Superficial Layer Meniscus Cells Migrate Faster and are Less Mechanosensitive than those in the Meniscus Body

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Introduction

The knee meniscus supports dynamic joint motion and load transmission via its specialized organization and extracellular matrix (ECM) constituents. The meniscus contains an outer vascularized zone, an inner avascular zone, and two horns that anchor the meniscus to the tibia. Covering the entire tissue is a thin, specialized superficial layer. Previous studies have shown differences in superficial layer meniscus cell phenotype, ECM constituents, alignment, density, and mechanical properties compared to the deeper tissue of the meniscus body[1]. Recent work also demonstrated the critical role of progenitor cells localized to the superficial layer in meniscal wound repair[2]. However, it has also been noted that the repair tissue that does form contains altered extracellular matrix structure and composition and this may compromise the mechanical properties of the repair[3]. Therefore, gaps exist in our understanding of the cells that reside within this specialized superficial layer, and how they are mobilized in response to injury. The purpose of this study was to investigate the mechano-response of superficial cells to changes in mechanical environment as well as their migration potential, relative to donor-matched cells from the central region of the meniscus body. An understanding of the difference between the superficial and body zones of the meniscus may help connect pathology with cellular behavior to inform novel therapies.

Methods

Juvenile bovine medial and lateral menisci were dissected from two donors. Biopsy punches (12 mm diameter) were used to generate samples from the meniscus.

Histology and Staining

Samples were processed and embedded in paraffin. Sections (7 m thickness) were stained with toluidine blue to visualize tissue morphology.

In vitro culture

A custom device (Figure 1) was used to isolate samples from the superficial zone (~100 m depth) and deeper (body) zones (1 mm depth). Tissue sections were minced and incubated at 37C in basal media (DMEM with 10% fetal bovine serum and 1% penicillin / streptomycin / fungizone) for two weeks, during which time cells migrated from tissue and onto the tissue culture plastic. Cells from each region were frozen for subsequent assays and used between passage 1 and 3.

Substrate stiffness, Immunofluorescent staining, Imaging, and Analysis

5, 10, and 55 kPa polyacrylamide gels were prepared on glass slides and coated with fibronectin as in [4] prior to seeding with cells from the superficial and body zones. Cells were stained with Hoechst 33342 (nuclear stain), phalloidin (f-actin stain), and goat anti-YAP/TAZ (1:1000 in 1% BSA). Confocal microscopy was used to acquire images for each channel using the same imaging parameters across the samples, followed by quantification of cell area, aspect ratio, and YAP/TAZ nuclear to cytoplasmic ratio (Cell Profiler).

2D Wound Healing Assay

Cells were plated in six-well tissue culture dishes at 4 x cells per well and cultured to confluence. Next, a 200 ml pipette tip was used to scratch the cell monolayer. Images were taken using a

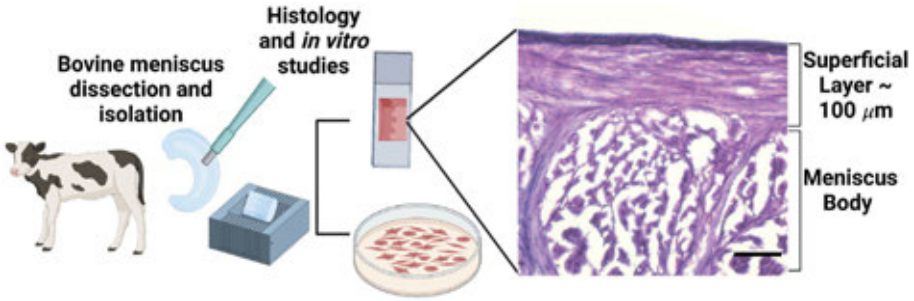

Figure 1. Study design. Juvenile bovine menisci were isolated for histology and in vitro cell culture studies. Images shows toluidine blue staining of superficial and deeper body zones. Scale bar: 100 m.

brightfield inverted microscope every 2 – 4 hours after scratching. Wound closure was computed and analyzed using ImageJ.

Statistics

Figure 2B, 2C: ANOVA. Figure 3B: t-test.

Results

Histological analysis confirmed that the superficial layer was distinct from the deeper meniscus body, as previously reported[5] (Figure 1). When placed on substrates of differing stiffness, superficial layer cells showed differences in mechano-response (Figure 2A). Specifically, superficial meniscus cells were smaller on stiffer substrates (10 and 55 kPa) and had a decreased level of nuclear YAP/TAZ in comparison to body cells. The spread area of superficial cells showed little change with increasing stiffness (Figure 2B), while there was a marked increase in spread area for body meniscus cells with increasing stiffness (Figure 2B). Notably, the superficial and body meniscus cells had similar areas at the smallest stiffness, but a significant difference in spread area at higher stiffnesses (Figure 2B). Similarly, body cells had more nuclear YAP translocation as compared to superficial cells (Figure 2C), indicating that superficial were not as mechanoresponsive as body cells. Superficial cells also migrated faster than body cells (Figure 3A-B). At 36hrs, the scratch wound completely closed for superficial zone cells, whereas the wound remained nearly 50% open for body zone cells. These results indicate that superficial cells have a higher migration potential than body cells on tissue culture plastic.

Discussion

This study shows mechanobiological differences between superficial cells and the rest of the meniscus, specifically in mechanosensation and migration potential. Previous work characterized superficial cells as progenitors for meniscus regeneration [2]. Our findings suggest that the fast migration and decreased response to substrate stiffness could account for their rapid filling of meniscus defects and may impact how they form new repair matrix. Further characterization of meniscus repair matrix is needed to elucidate the role of superficial layer cells in regeneration. Future studies will assess the proliferation rate, cytoskeletal regulation, and matrix formation potential of superficial layer cells.

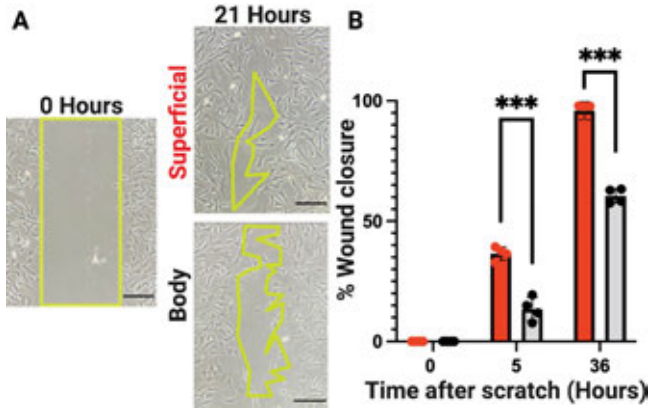

Figure 3. 2D Migration Assay. Superficial cells closed the wound faster than cells from the body of the meniscus. A) Image at left shows wound at time zero. Middle images show wound after 21 hours. B) Quantification of wound closure over 36 hours. Sample number: 5 per group, ***$p < 0.001$. Scale bar: 150 m.

Significance

This work shows key mechanobiological distinctions between the superficial layer cells and the rest of the meniscus. The lower mechanosensitivity of superficial layer cells despite their high migration potential suggests that, albeit their progenitor nature, those cells respond less to micromechanical cues. These results may connect the progenitor nature of superficial layer cells with the poor intrinsic healing potential of this progenitor population in the meniscus.

Acknowledgements

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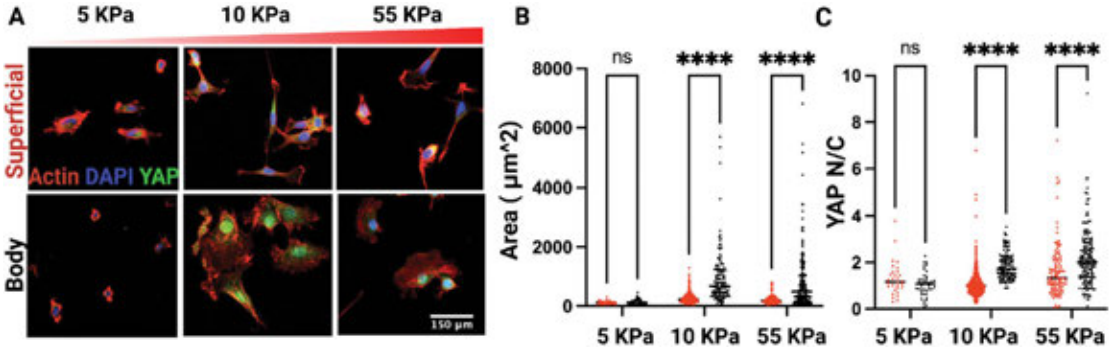

Figure 2. Response to Mechanical Environment. Superficial cells did not show a significant change in area or nuclear YAP with an increase in stiffness, unlike cells of the meniscus body. N/C: Nuclear/Cytoplasmic, sample number: 5 per group, ****$p < 0.0001$. Scale bar: 150 m.