Gradually Softening Hydrogels for Understanding Cell Behavior During Fibrosis Regression

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Introduction: There is a critical unmet need for hydrogel systems that can recapitulate salient features of native tissues during processes such as morphogenesis, aging, and disease. The extracellular matrix (ECM) of tissues is dynamic, presenting an evolving set of mechanical and compositional cues to resident cells. *In vivo* studies suggest that during the regression of liver fibrosis hepatic stellate cells (HSCs), the primary source of fibrogenic myofibroblasts, either undergo apoptosis or acquire a phenotype that appears similar to healthy quiescent HSCs but is characterized by abnormal gene expression and unusually rapid myofibroblastic differentiation after a repeat insult [1]. However, the role of liver mechanics in mediating this process is unknown. This work introduces a methacrylated hyaluronic acid (MeHA) hydrogel system containing a combination of stable and hydrolytically degradable crosslinks that presents a gradually softening (but not fully degradable) milieu to mimic physiological softening events such as fibrosis regression.

Results: MeHA hydrogels (3 wt%) were crosslinked with a combination of thiol-based crosslinkers via Michael-type addition. Stable crosslinks were introduced with dithiothreitol (DTT) while PEG-based thiolated crosslinkers containing ester groups [2] susceptible to hydrolysis were also incorporated. These gels displayed relatively high initial stiffness (~15-20 kPa) and gradually softened over the course of 14 days to a stable stiffness of ~2-3 kPa. These values compare favorably to fibrotic and healthy liver respectively. Rat HSCs that were mechanically primed on tissue culture plastic initially remained spread on stiff gels (both non-degradable and degradable variants) with significantly larger cell area, loss of lipid droplets, and expression of the myofibroblast marker α-SMA. However, following 14 days of culture HSCs on softening gels showed significantly reduced cell area and more lipid droplets compared to HSCs on stiff gels. Human mesenchymal stem cells (hMSCs) showed a similar response when cultured on softening gels, displaying gradually decreasing cell area (Day 1: 3110 ± 400 μm^2, Day 7: 842 ± 126 μm^2) and reaching a similar area to hMSCs cultured on soft static gels after 7 days (743 ± 86 μm^2).

Conclusions: We describe a softening hydrogel system to explore the influence of dynamic changes in mechanical properties on cell phenotype during fibrosis regression. Ongoing work is developing gel formulations with a wider range of degradation profiles and evaluating HSC and hMSC phenotype in response to different softening dynamics.

References:

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