Mechanically Dynamic Hyaluronic Acid Hydrogels for Investigating Cellular Mechanotransduction

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Statement of Purpose: Hyaluronic acid (HA) is a natural component of the extracellular matrix (ECM) involved in many biological processes. HA can be chemically modified in a variety of ways to obtain hydrogels with tunable properties such as degradation, mechanics, and integrin binding. HA and other hydrogel systems have provided insight into the role of mechanics in controlling cell behavior; however, most of these platforms have been static and could not recapitulate the temporal dynamics of disease states (e.g., liver fibrosis). We have addressed this problem by developing HA hydrogels that dynamically change their crosslink density with time, either through the introduction of new crosslinks via a light-mediated polymerization process for stiffening gels or through decreasing crosslink density via controlled degradation for softening gels. We then used these substrates to model cell behavior during the mechanical progression (stiffening) and regression (softening) of liver fibrosis.

Methods: Hydrogels were fabricated from methacrylated hyaluronic acid (MeHA). Stable MeHA gels were made by tuning the amount of dithiothreitol (DTT) crosslinker. Soft-to-stiff gels were fabricated by an initial Michael-type reaction followed by secondary photocrosslinking of unreacted methacrylates (Fig 1a). Stiff-to-soft gels incorporated a mix of DTT and ester-containing hydrolytically-degradable crosslinkers (Fig 1d). Softened gels could be re-stiffened via photopolymerization of unreacted methacrylates. Gel mechanics were measured by atomic force microscopy (AFM), rheometry, and compression testing. Hepatic stellate cells (HSCs), the main liver cell type responsible for fibrosis, were isolated from rats by in situ perfusion and density gradient centrifugation. Expression of α-smooth muscle actin (α-SMA) and Yes-associated protein (YAP) were assessed via immunostaining.

Results: Soft-to-stiff gels were fabricated with visible light-mediated secondary crosslinking at user-defined time point(s). Stiffness increased from ~ 2 kPa to ~ 30 kPa with no negative impact on HSC viability (Fig 1a-b). HSC myofibroblast differentiation was correlated with substrate stiffness. 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-SMA expression compared to early stiffening (day 1). After 14 days, ~ 95% of HSCs cultured on stiffened gels expressed α-SMA and ~ 90% showed nuclear localization of YAP, regardless of early (day 1) or late (day 7) stiffening. Up-regulation of α-SMA and collagen I genes was also observed on stiffened substrates. Although α-SMA expression was eventually observed in almost all HSCs cultured on stiffened substrates, 48 h after stiffening only half of cells express α-SMA while ~ 90% show nuclear localization of YAP (Fig 1c). Stiff-to-soft gels were formed by incorporating ester-containing hydrolytically-degradable crosslinkers as well as stable DTT crosslinks. Gel modulus (initial modulus 20.3 ± 7.0 kPa) decreased over time

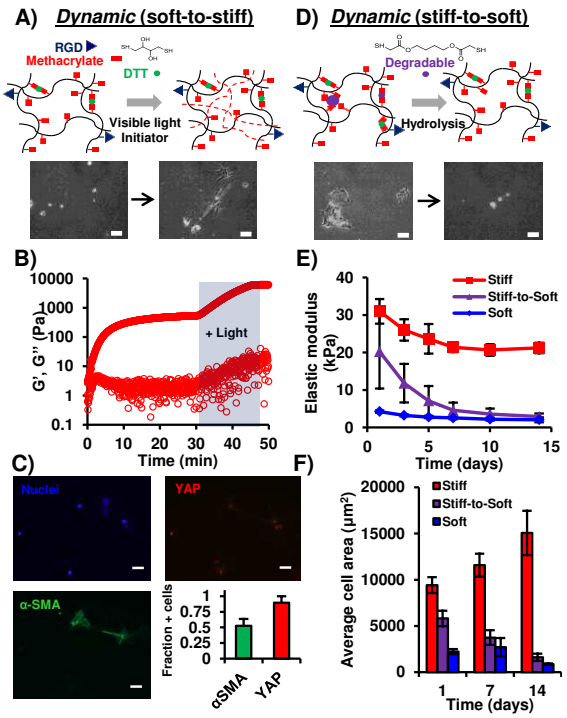

Figure 1. A) Fabrication approach for soft-to-stiff gels with resultant changes in HSC spreading. B) Rheometry shows order of magnitude increase in gel stiffness in response to LAP and visible light-mediated secondary crosslinking. C) HSC α-SMA expression lags behind YAP nuclear localization after stiffening. D) Fabrication approach for stiff-to-soft gels with resultant changes in HSC spreading. E) Stiff-to-soft gels display gradually decreased mechanics after 14 days, reaching original level of soft gels. F) HSCs on stiff-to-soft gels show reduced cell area over 14 days. Scale bars: 50 μm.

to a final stable modulus of 2.9 ± 0.8 kPa (Fig 1e). Mechanically-primed HSCs initially spread on stiff-to-soft gels but then gradually showed decreased cell area (Fig 1f). Mechanics could also be cycled to model repeated injury. Stiff-to-soft gels could be re-stiffened by radical polymerization of the remaining gel methacrylates (re-stiffened modulus: 30.0 ± 3.7 kPa).

Conclusions: We present a hydrogel platform based on dynamic crosslinking of the natural polysaccharide HA. We show that visible-light mediated secondary crosslinking results in an order of magnitude change in stiffness while maintaining HSC viability and promoting myofibroblast differentiation. HSCs show nuclear localization of YAP before α-SMA expression. Softening gels with a mix of stable and hydrolytically degradable crosslinks were also synthesized. These gels support reduced spreading of mechanically-primed HSCs over time and could also be re-stiffened to mimic a second injury event. Together, the dynamic substrates developed here will be useful as streamlined models of disease and as tools to identify potential targets for therapeutic intervention.