Characterizing Meniscus Cell Mechano-Activation During Microgel Assisted Tissue Bulking

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Introduction: Due to limited vascular presence, inner zone tears of the meniscus have poor intrinsic healing capacity. Further, additional tissue deterioration following a partial meniscectomy is common, and has no clinical treatment options. Preliminary data shows the use of novel mechano-instructive hydrogel microparticles for facilitating meniscus wound closure via tissue bulking, an FDA approved acelluar therapeutic strategy. This strategy uses biocompatible hydrogels that are injected into a lesion to facilitate new tissue growth. Previous data showed that meniscus cells had increased cell spread and nascent matrix deposition when seeded on composite gels of norbornene-modified hyaluronic acid (NorHA) hydrogel microparticles encapsulated in bulk collagen. In this project, we further characterized the mechano-responsive activation of meniscus cells to NorHA microgels in order to enhance the clinical translation of this potential meniscus injury repair strategy.

Materials and Methods: 2D meniscus cell mechano-sensitivity confirmation: primary bovine meniscus cells (bMFCs) were cultured on polyacrylamide (PA) hydrogels with stiffnesses of 5 kPa, 55 kPa, and a glass positive control. Following 1d, cells were stained for Yes-associated protein (YAP) mechano-active transcriptional activator, actin (phalloidin-564), and nuclei (hoechst 33342) via immunofluorescence and imaged using confocal microscopy. Images were quantified for YAP nuclear localization, and cell shape, number, and density using Dragonfly software. Composite gel formulation: Soft (~5 kPa), stiff (~8 kPa), or no NorHA microgels (3wt%, NorHA, 2wt% fluorescein isothiocyanate green fluorophore, 0.05% LAP, 1mM RGDS, and variable DTT concentrations to control gel stiffness; fabricated using batch emulsion[2]) and bMFCs were encapsulated within collagen hydrogel (~2 kPa). Confocal: following 1d of culture, composite gels were stained, imaged, and analyzed as described above for PA gel experiments. Western blotting: following 1, 3, and 7d of culture, proteins were extracted from composite gels with RIPA buffer and separated on SDS-PAGE gels. Blots were transferred to PVDF membranes, treated with primary antibodies (YAP, anti-rabbit, ColI, anti-rabbit, Histone 3, anti-mouse), incubated in species-specific HRP-conjugated secondary antibodies, imaged using a ChemiDoc XRS Imaging System (BioRad), and analyzed using FIJI software. All statistical analysis was performed using PRISM software.

Results and Discussion: YAP nuclear localization is an indicator of cellular mechanoactivity.[2] There was a clear localization of YAP to the nucleus on the glass and 55 kPa substrates, as well as increased cell spreading on these substrates, compared to the soft substrate condition (Fig 1). Composite gels with stiff NorHA microgels had increased total cell number and cell spreading compared to gels with soft or no microgels (Fig 1). YAP nuclear localization increased when in proximity to both soft and stiff microgels, indicating meniscus cell mechano-activation in composite gels containing microgels (Fig 1). Global YAP protein expression did not significantly differ between conditions, which is potentially explained by heterogeneity in YAP subcellular localization in each composite gel. COL1 extracellular matrix (ECM) protein expression trended higher in composite gels with stiff microgels at 1d and 3d time points, indicating potential ECM repair capabilities of stiff NorHA microgels (data not shown).

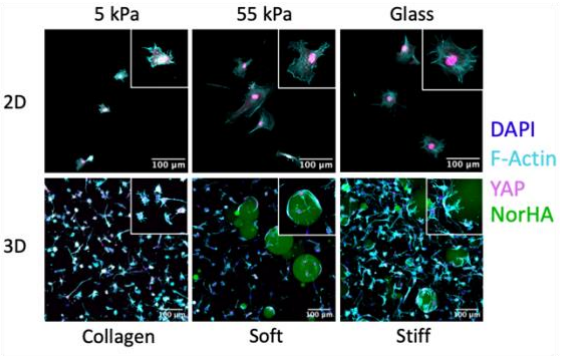

Figure 1. Meniscus cell mechano-activation on stiffer substrates. (Top row) bMFCs seeded on 2D substrates of varying stiffnesses. (Bottom row) bMFCs encapsulated in bulk collagen with no, soft, or stiff NorHA microgels.

Conclusions: Mechano-instructive hydrogels have the potential to activate meniscus cells, facilitating meniscus wound repair through increased cell proliferation, cell spread, and ECM protein production. This therapeutic strategy has high clinical translatability due to its simplicity of design and represents a potential treatment for dense connective tissue injuries.

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