The Use of Autologous Adipose and Bone Marrow Derived Stem Cells in a Point of Care Goat Non-Instrumented Posterolateral Lumbar Spinal Fusion Model

INTRODUCTION
With over 180,000 spinal fusions performed each year in the United States, clinical therapies are searching for ways to bypass the current gold standard of the use of iliac crest bone graft (ICBG) for generating spinal fusion. One of the benefits of ICBG is that it contains all three necessary components to generate a solid fusion: osteogenic, osteoinductive, and osteoconductive. However, the use of ICBG is linked to donor site morbidity, necrosis, and long-term pain for the patient. Bone marrow harvesting and isolation through centrifugation is now common among orthopaedic therapists with the use of an appropriate scaffold to bypass ICBG. It is believed this treatment also contains the necessary components for solid bone fusion. However, bone marrow is limited in the volume that can be extracted without dilution of peripheral blood. Recent work by Zak et al (2001, 2002) have shown that mesenchymal stem cells (MSC) isolated from adipose tissue may be an alternative source of stem cells that contain more cells, with easier access and less complications than those seen with ICBG and bone marrow harvest. Additionally, a proper large animal model had to be selected to closely mimic that of a human being. The goat was chosen due to their similarities to humans in loading conditions of the spine, trabecular bone structure of the vertebral, and their common use in testing orthopaedic therapies as a clinically relevant model.

The aim of this study is to characterize and compare goat bone marrow stem cells (BMSC) and adipose derived stem cells (ADSC) as well as utilize them in a “Point-of-Care” orthopedic therapy in goat non-instrumented posterolateral lumbar spinal fusion.

METHODS
Skeletally mature domestic goats (n=15) (age 24-36 months) were obtained from Lone Star Labs (LSL) (Seguin, TX). The Institutional Animal Care and Use Committee (IACUC) at LSL approved all animal protocols prior to initiation of the study and LSL was the site of all surgical procedures and provided care for the animals postoperatively and throughout the duration of the study.

*In vivo* Implantation: Animals were sedated and prepared for surgery at LSL. Bone marrow was harvested through percutaneous aspiration of the iliac crest and isolated on a proprietary bone marrow separating device to isolate the BMSCs. Adipose tissue was harvested through excision of a portion of subcutaneous sternal fat, digested with a proprietary collagenase to release the stem cells from the tissue and isolated through centrifugation, red blood cell lysis and filtration to obtain the ADSCs, following the Zuk protocols. Cell viability for *in vivo* implantation was performed on a NucleoCounter cell counter (New Brunswick Scientific, Edison, NJ) and on Countess (Invitrogen, Carlsbad, CA) for *in vitro* analysis. Both cell counters were cross- compared and verified, similar in cell counting abilities. Cell isolations for both BMSCs and ADSCs were resuspended in 10 mL of plasma and DPBS respectively prior to injection into the scaffold. All cell harvests, isolations, and implantations were performed within the time limits of the spinal fusion surgery thus described as “Point-Of-Care.”

Goats were subjected to a two independent level non-instrumented posterolateral spinal fusion with a proprietary hydroxyapatite/tricalcium phosphate (HA/TCP) scaffold. The upper and lower level fusions were randomized into three groups: Scaffold alone + DPBS; Scaffold + BMSCs; Scaffold + ADSCs. After implantation and suturing, animals were monitored for 48 hours for infection then released into their normal setting and resumed normal activity. The goats were monitored for a 6 months post-operatively at LSL. Radiographs were taken at the 3-month time point. At 6 months, animals were euthanized and spines harvested and subjected to radiographs, CT scans, and analysis to grade fusion levels on a 0 to 4 graded scale of fusion, with grade 4 fusions exhibiting solid, bilateral, trabeculated stout fusion mass.

In *vivo* Analysis: All *in vitro* testing was performed at The University of Texas at Austin (UTA). Samples of BMSCs and ADSCs were transported to UTA for culturing and expansion. Cells from passages 1-4 were used for *in vitro* analysis. Flow cytometry was used to determine presence of cell surface markers CD34 and CD45 at The UTA CORE facility on the FACSCalibur (BD, San Jose, CA). For multiligneage differentiation, cells were cultured in either adipogenic (AM) or osteogenic media (OM) for 2 weeks, after which, cells in AM were stained with Oil Red O to determine presence of lipid droplets within cells and those in OM were stained with Alizarin Red to determine presence of calcium deposits, both using microscopy. Statistics: ANOVA was used to compare cell viabilities for live, dead, and total cell count as well as viability. Fischer’s exact test was used to compare union scores of fusion.

RESULTS
Cell viability was determined from bone marrow & adipose tissue at time of isolation on the NucleoCounter. There was a significant difference in cell viability (p < 0.05) between BMSCs (82 ± 11%) and ADSCs (69 ± 14%). MSCs were isolated in culture and cell counts were determined using the Countess. There was no difference in cultured cells in total cell count, non-viable cells, total viable cells and viability between BMSCs and ADSCs. Both BMSCs and ADSCs lacked expression of stem cell markers CD34 and CD45. Additionally, both BMSCs and ADSCs differentiated into both the osteogenic and adipogenic lineages with positive staining for calcium deposits and lipid droplets, respectively. At the 3-month time point, there were no differences between implantation groups in radiographic analysis. There were significant differences in the radiographs between the 3 and 6 month time period: Scaffold only (p < 0.01), Scaffold and BMSCs (p < 0.005), and Scaffold and ADSC (p < 0.005). There was no difference between groups at the 6-month time point for radiographic analysis. There was no difference between preliminary CT scans at the 6-month time point between the right and left side fusion grades within each group or fusion grades between groups.

DISCUSSION
The lack of CD34 and CD45 expression confirms the isolated cells were MSCs, since only hematopoietic stem cells express these markers and confirms that these cells were present on the scaffolds when implanted into the goat. Additionally, the multilineage differentiation of both cell types to the osteogenic and adipogenic lineages confirms the cells were MSCs. Although we had positive *in vitro* results, we had limited *in vivo* results. Possible explanations to this may be that the surgical procedure was non-instrumented and that the time period we examined was not long enough to obtain full fusion. A 9-month or 12-month follow up may be more indicative of true fusion ability of these cells. Additionally, the comparisons of the radiographs from 3 to 6 months do indicate significant bone formation, indicating that osteogenesis has been established, but it is unknown at this time to what extent. The scaffold did not remain present throughout the duration of the study as indicated through the preliminary CT scans, and the majority of the bone growth was seen between the transverse processes and the implant, however, it was not fully trabeculated and fused bone. Further examination of the CT scans will be needed to determine yield of bone formation and fusion mass. Additionally, final CT results, biomechanical testing, and histology results were not available at time of submission.

This study is the first of its kind to investigate a large animal model comparison of BMSCs and ADSCs in spinal fusion and demonstrated that “Point-of-Care” stem cells derived from either bone marrow or adipose tissue demonstrated the potential for bone formation.