

Regulating Fibrous Tissue Contraction via Hydrogel Presentation of Cell-Cell or Cell-ECM Adhesive Ligands

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INTRODUCTION: Fibrous tissue (tendon, annulus fibrosus, meniscus) growth and development is exemplified by dramatic transitions from cell rich-disorganized aggregates to ones that are cell-poor but rich in organized extracellular matrix (ECM) [1]. As such, development of these tissues includes a transition from a state in which information is relayed through cell-to-cell contacts to one governed by cell-to-matrix contacts. Further, forming tissues often use boundaries as mechanical anchorage points against which cell-mediated contraction occurs [2]. Not only are these growth and remodeling events necessary to achieve the final functional form of these fibrous tissues, but these same physical cues can regulate resident cell differentiation and function to drive regional tissue specialization [3]. How, and to what extent, the crosstalk between cells, their deposited ECM and evolving forces in the developing tissue governs cell function is difficult to study given the complexity of cellular interactions in vivo and the inability to decouple these cues. To that end, we developed a fully-defined, tunable composite fibrous hydrogel system that can undergo cell-mediated contraction. Using this tunable material, we presented adhesive ligands representative of cell-cell interactions (via HAVDI, an N-cadherin based peptide) or cell-ECM (via RGD, a fibronectin-based peptide) and evaluated how these interactions impacted neo-tissue contraction, cell alignment and tissue formation.

METHODS: Material Fabrication: The composite material (**Fig. 1A**) consists of a continuous phase (CP) of acrylated hyaluronic acid (AHA) and a fragmented fiber (FF) phase consisting of short RGD or HAVDI-modified [4] methacrylated HA fibers (produced by electrospinning followed by mechanical fragmentation [5]). In this composite, crosslinking occurs via a Michael addition reaction with the sequential addition of dithiothreitol (DTT) at a basic pH. Cell-Mediated Contraction Assays: Constructs were created by mixing juvenile bovine meniscal fibrochondrocytes (MFCs) or porcine MFCs at varying developmental stages with the prepolymer solution, pipetting into polydimethylsulfoxide (PDMS) molds (plug: unconstrained, wells with middle post: constrained), and allowing constructs to partially crosslink for 1 hour at pH 8 and 37°C before adding media. In this phase, a specific proportion of the acrylates are consumed, leaving the remaining groups free for subsequent crosslinking steps. To fully crosslink constructs, excess additional DTT was added at defined time points and allowed to crosslink as above. Imaging: Fluorescent fibers and cells were visualized by actin or CellTracker Red stain and imaged on a confocal microscope. Bulk hydrogel images were taken with a brightfield microscope at defined time points and contraction extent was quantified using ImageJ. Statistics: Outcomes were compared using one-way ANOVA with Tukey posthoc, with significance set at $p < 0.05$. For all quantified samples, $n = 3-10$, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

RESULTS: In initial studies, the composite system was evaluated using juvenile bovine meniscus cells. With a 5 wt% fragmented fiber (FF) concentration and a 0.7 wt% CP concentration, gels contracted similarly to collagen gels (3 mg/mL), with area decreasing by 66% over the first day, and contraction persisting through day 3 (**Fig. 1B,C**). Notably, cells transitioned from a rounded to an elongated morphology during this time (**Fig. 1D**). The extent of contraction could be modulated by changing density of the CP (**Fig. 1E**), the FF (**Fig. 1F**), and the cell concentration (**Fig. 1G**). Notably, contraction could be fully arrested if constructs were transitioned from partial to full crosslinking (**Fig. 1H**). To determine how cell-cell versus cell-ECM interactions impacted tissue contraction, porcine meniscus cells (either embryonic day 42 (E42) or juvenile (9 months)) were seeded into composites where fibers were modified with either RGD or HAVDI and allowed to contract around PDMS posts (**Fig. 2A**). For E42 cells, constructs contracted rapidly when fibers were modified with RGD, but showed slower contraction with HAVDI modified fibers (**Fig. 2B,C**). For juvenile cells, constructs rapidly contracted in RGD-modified fiber composites, but did not contract in composites presenting HAVDI (**Fig. 2D,E**).

DISCUSSION: Here, we introduce a novel fibrous material and explored how cell interactions with RGD (mimicking ECM) versus HAVDI (mimicking cell-cell interactions) modified fibrous elements influenced contraction. In E42 cells, but not juvenile cells, adherence to HAVDI supported contraction, suggesting that embryonic cells adhere to and/or mechanosense cell-to-cell contacts to a greater extent than cells from later developmental states. Interestingly, the E42 cells also rapidly contracted RGD-presenting constructs, suggesting that, at this developmental stage, the cells can utilize nascent ECM to contract and form an organized tissue. Because of maturation and matrix deposition, the juvenile meniscus is relatively cell-poor, with cells interacting almost exclusively with the ECM. Interestingly, constructs formed from more mature cells rapidly contracted RGD-presenting fibrous materials, but not HAVDI, suggesting a transition in phenotype in these cells. Future studies will explore the effect of adhesive moieties on the progression of meniscus cell phenotype during patterning, as well as how varying combinations of RGD and HAVDI-presenting extracellular environments impact matrix elaboration and regional specification.

SIGNIFICANCE: This novel material system enables the exploration of key developmental inputs that guide meniscus formation, providing new insight into regenerative strategies that may be leveraged towards meniscus repair.

REFERENCES: [1] Clark et al. *JBS*, 65(4), 538-547, 1983. [2] Ma et al. *AJP-Cell Physiology*, 323 (6), 1652-1663, 2022. [3] Bonnevill et al, *Nat. Biomed Eng.*, 3, 998-1008, 2019. [4] Cosgrove et al. *Nat. Mater.* 15(12), 1297-1306, 2016. [5] Davidson et al, *Sci Adv.* 7 (46), 2021.

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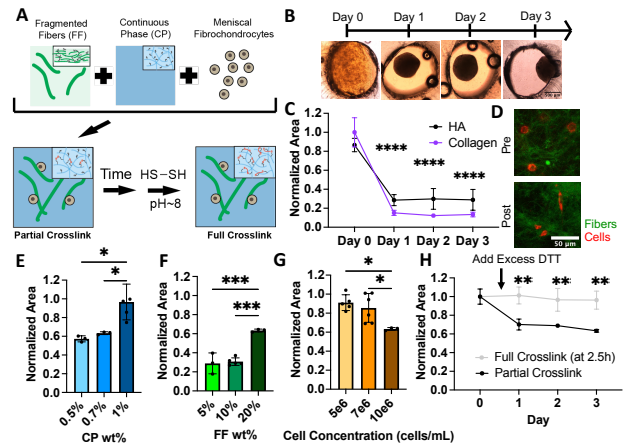


Figure 1: A) Schematic of Contractile Hydrogel. B,C) Representative images of construct (0.7% CP, 5% FF, 10⁶ cells/mL) plug contraction (B) and corresponding quantification (C, asterisks denote time points significantly different from corresponding Day 0 group, HA - hyaluronic acid-based fibrous hydrogel). D) Cellular morphologies and interactions with fiber population before contraction (Pre) and after (Post). E-G) Quantification of contraction with varying CP wt% (E), FF wt% (F), and cell concentration at day 3 (G, unless parameter of interest, constructs were 0.7% CP, 20% FF, 10⁶ cells/mL). H) Comparison of contraction when partially crosslinked (allowing cell remodeling) compared to fully crosslinked (halting remodeling), asterisks denote significance between partially and fully crosslinked (0.7% CP, 20% FF, 10⁶ cells/mL).

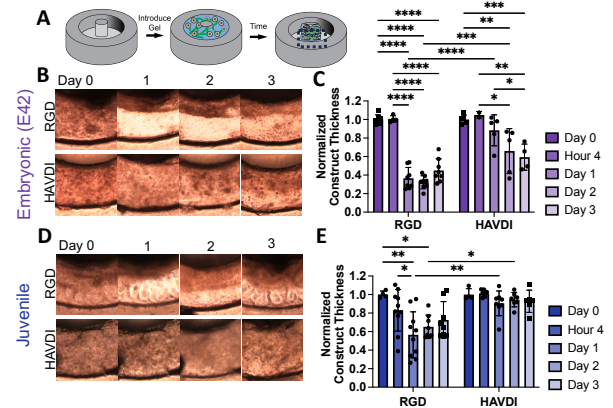


Figure 2: A) Schematic of PDMS mold with construct (0.7% CP, 5% FF, 10⁶ cells/mL) contraction over time. Dotted black line represents area shown in B and D. B-E) Representative images and quantification of E42 (B,C) and juvenile (D,E) porcine cell construct contraction.