Osteoinductivity of LifeNet Readigraft®
BLX Demineralized Fibers in the Athymic Mouse Model

Michael G. Dunn, Ph.D., Director, Orthopaedic Research Laboratory
Rutgers University – Robert Wood Johnson Medical School,
1 Robert Wood Johnson Pl., New Brunswick, NJ 08903

SUMMARY

The objective of this study was to characterize the osteoinductive properties of a demineralized bone matrix: Readigraft® BLX Fibers (LifeNet). 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:

Readigraft BLX Fibers were marginally osteoinductive in this model; only 48% of the samples were osteoinductive, with an average osteoinduction score (pooling data from 3 donors) of 0.57 ± 0.59.

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 Readigraft BLX Fibers, which is a commercially-available product from LifeNet containing DBM fibers.

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: **LifeNet Readigraft BLX Fibers** (3 lots; *Table 2)*. For comparison, this study references osteoinductivity data on ENHANCE® demineralized cortical fibers collected by MTF.[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.

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

Readigraft BLX Fibers were marginally osteoinductive in this model; only 48% of the samples were osteoinductive, with an average osteoinduction score (pooling data from 3 donors) of 0.57 ± 0.59 (*Tables 2 & 3*).

The osteoinductivity scores of Readigraft BLX are significantly lower than the osteoinductivity scores for the ENHANCE demineralized cortical fibers. In all cases, 100% of ENHANCE demineralized cortical fiber samples were osteoinductive when assessed using this model.[2]

Readigraft BLX (LifeNet)			
Test Article	Lot #	Average OI Score	Group Std Dev
Readigraft BLX Fibers – TA I	1211781	0.38	0.52
Readigraft BLX Fibers – TA II	1216834	1.00	0.58
Readigraft BLX Fibers – TA III	1216810	0.38	0.52

Table 2: *Readigraft BLX Fibers osteoinduction scores*

Summary Statistics - Readigraft BLX			
# of Samples Ranked	Osteoinductive Samples	Mean OI Score (0 to 4)	Std Dev
23/24	11/23 (48%)	0.57	0.59

Table 3: *Summary statistics for Readigraft BLX 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: Readigraft BLX Fibers. *H&E stain; 100X magnification; BAR = 100 MICRONS. Sample received a score of 1. < 25% area of new calcified cartilage (top arrow) and bone formation (bottom arrow) among the residual demineralized fibers.*

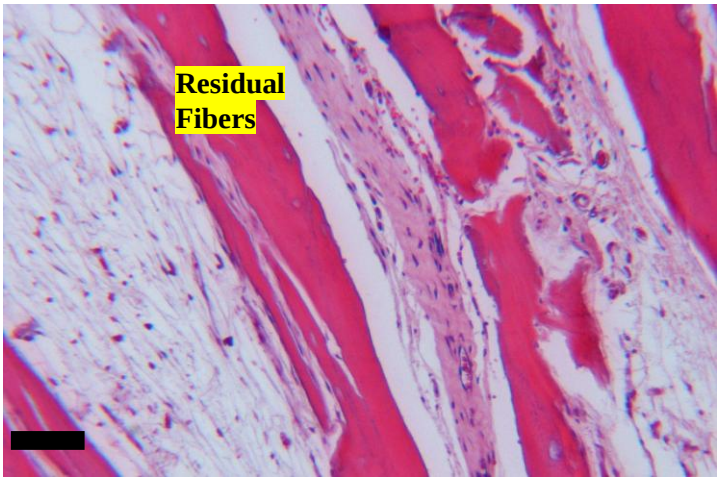

Figure 2: Readigraft BLX Fibers*. H&E stain; 100X magnification; BAR = 100 MICRONS. Sample received a score of 0, no new cartilage or bone formation among the residual demineralized fibers of the implant.*

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 of Cortical Fibers alone and combined with PRP.*[2]

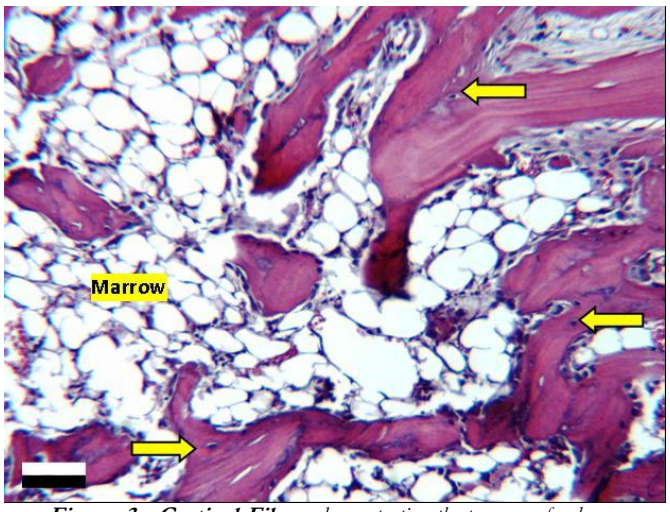

Figure 3: Cortical Fibers *demonstrating the presence of a large region of new bone formation with marrow, and osteocytes embedded in the newly formed bone (arrows). H&E stain; 100X magnification; BAR = 100 MICRONS.*

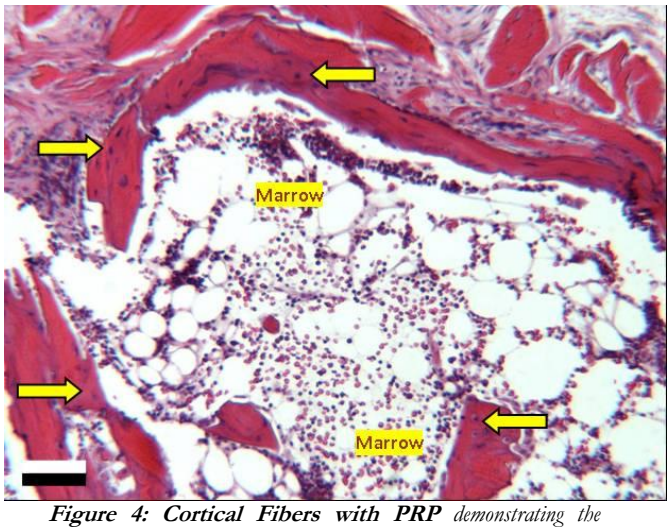

Figure 4: Cortical Fibers with PRP *demonstrating the presence of new bone formation with marrow, and osteocytes embedded in the newly formed bone (arrows). H&E stain; 100X magnification; BAR = 100 MICRONS.*

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 5-8-200.
4. Data on file, MTF