Peripheral Stem Cells Homing to Bioengineered Bone Grafts in a Posterolateral Lumbar Fusion Model

Justin J. Lee MS[1], Freddy Jacome BS[1], David M. Hiltzik MD[1], Erika Li[1], Manasa Pagadala BA[1], Sia Cho BA[1], Jacqueline Inglis BA[1], Alyssa Goodwin BS[1], Steven Kurapaty MD[1], Hogan Brecount MD[1], Rahim Laiwalla MS[1], Charlotte Chen PhD[2], Nick Sather PhD[2], Jinyuan Li PhD[1], Samuel Stupp PhD[2], Wellington Hsu MD[1], Romie F. Gibly MD/PhD[1,3], Erin Hsu PhD[1, 2]
Corresponding author email: justin.lee@northwestern.edu

[1]Northwestern University, Department of Orthopaedic Surgery, Chicago, IL
[2]Simpson Querrey Institute for BioNanotechnology (SQI), Northwestern University 303 E. Superior, Chicago, IL
[3]Ann & Robert H. Lurie Children's Hospital of Chicago, Division of Orthopaedic Surgery and Sports Medicine, Chicago, IL

DISCLOSURES: **N. Sather**: 3A; Amphix Bio. 4; Amphix Bio. **W. Hsu:** 1; Stryker. 3B; Asahi, Medtronic, Promimic, ZSFab, Amphix Bio. 5; Inion. **E. Hsu**: 5; Inion, Amphix Bio. **S. Stupp**: 4; Amphix Bio. For all other authors none were declared.

INTRODUCTION: Current methods of spinal fusion exhibit significant rates of pseudoarthrosis (failed fusion) that are associated with significant morbidity. BMP-2 is currently used as an adjunct to help increase rates of fusion; however, it is associated with significant side effects at the dosages required to achieve successful fusion. Peptide amphiphiles (PA) are a class of self-assembling molecules that are highly modifiable and can be used to fabricate scaffolds with specific biologic activity. We previously reported the development of a peptide amphiphile nanofiber scaffold capable of binding to BMP-2, which achieves successful spine fusion in rats using recombinant human BMP-2 (rhBMP-2) at significantly lower doses than those required without PA. We hypothesize in addition to potentiating growth factor signaling by prolonging half-life, the BMP-2 binding PA can promote stem cell homing to the implant. To test this hypothesis, we examined homing of syngeneic bone marrow- and periosteum-derived stem cells (BMSCs and PDSCs, respectively) to BMP-2 binding PA implants in the pre-clinical setting of posterolateral spinal fusion in rats.

METHODS: This study was approved by the Northwestern University IACUC with Animal Welfare Assurance from the Office of Laboratory Animal Welfare. PDSCs were harvested from female Sprague-Dawley rats that endogenously express GFP, while RFP-BMSCs were purchased from Cyagen Biosciences Inc. Cells were expanded in culture out to passage 7, and GFP and RFP expression was verified via fluorescent microscopy. Twelve 16-week-old female Sprague-Dawley rats then underwent posterior lumbar spinal fusion with bilateral placement of absorbable collagen sponge (ACS) scaffolds loaded with 130 µl of 1 wt.% BMP2 binding PA. After implantation, rats received a single tail vein injection of either PBS only (negative control) or a suspension of 3 x 10^6 syngeneic RFP-BMSCs and GFP-PDSCs in PBS. Implants were harvested at 1- and 10-days post-operation along with lung, spleen, and liver. Confocal microscopy was used for visualization of fluorescently-labeled cells. In vitro experimentation of fluorescent stem cells directly inoculated onto ACS scaffolds for 1 and 10 days were used to confirm the survival of target cells on ACS.

RESULTS SECTION: Peripherally injected BMSC and PDSCs were found to migrate to collagen/PA implantable scaffolds at 1 day postoperatively, and cells remained viable through 10 days. The negative control (figure 1A) shows some autofluorescence of the collagen/PA scaffold at baseline. 1-day post operation (figure 1B), there is GFP and RFP signal indicating presence of PDSC and BMSCs, respectively. At 10-days post operation (figure 1C), GFP and RFP positive cells were still present in the scaffold.

DISCUSSION: This study shows that peripherally injected stem cells are capable of migrating through the circulation and homing to a collagen-PA matrix and that these cells survive on that matrix for at least 10 days. Bone morphogenetic protein (BMP-2) is currently used in clinical practice following lumbar fusion; however, its use is limited by the high dose requirements which lead to many complications including localized edema, ectopic bone formation, vertebral body resorption, and new cancers. Here, we provide evidence that a scaffold containing collagen and a BMP-2 binding PA may potentiate bone healing by recruiting multipotent progenitor cells. Work is ongoing to quantify the differences in homing capacity of BMSC and PDSCs to collagen only and PA-containing scaffolds with the eventual goal of quantifying preferential migration of progenitor cells to various PA-containing implants.

SIGNIFICANCE/CLINICAL RELEVANCE: This work suggests that bioactive epitopes which can bind and potentiate growth factor signaling may improve regenerative capacity by enhancing progenitor cell recruitment to the site of spinal fusion. The capacity for rationally-designed scaffolds to potentiate stem cell migration to a distant site of regenerative activity could be leveraged not only in the setting of bone healing and spinal fusion, but to other target tissues as well.

IMAGES AND TABLES:

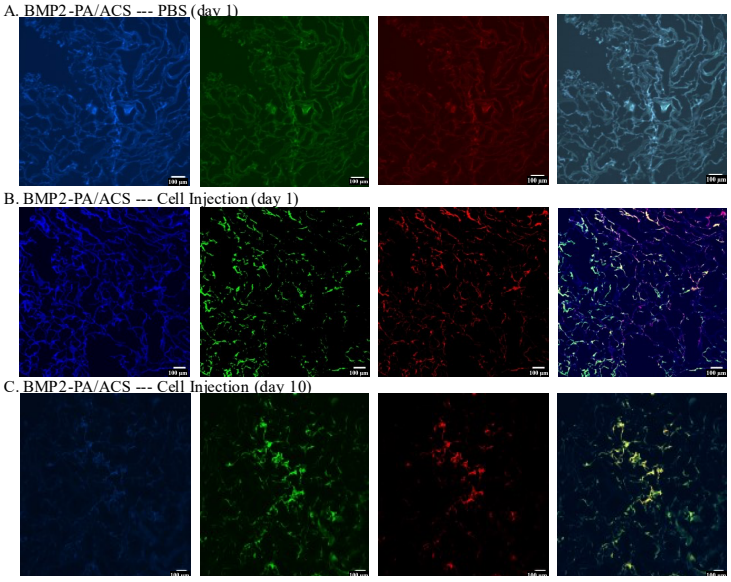

Figure 1: Confocal imaging of BMP-2bPA/ACS scaffold implants harvested at 1 day post operation viewed in DAPI, GFP, RFP, combined channels in rats that underwent posterior lumbar fusion followed by (A) sham injection with PBS at Day 1(B) tail vein injection with GFP-PDSC and RFP-BMSC at Day 1 (C) tail vein injection with GFP-PDSC and RFP-BMSC at Day 10.