Mechanically Dynamic Hydrogels for Probing Hepatic Stellate Cell Response in Fibrosis

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Introduction: Chronic liver injuries drive hepatic stellate cell (HSC) differentiation into myofibroblasts. This activation process leads to excessive extracellular matrix (ECM) deposition, resulting in fibrosis and loss of liver function. While static model systems have provided insight into the role of mechanics in the development of fibrosis, there is a paucity of engineered model systems that can recapitulate the temporal dynamics of disease states in order to understand relevant cellular signaling events. We are addressing this issue by developing a suite of static and dynamic hyaluronic acid (HA) hydrogel substrates to model the mechanical progression and regression of liver fibrosis.

Results: Stiffening and softening methacrylated HA (MeHA) systems were developed to mimic the progression and regression of liver fibrosis respectively. Soft-to-stiff MeHA gels were fabricated by an initial Michael-type addition reaction followed by visible light-mediated secondary radical crosslinking at user-defined time point(s) with no negative impact on HSC viability observed. HSC myofibroblast differentiation was correlated with substrate stiffness, not the generation of radicals during secondary crosslinking. The timing of stiffening significantly affected HSC phenotype with later stiffening (day 7) resulting in more rapid cytoskeletal re-organization as measured by cell spread area and α-smooth muscle actin (α-SMA) expression compared to early stiffening (day 1). After 14 days, ~ 95% of HSCs cultured on stiffened gels expressed α-SMA regardless of early (day 1) or late (day 7) stiffening. Stiff-to-soft gels mimicking the regression of fibrosis were formed by incorporating ester-containing hydrolytically-degradable crosslinkers. Mechanics could also be cycled to model repeated injury. Stiff-to-soft gels (Day 0 stiffness: 17.3 ± 4.5 kPa, Day 14 stiffness: 3.4 ± 1.0 kPa) could be re-stiffened by radical polymerization of the remaining gel methacrylates (re-stiffened modulus: 30.0 ± 3.7 kPa).

Conclusions: We describe a series of stiffening and softening hydrogel constructs to explore the influence of dynamic changes in mechanical properties on HSC phenotype during fibrosis activation and regression. Critically, this platform enables nuanced interrogation of HSC phenotype in response to an evolving set of microenvironmental cues. Ongoing work is tracking *in vivo* liver mechanics during fibrosis induction and recovery to guide rational biomaterial design. Together, the dynamic substrates developed here will be useful as streamlined models of other disease states in the wound healing-fibrosis-cancer triad and as *ex vivo* tools to identify potential targets for therapeutic intervention.