**Acceleration of Spinal Fusion with Adipose Tissue Derived Adult Stem Cells**

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**Introduction:** Back pain affects over 75% of Americans during their lifetime. Posterolateral spinal fusion is the standard treatment for lumbar compression fractures at an annual direct medical cost of over $746 million in the US. Failure rates associated with spinal fusion are as high as 44% and increase costs substantially. Higher success rates will have significant economic, medical and societal implications.

Bone grafts are used to facilitate and accelerate spinal fusion. Autografts are the current “gold standard”, but there are complications associated with graft harvest. Regenerative medicine employs stem cells to promote tissue healing. Adipose tissue is an abundant and accessible source of adipose tissue-derived stromal cells (ASCs) which promote osteogenesis both in vitro and in vivo. This study was designed to test the hypothesis that syngeneic and allogeneic ASCs combined with a biomaterial scaffold will accelerate spinal fusion compared to no treatment or scaffold alone in a rat model.

**Materials and Methods:** Subcutaneous adipose tissue was harvested from 10 week old male Fischer and ACI rats (n=16/cohort). Tissue was minced, washed, and suspended in phosphate buffered saline containing 1% bovine serum albumin and 0.1% collagenase type I. Following a 60-minute digestion at 37°C, the suspension was centrifuged. The pelleted stromal vascular fraction cells were plated in stromal media (DMEM/F-12 Ham’s Media with 10% fetal bovine serum and 1% antibiotic/antimycotic) and incubated in a humidified 5% CO2 incubator to 75% confluency. The ASCs were then harvested and expanded up to 2 passages. Cell aliquots (5 x 10⁶ cells/ml) were cryopreserved in liquid nitrogen. Cell fractions were evaluated in vitro for their osteogenic and adipogenic capacity using standard assays. Prior to surgery, cells were defrosted, rinsed, suspended in stromal media and loaded onto 0.5 x 0.2 x 0.7 cm scaffold blocks (Vitoss, Orthovita, Malvern, PA) blocks. Blocks loaded with cells or media alone were cultured in stromal media for 48 hours prior to surgery.

Sixty-four male Fischer rats (200g) were randomly assigned to one of 4 cohorts (n=16/cohort): 1) No treatment; 2) Scaffold only; 3) Scaffold + syngeneic ASCs; or 4) Scaffold + allogeneic ASCs. Following humane euthanasia, spines were harvested from half of each cohort 4 or 8 weeks after surgery. The surgical procedure was performed according to published methods. Rats were anesthetized with isoflurane. The lumbar region was aseptically prepared, and the posterior lumbar spine was exposed. The L4 and L5 transverse processes were decorticated bilaterally. Following decortication, routine closure was performed in the no treatment cohort while scaffold blocks with or without ASCs were placed at the level of the L4-L5 intervertebral space prior to closure in scaffold cohorts. Cell loading on the blocks was confirmed with scanning electron microscopy (SEM). Following harvest, spines were imaged with micro-CT. They were then fixed in formalin, decalcified and embedded in paraffin. Coronal sections at the level of the L4-L5 intervertebral space were stained with H&E and Masson’s trichrome. The quality and quantity of spinal fusion was evaluated with radiographs, micro-computed tomography (micro-CT), and light microscopy. Outcome measures were statistically evaluated with MANOVA models that tested the four group comparisons multivariately. Significance was considered at $p < .05$.

**Results:** All animals survived the surgical procedure and post-operative period. Cells were evident on loaded scaffolds prior to surgical implantation with SEM. The cells demonstrated both adipogenic and osteogenic potential in vitro. Spines without scaffolds did not have evidence of callus formation 4 or 8 weeks after surgery. There was radiographic and micro-CT evidence of callus formation at the level of the L4-L5 intervertebral space in all three scaffold treatment groups 4 and 8 weeks after surgery. Based on light and polarized light microscopy in ASC cohorts. There was no evidence of callus calcification in the scaffold only cohort 8 weeks after surgery.

The observations were further confirmed by callus calcification evident with light and polarized light microscopy in ASC cohorts. There was no evidence of callus calcification in the scaffold only cohort 8 weeks after surgery.

**Discussion:** The results of this study support ASC acceleration of posterior lumbar spinal fusion in a rat model compared to no treatment or scaffold alone. Bone formation in the L4-L5 intervertebral space was promoted by scaffolds with and without ASCs. However, callus formation was more organized and mature in the ASC loaded scaffold treatment groups 8 weeks after implantation. Additionally, both syngeneic and allogeneic ASCs appeared to accelerate the spinal fusion process equally. Lack of differences in outcome parameters 4 weeks after surgery may be due to scaffold influences on bone formation that masked ASC effects. Based on the results of this study, further investigation of syngeneic and allogeneic ASCs to promote spinal fusion in a large animal model is warranted. The use of ASCs to accelerate spinal fusion has potential to significantly reduce morbidity and complications associated with the procedure.

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**Figure 1:** Three-dimensional micro-CT reconstructions of the L4-L5 intervertebral space (anterior surface) 4 or 8 weeks after surgical implantation of Vitoss blocks (Vitoss only) or Vitoss blocks that were loaded with syngeneic or allogeneic ASCs.

**Figure 2:** Representative sections (2.5x) of L4-L5 intervertebral space callus 8 weeks after surgery. (A) Masson’s trichrome photomicrographs; (B) composite photomicrographs of sections viewed under nonpolarized and polarized light. Light diffraction due to tissue calcification is pseudocolored red in composite images. P = parent bone.