DBM Fibers and Cancellous Bone Induce Spinal Fusion in the Athymic Rat PLF Model

Sherry Triplett, BS; Katrina Gonzales, BS; Silvia Chen, Ph.D

INTRODUCTION

Bone void fillers containing demineralized bone matrix (DBM) and cancellous bone derived from human tissues have been used in orthopedic and spinal fusion surgery with encouraging results. DBM contains osteoinductive proteins and has demonstrated the ability to stimulate new bone formation and spinal fusion[1,2]. Allograft cancellous bone has been recognized to have osteoconductive properties.[3] The athymic rat is an ideal model to test bone void filler containing human DBMs since the animal will not reject the human tissue implants. The purpose of this study was to assess the ability of the human DBM fibers and cancellous bone to induce posterior-lateral intertransverse process spinal fusion (PLF) in an athymic rat model. This model has been extensively characterized to ensure that there is no instance of spontaneous fusion, instead fusion results from the osteogenic, osteoconductive, and/or osteoinductive nature of the graft materials.[4,5]

MATERIALS AND METHODS

Graft Production, Processing, and Irradiation: Research authorized donors were processed to produce cortical bone fibers and cancellous bone chips according to the LifeNet Health Allowash® technology. LifeNet Health's Pulsatile Acid Demineralization (PAD) technology was used to demineralize the cortical bone fiber to produce DBM fibers.

For proper graft fit in the small defect size in the rats, the DBM fibers and cancellous chips were ground to 1-2 mm. DBM fiber and cancellous chips were mixed at a ratio of about 2g DBM fiber to about 3g of cancellous chips. Samples were shipped and irradiated on dry ice at an absorbed dose of 22 kGy, and stored at ambient temperature until time of implant.

Surgery: All animal procedures and surgeries were performed under an approved IACUC protocol at a contract laboratory. Seven animals were used for bilateral implantation of 0.2 cc graft material in between the L4 and L5 transverse processes. Graft materials were prepared prior to implantation by adding saline and/or autologous blood taken via an ocular blood draw. Animals were euthanized eight weeks post-surgery. Samples were excised one level above and below the fusion mass, and analyzed for fusion by manual palpation, radiographs, microCT and histology.

Manual Palpation: After trimming the soft tissue from the spine segments, each spine segment was independently evaluated by three observers. A three point scoring system (0: not fused; 1: unilateral fusion; 2: bilateral fusion) was used, and observations noted. After evaluation, the spinal segments were placed in 10% neutral buffered formalin for fixation.

Radiography: One Faxitron radiograph was taken for each specimen and graded (0: not fused; 1: unilateral fusion; 2: bilateral fusion) twice by a single observer for fusion. The two scores were averaged, and rounded down to obtain a single fusion score per animal.

MicroCT: Each side of the sample was scored (0: not fused; 1: fusion), and added together to get a fusion score per animal.

Histology: Each fusion site was isolated and trimmed of soft tissue, decalcified, and embedded in paraffin. Three step sections were taken from each block, hemotoxylin and eosin stained, and analyzed.

RESULTS

One rat did not survive the procedure. Death was not attributed to the test article. No abnormal clinical observations were noted during the course of the study relating to the test articles.

Manual Palpation: Fusion was observed in five out of the six animals, with 3 animals demonstrating bilateral fusion (**Table 1**).

Analysis	Fusion Rate	Total Fusion Frequency	No Fusion	Unilateral Fusion	Bilateral Fusion
Manual palpation	83.3%	5/6	1/6	2/6	3/6
Radiograph	100%	6/6	0/6	2/6	4/6
Micro CT	100%	6/6	0/6	1/6	5/6

Table 1: Fusion frequency

Radiography: The DBM fibers/cancellous chip induced a 100% fusion rate, with four out of the six animals demonstrating bilateral fusion (**Table 1**). A representative radiographic image is shown in **Figure 1**.

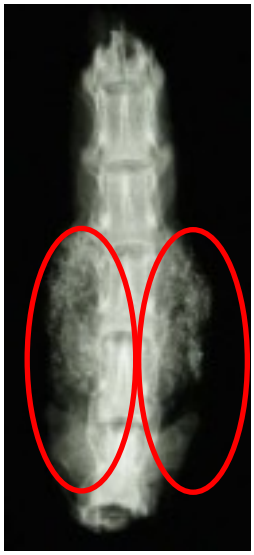

Figure 1: Representative radiograph image with the fusion masses highlighted in red.

MicroCT: Similar to the radiography, a 100% fusion rate was observed, with five out of the six animals had bilateral fusion (**Table 1**). Representative MicroCT images showing dense fusion mass are shown in **Figure 2**.

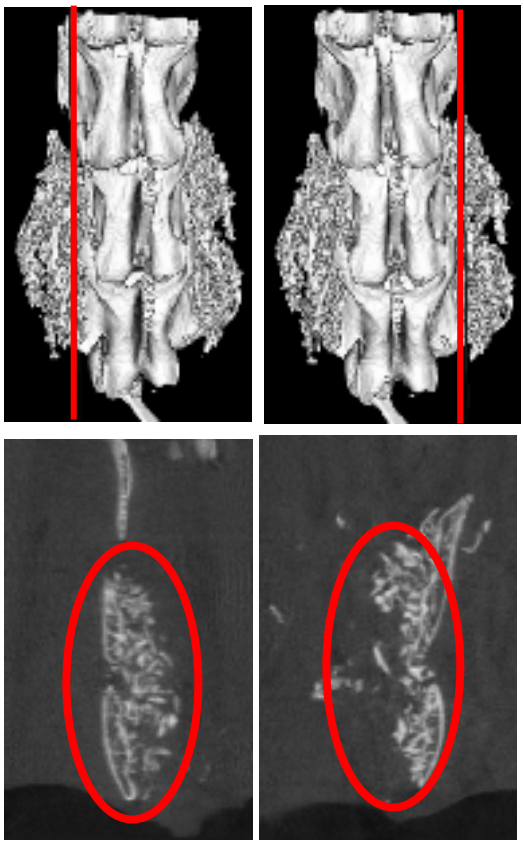

Figure 2: Representative MicroCT Images showing bilateral fusion. Top row - the red line indicates the plane of the cross sectional image. Bottom row - cross sectional image of the image above with the red circle highlighting the fusion mass.

Histology: Complete spinal fusion was seen in all samples. The DBM fibers proved to be osteoinductive, inducing new bone and bone marrow formation (**Figure 3**). A higher level of new bone, and/or bone marrow formation was found in areas where elevated concentrations of DBM fibers were presented in the defect site.

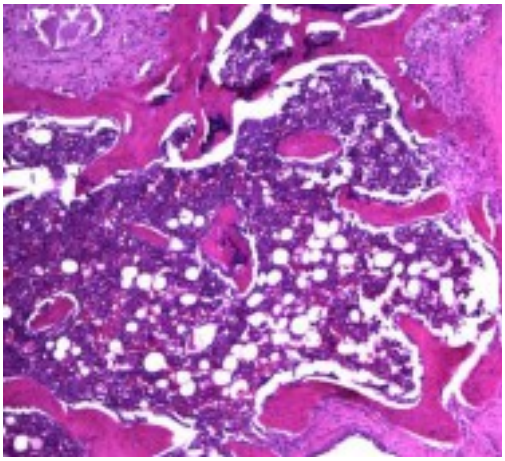

Figure 3: Representative histology of DBM fibers explant fusion mass, H&E stained, which shows new bone and bone marrow formation.

CONCLUSION

The bone void filler containing DBM fibers and cancellous chips showed successful spinal fusion in the athymic rat PLF model. A 100% fusion rate was observed in both radiography and MicroCT. Additionally, histology analysis indicated that the amount of new bone was directly related to the amount of DBM fibers present, indicating the importance of the osteoinductive potential of the DBM fibers in spinal fusion.

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