Meniscus-Mimetic Platform Recapitulates Zone- and Age-Specific Microenvironmental Effect on MSC Behavior

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INTRODUCTION: The meniscus exhibits distinct zone-dependent biological and mechanical properties (**Fig. 1a**) that are critical for load distribution and joint homeostasis **[1]**. These zonal differences arise during development, yet the mechanisms that establish and regulate them remain poorly understood. To address this gap, in this study, we developed an *in vitro* model system that mimics development to investigate how zone-dependent mechanical environments, particularly hoop stresses that emerge as compressive loads are redirected into tensile forces along the collagen fiber network **[2]**, influence cell behavior. In addition, prior studies have shown that stem cell behaviors are regulated by chemical cues from fetal and adult bovine meniscus-derived extracellular matrix (ECM) **[3, 4]**. Since aging profoundly alters meniscus ECM composition and mechanics, we further examined how age-dependent ECM properties modulate mesenchymal stem cell (MSC) morphology under hoop stress conditions. This approach provides a platform to explore the interplay between zonal mechanics, ECM aging, and stem cell responses, offering insight into mechanisms that govern meniscus maturation and degeneration.

METHODS: Fetal (3rd trimester) and adult (~30 months) bovine menisci were decellularized (d) following previous protocols **[3]**. A curved mold with a 7.5 mm radius was fabricated using a stereolithography 3D printer with a high-temperature resin (**Fig. 1b**). Juvenile bovine mesenchymal stem cells (bMSCs; Passage 2) were encapsulated in fetal dECM (FDEM) or adult dECM (ADEM) hydrogels and deposited onto the mold surface through a nozzle using a custom rotation system integrated into a 3D bioprinter (**Fig. 1b**). The constructs were then cross-linked at 37 °C for 30 minutes. The geometry of the 3D-printed dECM gels was designed to undergo both tension and compression to generate a hoop stress (**Fig. 1c**). Stress distribution within the designed hydrogel model was analyzed following simulation of boundary and loading conditions to simulate Hoop stress using finite element analysis (FEA; Abaqus, Simulia, USA). Samples were cultured in basal growth medium for 7 days. For additional validation, cylindrical dECM hydrogels (5 mm inner diameter) containing bMSCs were fabricated and cross-linked under the same conditions. To test the role of cell contractility, cylindrical dECM hydrogels were cultured in basal medium supplemented with 20 μM Y-27632 (Y27). At day 7, encapsulated cells were stained with Phalloidin (Invitrogen, USA) and imaged. Cell aspect ratio was quantified using ImageJ, and cellular alignment was analyzed using FiberFit software **[5, 6]**.

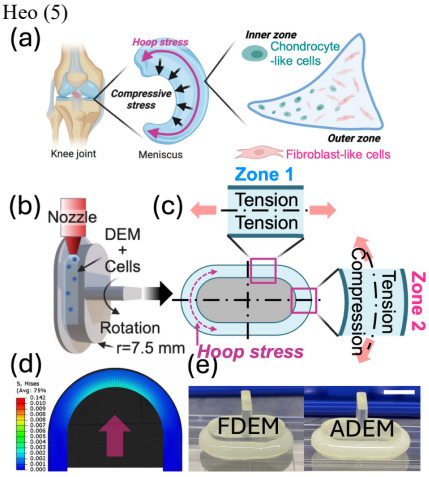

Figure 1: (A) Illustration of hoop and compressive stresses in the meniscus. (B) Fabrication method of the meniscus-mimetic model. (C) Schematic of stress types within the model. (D) Strain distribution and boundary conditions from FEA analysis. (E) Meniscus-mimetic constructs fabricated using bovine fetal and adult ECM (Scale bar: 10 mm).

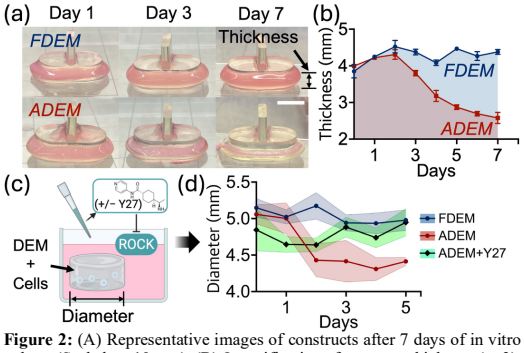

Figure 2: (A) Representative images of constructs after 7 days of in vitro culture (Scale bar: 10 mm). (B) Quantification of construct thickness (n=3). (C) In vitro test using cylindrical models. (D) Quantified diameter of cylindrical constructs after culture (n=5, showing standard deviation).

RESULTS: Finite element analysis (FEA) confirmed the distribution of hoop stress within the meniscus mimic model, showing localized compressive stress in the inner region of Zone 2 associated with hydrogel contraction, likely driven by cell-mediated hydrogel compaction forces (**Fig. 1d**). Meniscus-like constructs were successfully fabricated using FDEM and ADEM hydrogels on the system (**Fig. 1e**). After 7 days of culture, the ADEM constructs exhibited a significant reduction in thickness compared to the FDEM group **(Fig. 2a, b)**. To determine whether this contraction was driven by cell activity, cultures were treated with the ROCK inhibitor Y-27632 (Y27) (**Fig. 2c**). By day 5, Y27-treated ADEM constructs showed no evidence of ECM contraction, indicating that cell contractility contributed to the observed thickness changes **(Fig. 2d)**. Phalloidin staining revealed zone-specific cell morphology: in the inner region, encapsulated cells displayed a circular shape, while in the outer region they were significantly elongated and aligned along the hoop stress axis generated by hydrogel contraction (**Figs. 3a-e**). Quantitative analysis showed that the mean orientation angle (Based on neutral axis) of cells in the outer region of Zone 2 within ADEM constructs was 161.6 ± 43.2 degrees (**Fig. 3f**).

DISCUSSION: These findings demonstrate that the zonal compressive and tensile stress patterns predicted by FEA were successfully recapitulated in both the FDEM and ADEM constructs. Limiting contraction induced circumferential hoop stress, which likely acted as a mechanical cue guiding cell and fiber alignment **[7]**. The significant thickness reduction observed in ADEM hydrogel indicates that cell-mediated matrix contraction played a dominant role, as confirmed by the absence of cellular contraction under ROCK inhibitor treatment. Furthermore, region-specific differences in cell morphology and orientation highlight the ability of this platform to provide spatially relevant mechanical and structural cues that mimic the native meniscus microenvironment. Cells in the inner region adopted a rounded, chondrocyte-like morphology, whereas cells in the outer region elongated and aligned along the hoop stress axis, reflecting the fibroblast-like phenotype typical of the native meniscus. Importantly, the hoop stress environment reproduced the mechanically heterogeneous state of the meniscus, where compressive forces dominate the inner zone and tensile forces the outer zone. The stronger contractile response in ADEM compared to FDEM suggests that age-associated ECM changes amplify matrix remodeling and alignment cues, potentially biasing the tissue toward fibrotic remodeling rather than regeneration. Collectively, this study provides a promising platform to investigate age- and zone-specific mechanobiology of the meniscus, while informing the design of bioinspired regenerative strategies that recapitulate developmental and zonal microenvironments.

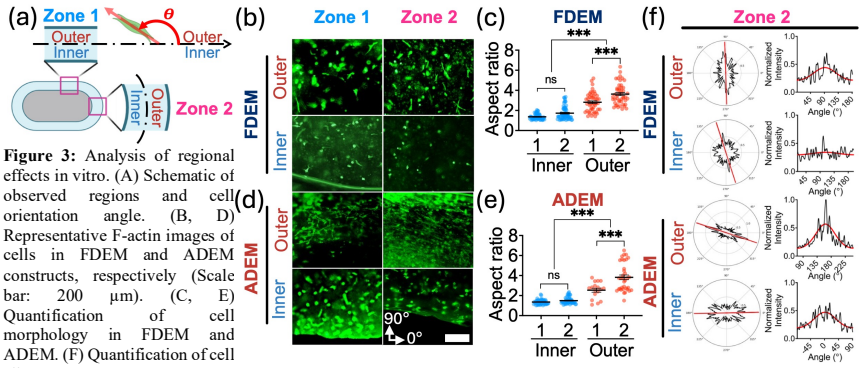

Figure 3: Analysis of regional effects in vitro. (A) Schematic of observed regions and cell orientation angle. (B, D) Representative F-actin images of cells in FDEM and ADEM constructs, respectively (Scale bar: 200 μm). (C, E) Quantification of cell morphology in FDEM and ADEM. (F) Quantification of cell alignment.

SIGNIFICANCE/CLINICAL RELEVANCE: This study establishes a bioinspired meniscus-mimetic platform that reproduces native region-specific mechanical and structural cues, enabling precise evaluation of cell behavior under physiologically relevant hoop stresses. This approach offers a translational framework for regenerative strategies tailored to the zonal heterogeneity of the meniscus, with the potential to improve clinical outcomes in meniscal repair.

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