductive scores for Cortical Fibers alone and combined with PRP.²

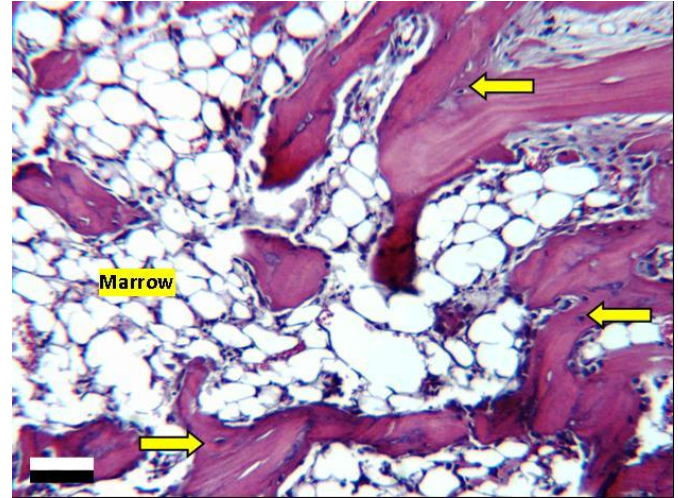


Figure 3: Cortical Fibers demonstrating the presence of a large region of new bone formation with marrow, and osteocytes embedded in the newly formed bone (arrows). H&E stain; 100X magnification; BAR = 100 MICRONS.

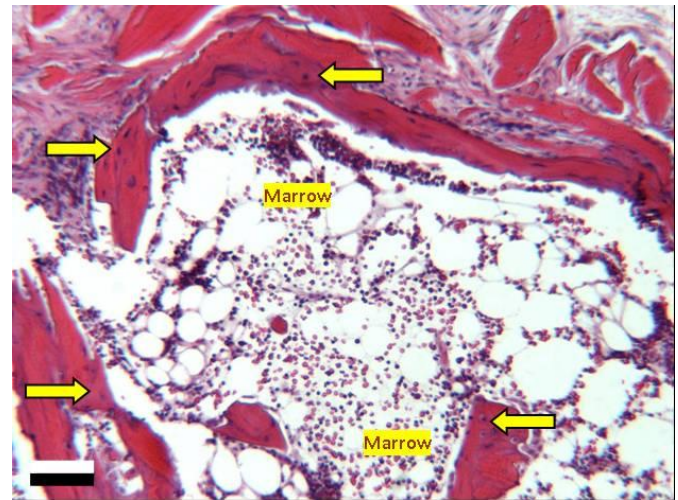


Figure 4: Cortical Fibers with PRP demonstrating the presence of new bone formation with marrow, and osteocytes embedded in the newly formed bone (arrows). H&E stain; 100X magnification; BAR = 100 MICRONS.

REFERENCES

1. Schwartz, et al., J. Periodontol Surg. 69: 470 – 478, 1998.
2. Dunn, Osteoinductivity of MTF Demineralized Cortical Fibers with CASCADE® Platelet Rich Plasma in the Athymic Mouse Model, 2013.
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.
4. Data on file, MTF