Dynamically Softening Hydrogels for Probing Hepatic Stellate Cell Response in Fibrosis and Regression

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Statement of Purpose: Chronic injury to the liver drives 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. Local tissue mechanics influence HSC behavior, with HSCs retaining a quiescent phenotype in soft environments and undergoing myofibroblast differentiation in stiffer environments. While static model systems have provided insight into the role of mechanics in the development of fibrosis, there is a paucity of *in vitro* model systems that can recapitulate the temporal dynamics of disease states. We are addressing this issue by developing a suite of static and dynamic hydrogel substrates to model the mechanical progression and regression of liver fibrosis.

Methods: Hydrogels were fabricated from methacrylated hyaluronic acid (MeHA) as previously described [1]. RGD containing peptides (GCGYGRGDSPG, Genscript) were coupled to MeHA macromers via a Michael-type addition reaction (*Figure 1a*). Stable MeHA gels over a range of stiffnesses were fabricated by tuning the amount of the non-degradable crosslinker dithiothreitol (DTT) (*Figure 1b*). Softening hydrogels incorporated ester-containing hydrolytically-degradable crosslinkers [2]. Hydrogel thin films were fabricated in PDMS molds covered with thiolated coverslips. Hydrogel mechanics were measured by atomic force microscopy (AFM). HSCs were isolated by *in situ* perfusion followed by density gradient centrifugation [3]. HSCs were seeded onto MeHA substrates at $5x10^3$ cells per cm^2 with media replaced twice a week. Cell spread area was measured every other day (ImageJ) from bright field images. Expression levels of α-smooth muscle actin (α-SMA) and peroxisome proliferator-activated receptor-γ (PPAR-γ) were assessed via immunostaining. ANOVA with Tukey's HSD post hoc tests were used to make comparisons between groups (n = 3 samples per group).

Results: Soft and stiff MeHA gels (~85% modification with DTT and/or degradable crosslinkers) were fabricated with starting elastic moduli of ~ 2-3 kPa and 10-15 kPa, respectively. Stiff to soft gels displayed decreased mechanics after 14 days of incubation in media at 37°C with elastic modulus similar to the original levels measured in soft gels (*Figure 1c*). HSCs that were mechanically primed on tissue culture plastic for 14 days 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 stiff to soft gels showed significantly reduced cell area (Day 1: 5818 ± 834 μm^2, Day 14: 1618 ± 390 μm^2) compared to HSCs on stiff gels (Day 1: 9422 ± 860 μm^2, Day 14: 15062 ± 2392 μm^2) (*Figure 1d,e*).

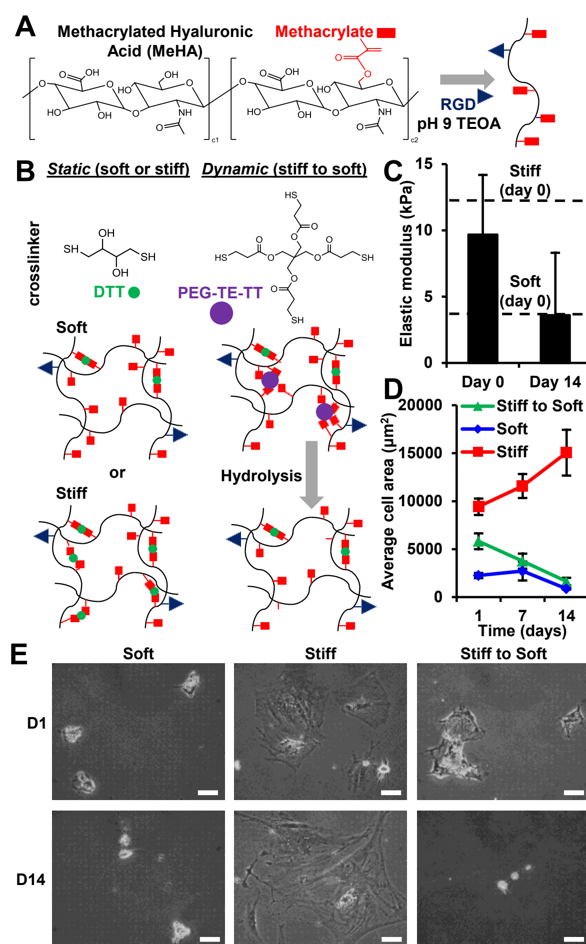

Figure 1. A) Chemical structure of MeHA and coupling of RGD. B) Various routes to alter gel mechanics (left, static systems; right, stiff to soft dynamic system). C) Stiff to soft gels display decreased mechanics after 14 days, reaching the original level of the soft gels. D, E) Mechanically primed HSCs remain spread on stiff gels, rounded within a day on soft gels, and gradually showed decreased cell area and new lipid droplets (bright spots) when cultured on stiff to soft gels. Scale bars: 50 μm.

Conclusions: We describe a series of hydrogel constructs to explore the influence of dynamic changes in mechanical properties on HSC phenotype during fibrosis activation and regression. This tunable system allows interrogation of mechanical instructive cues on HSC phenotype, and more broadly should be useful for future studies of fibrosis in other organs and of the stroma in wound healing and cancer. Ongoing work is tracking *in vivo* liver mechanics during fibrosis induction and recovery to guide rational biomaterial design.

References: 1. Burdick JA. Biomacromolecules. 2005;6:386-91. 2. Zustiak SP. Biomacromolecules. 2010;11:1348-57. 3. Uemura ME. Mol Bio Cell. 2005;16:4214-24.

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