Introduction: While meniscus tears in the peripheral vascularized region can be successfully repaired, the success rate for repair of the avascular region is poor. In pursuit of sources for regenerative therapy, undifferentiated cells have been successfully isolated from adult skeletal muscle, and their origin has been recently associated to vascular derived cells. It has also been attempted to isolate and characterize stem cells derived from the meniscus of the knee. We believe that the vascular stem cells within the vascular zone of the meniscus represent a source of stem cells. The cell surface markers CD31 and CD146 are well known markers of vascular endothelial cells, and CD34 has been shown to be closely associated with hematopoietic progenitor cells. Given the advantages for self-repair inherent in the more highly vascularized peripheral region of the meniscus, we hypothesize that this region of the meniscus has a richer supply of vascular stem cells when compared to the avascular zone of the meniscus.

Materials and Methods: Sample: Complete, visually intact human lateral adult menisci were harvested from subjects undergoing total knee arthroplasty, and fetal menisci were harvested from spontaneously or therapeutically aborted fetuses with less than 24 weeks gestational age following IRB approved protocols. Cell isolation: Menisci were separated into 2 regions, the peripheral one third and the inner two thirds. Each tissue was minced and then digested with collagenase in DMEM. Immunohistochemical staining: Menisci were snap-frozen and were immuno-stained for CD31 (endothelial cell marker), CD34 (stem cell marker), and CD146 (pericyte marker), coupled with smooth muscle actin (SMA) to detect various cells around the arterioles. Characterization of meniscus-derived cells: Meniscus-derived cells were characterized by flow cytometry for CD34, CD146, and CD31 expression. The meniscus cells were sorted for expression of CD34 and CD146 after gating out hematopoietic (CD45+) cells (4 populations from adult, 4 populations from fetal). CD146+ cells from the adult peripheral and inner meniscus did not expand well, leaving 6 populations to be tested for their multilineage potential. Chondrogenic assay: Cells (n=2.5x10^5) were placed in a 15ml conical tube, centrifuged at 600g, and cultured in chondrogenic medium (Lonza) supplemented with BMP-4 (100ng/ml) and TGF-β3 (10ng/ml). Pellets were assessed macroscopically at day 21, and stained with Alcian blue/nuclear fast red. Osteogenic assay: Cells (n=1.0x10^5) were cultured in 6-well-plates in osteogenic medium supplemented with BMP4 (100ng/ml) and stained for alkaline phosphatase (ALP) at day 6. Cells were also cultured as pellets for 21 days in osteogenic medium and evaluated with a micro-CT (Visio) and stained with von Kossa for assessment of mineralization. Adipogenic assay: Cells (n=1.0x10^5) were cultured in 6-well-plates for 14 days in adipogenic medium and stained for Oil Red O.

Results: Immunohistochemical staining: Adult and fetal tissues showed more positive staining for CD31, CD34, and CD146 in the periphery than in the inner meniscus (Fig.1). Chondrogenic assay: The percentage of positive cells for CD31, CD34, and CD146 were, respectively, 0.13, 12.87, and 0.58% in the adult peripheral cells, 0.28, 2.67, and 0.47% in the adult inner cells, 0.0, 14.5, and 15.6% in the fetal peripheral cells, and 0.03, 11.18, and 21.48% in the fetal inner cells (CD34 in adult peripheral and inner cells, P <0.01). Chondrogenesis: Adult pellet size did not differ significantly between the peripheral CD34+ cells (P34) and the inner CD34+ cells (I34) (data not shown). P34 pellets showed more positive staining with Alcian blue compared to I34 pellets (data not shown). Fetal pellets made of peripheral CD146+ (P146) cells showed the largest size among all populations from fetal samples (Fig.2a, c). Pellets of P34 and P146 cells showed a greater degree of staining with Alcian blue (Fig.2b). Osteogenesis: Adult-ALP staining - P34 cells displayed significantly more ALP staining compared to I34 cells (P <0.05). Adult-Micro CT - Bone volume from P34 pellets was significantly larger than in I34 pellets (P <0.05). Adult-von Kossa staining - P34 cells displayed more positive staining compared to I34 cells (P <0.05). Fetal-ALP staining - P34 and P146 displayed more positive staining compared to the inner cells (Fig. 2d, e). Fetal-Micro CT - Bone volume from P34 pellets was the largest among fetal populations (Fig. 2f). Fetal-von Kossa staining - Pellets made with peripheral cells displayed more positive staining compared to pellets from inner cells (Fig.2g). Adipogenesis (adult and fetal): Oil red O staining demonstrated that peripheral cells showed more characteristic lipid droplets compared to inner cells (P <0.01) (Fig.2h, i).

Discussion: Our study showed that CD34+ and CD146+ cells, which were found more prevalent in the peripheral vascular region than in the inner avascular region, exhibited potential for multilineage differentiation, suggesting that these populations exhibit stem cell characteristics and may contribute to meniscal regeneration. Since adult cells did not express as much CD146 than fetal cells, and they did not display as great a degree of multilineage differentiation, it would appear to suggest that CD146 is important for meniscal healing. The present findings may provide important clinical insight for cell-based therapy aimed at enhancing meniscal repair and regeneration following injury.