Osteoinductivity of MTF Demineralized Cortical Fibers with CASCADE® Platelet Rich Plasma in the Athymic Mouse Model

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SUMMARY

The objective of this study was to characterize the osteoinductive properties of the ENHANCE® demineralized cortical bone fibers from the Musckuloskeletal Transplant Foundation (MTF) alone and combined with Platelet Rich Plasma (PRP) from the CASCADE® Autologous Platelet System. PRP is routinely used as an adjunct in orthopedic surgery applications. In this study, human PRP was obtained at the time of surgery. 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 the test groups. Results of this study suggest:

Cortical Fibers were consistently osteoinductive in this model; 100% of the samples were osteoinductive, with an average osteoinduction score of 2.50 ± 1.07.

Cortical Fibers + PRP were consistently osteoinductive in this model; 100% of the samples were osteoinductive, with an average osteoinduction score of 3.25 ± 0.96

INTRODUCTION AND BACKGROUND

Demineralized bone matrices (DBMs) have been used in orthopedic surgery as an osteoinductive and osteoconductive scaffold for many years.[1] Autologous Platelet Rich Plasma from commercially available systems employs concentrated platelets and their associated growth factors that can be clinically applied to stimulate a reparative healing response.[2] Han *et. al.* evaluated DBM combined with PRP with and without thrombin in an athymic rat model. PRP without thrombin stimulated chondrogenesis and osteogenesis whereas PRP with thrombin inhibited healing in the same model.[3] The ENHANCE demineralized cortical bone fibers from MTF have known wicking capabilities while the Cascade Autologous Platelet System provides a reproducible method for processing PRP.[4] When mixed, the cortical fibers become hydrated with the PRP providing a stable matrix for delivering DBM and PRP into a bony defect without the use of thrombin.

The purpose of this study was to characterize the osteoinductivity of the ENHANCE demineralized cortical bone fibers from MTF alone and combined with the PRP produced from the CASCADE System in an athymic mouse model. When implanted into normal animals, human demineralized bone fibers are xenogeneic and 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 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.*[5]

Samples of the test groups were implanted bilaterally into the mouse hamstring muscle. Intramuscular implantation of active demineralized bone fibers 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 two test groups: **Cortical Fibers and Cortical Fibers + PRP** (a single donor lot was used for all implants in the test groups; *Table 1*).

Group	*Lot #*	Composition
Cortical Fibers	0651005976	31% (w/w) Fiber in NaCl
Cortical Fibers + PRP	0651005976	31% (w/w) Fibers in PRP

Table 1: *Donor lot numbers and compositions for the test groups.*

Samples (weighing 25 mg each) from each group (N= 4-8 per group) were prepared for implantation. Implants for Cortical Fibers alone were prepared by mixing with NaCl prior to surgery. Implants for the Cortical Fibers + PRP group were prepared by mixing the Cortical Fibers with PRP immediately prior to implantation (ratio of 31:69 of fibers to PRP). The samples were randomized and implanted bilaterally in the hamstring muscles of athymic nude mice. Animals were sacrificed at 28 days post-implantation. Decalcified histology was then performed on the explanted samples. Slides were stained with hematoxylin and eosin, and explanted samples were 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

industry[6], the scoring system in *Table 2* was utilized. The overall score for each test group were determined by averaging the scores from the individual explanted samples. The results are presented as a mean ± standard deviation.

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 2: *Osteoinductivity Scoring Scale and Criteria*

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

Cortical Fibers were consistently osteoinductive in this model; 100% of the samples were osteoinductive, with an average osteoinduction score (pooling data from 1 donor lot) of 2.50 ± 1.07.

Cortical Fibers + PRP were consistently osteoinductive in this model; 100% of the samples were osteoinductive, with an average osteoinduction score (pooling data from 1 donor lot) of 3.25 ± 0.96

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 Cortical Fibers with or without PRP. Additionally, when periodically re-tested in this model, MTF demineralized bone is always osteoinductive.[7]

In conclusion, these results suggest that under the conditions of this study, and for the batches (donors) tested, that ENHANCE Demineralized Cortical Bone Fibers, both alone and in combination with CASCADE PRP, are consistently osteoinductive with every sample of fibers demonstrating osteoinductivity in the athymic mouse model.

Summary Statistics	Osteoinduction Score (0-4 Scale)		# Ranked Samples	Osteoinductive (Numbers & Percentages) Samples
	Mean	Std Dev		
Cortical Fibers	2.50	1.07	8	8 / 8 (100%)
Cortical Fibers + PRP	3.25	0.96	4	4 / 4 (100%)

Table 3: *Osteoinductive scores for Cortical Fibers alone and combined with PRP. Table includes 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.*

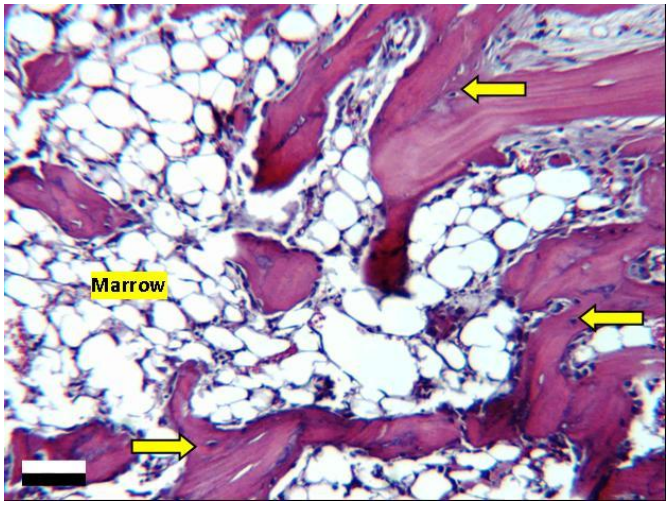

Figure 1: Cortical Fibers *demonstrating the presence of a large region of new bone formation with marrow, and osteocytes embedded in the newly formed bone (arrows). H&E stain; 100X magnification; BAR = 100 μm.*

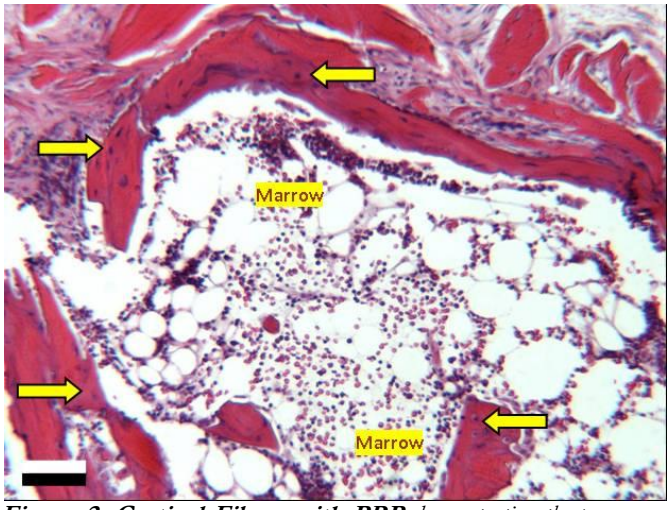

Figure 2: Cortical Fibers with PRP *demonstrating the presence of new bone formation with marrow, and osteocytes embedded in the newly formed bone (arrows). H&E stain; 100X magnification; BAR = 100 μm.*

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