# Modulating Human Meniscal Cell Phenotype via Matrix Adhesion and Stiffness

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**Introduction:** Stability and health of the knee joint relies on the integrity of the meniscus, a semilunar wedge-shaped disc of fibrocartilage that provides shock absorption and load distribution. Meniscus tears are the most common intra-articular knee injury, affecting one million people in the United States annually.[1] The only treatment options currently available to attempt repair and preserve the native tissue rely on suture techniques, and even the best results are characterized by formation of unorganized scar tissue. Because efficacy of suturing is a challenge, failure often results in the need for a partial meniscectomy, increasing the risk of developing osteoarthritis.[2] This shortcoming necessitates investigation of endogenous regeneration in meniscal tissue to facilitate the development of novel therapies. Regenerative cell behaviors, including migration, proliferation, and organized nascent extracellular matrix (ECM) deposition, are influenced by signals provided by the cellular microenvironment, namely macromolecule chemistry and mechanical properties. Additionally, these characteristics of the microenvironment can affect meniscal cell phenotype. *Our lab's single cell RNA sequencing of injured primary human inner meniscal tissue from young donors identified two subpopulations of matrix-synthetic cells that demonstrate a phenotypic range from more fibroblast-like cells (Cluster 0) clustering separately from more chondrocyte-like cells (Cluster 1) (**Figure 1A**).* We also have data that suggests the *stiffness of hyaluronic acid (HA)-based hydrogel substrates may play a role in controlling primary human meniscal cell phenotype when cells are cultured on the hydrogel surface (**Figure 1B**)*, with more balled up, chondrocyte-like morphology on lower stiffness gels and more spread, fibroblast-like morphology on stiffer gels.[3] In our HA-based hydrogels, initial adhesion and possible mechanotransducive signaling occurs via CD44-mediated cell-matrix adhesions, rather than integrin-mediated cell-matrix adhesions. CD44 is nearly ubiquitously expressed in meniscal cells, but because matrix-synthetic fibroblast-like meniscal cells differentially express integrin beta-1 (Cluster 0), it is important to understand how adhesion identity affects mechanotransducive signaling and subsequent meniscal cell phenotype. Taken together, the **overall goal** of this study is to determine the impact of cell-matrix adhesion and matrix mechanical properties on meniscal cell phenotype. We hypothesize that the presence of proteins with RGDS-containing adhesive sites (i.e., those suitable for integrin binding) and increased matrix stiffness will increase meniscal cell spreading, indicating a shift towards a more fibroblast-like and matrix-synthetic phenotype.

**Methods:** To investigate the impact of binding chemistry independent of substrate stiffness, primary young human meniscal cells (IRB Exemption #STUDY00000746) were cultured on tissue culture polystyrene (TCPS) coated to promote integrin binding (fibronectin, collagen) or CD44 binding (HA). Due to a thin coating, the cells experienced the same TCPS stiffness regardless of coating chemistry. HA coating (Lifecore Biomedical 500 kDA HA at 10 mg/mL in PBS overnight) was confirmed with Alcian Blue staining, fibronectin coating (Corning fibronectin at 10 µg/mL in water overnight) was confirmed with immunocytochemistry (ICC), and collagen-coated plates were purchased (Millipore). Meniscal cells (female age 14 and male age 17, passage 4) were cultured for 2, 5, and 7 days. ICC was performed to visualize nuclei, CD44, collagen, and the F-actin cytoskeleton. To investigate the impact of both binding chemistry and substrate stiffness, meniscal cells were cultured on the surface of HA-based hydrogels of low and high stiffness either with or without a fibronectin or collagen coating. Briefly, HA was functionalized with pentenoic anhydride via esterification and the resulting degree of reactive -ene group substitution (DoS) on the HA polymer backbone was determined via 1H NMR. This pentenoate-functionalized hyaluronic acid polymer was crosslinked into hydrogels of low and high stiffness (30% and 89% DoS, respectively) using a dithiol crosslinker (DTT) and LAP as the photoinitiator. Fibronectin coating (Corning fibronectin at 5 µg/mL in PBS for 2 hours) was again confirmed with ICC. Meniscal cells from the same donor as the 2D experiments were cultured for 24 hours and the same timepoints as 2D and ICC performed for same targets as in 2D. Images were taken using an Echo Revolution and analyzed using ImageJ. Statistical analyses were performed with outliers removed via ROUT (Q=1%), normality assessed via Shapiro-Wilk testing, and ANOVA (normally distributed data) or Mann-Whitney testing (non-normally distributed) conducted to determine significance.

**Results:** 2D Studies Preliminary ICC results show differential protein morphology depending on type of TCPS coating. Actin cytoskeleton signal appears diffuse in cells cultured on HA-coated plates compared to more linear stress fibers in fibronectin-coated and collagen-coated plates (**Figure 1C**), suggesting integrin binding is driving stress fiber formation. Additionally, CD44 is expressed in meniscal cells cultured in all conditions including uncoated TCPS. 3D Studies: Cells cultured without fibronectin coating on the surface of high stiffness HA gels showed more cell spreading compared to cells cultured on the surface of low stiffness gels (**Figure 1B**). Cells cultured on the surface of HA-based hydrogels with a fibronectin coating exhibit increased spreading (**Figure 1D**) compared to no fibronectin (HA alone), which is indicative of a more fibroblast-like cell phenotype compared to a more chondrocyte-like phenotype. These results support the use of binding site chemistry as a target for control of meniscal cell behavior, as well as implicate substrate mechanical properties as another method of modulating phenotypic shift. Ongoing experiments are expected to build on these results and further characterize cell phenotype based on adhesion mechanism, through ICC of an integrin matrix-adhesion related protein, integrin-beta-1, and collagen coating on HA gels modulating stiffness. Additionally, impact of binding site chemistry and substrate mechanics at initial substrate adhesion (~3 hrs) is currently being investigated with preliminary data suggesting increased stiffness impacts cell spreading and this is amplified with fibronectin coating.

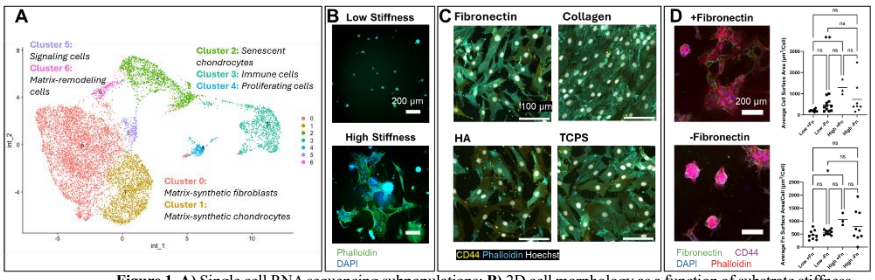


**Figure 1. A)** Single cell RNA sequencing subpopulations; **B)** 2D cell morphology as a function of substrate stiffness on HA gels; **C)** Meniscal cell morphology on coated TCPS substrates; **D)** 2D cell morphology on low stiffness HA gels as a function of fibronectin presence and semi-quantitative analysis of cell spreading and fibronectin presence

**Discussion:** This work builds from single cell RNA sequencing data to understand the impact of cell-matrix adhesion independently of matrix mechanical properties. Data generated during this study shows that meniscal cell adhesion to components of the surrounding microenvironment may impact cell phenotype and suggests that manipulating type and presence of adhesive sites may be a promising avenue for controlling this phenotypic shift. It is clear that microenvironment mechanics also play a role in meniscal cell phenotype, but this impact is currently linked to mechanosensing adhesion complexes and needs to be decoupled from binding site chemistry. **Clinical Relevance:** Understanding the impact of microenvironment composition and mechanics as well as cell adhesion to ECM components on meniscal cell behavior will contribute to the development of novel therapies for meniscal injury, such as injectable or implantable biomaterials with tailored composition, that will facilitate tissue repair and regeneration, enhancing the efficacy of current suturing techniques.