Hydrogels with Differential and Patterned Mechanics to Study Stiffness-Mediated Myofibroblastic Differentiation of Hepatic Stellate Cells

Murat Guvendiren[1], Maryna Perepelyuk[2], Rebecca G. Wells[2], Jason A. Burdick[1]
[1]Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA
[2]Department of Medicine, Perelman School of Medicine at the University of Pennsylvania, Philadelphia, PA 19104, USA

Statement of Purpose: Myofibroblastic differentiation of hepatic stellate cells (HSCs) is a key event in liver fibrosis.[1] Due to the local stiffening of the extracellular matrix (ECM) during fibrosis, it is of great interest to develop mimics that can be used to investigate the cellular response to changes in mechanics.[1,2] In this study, we used a two-step hydrogel crosslinking technique, where hyaluronic acid (HA) based macromers are crosslinked using a sequence of addition then UV light-mediated radical crosslinking, to generate hydrogels with tunable (both differential and patterned) stiffness.

Methods: Methacrylated HA (MeHA) substrates were fabricated via introduction of DTT to a 3wt% solution of MeHA in PBS buffer containing 0.2M triethanolamine at pH10, and initial moduli were controlled by %DTT. Prior to the formation of hydrogels, an RGD adhesion moiety (GCGYGRGDSPG) was coupled to MeHA macromers. Soft hydrogels (E~2kPa) were fabricated at 18% DTT. To fabricate stiff (E~24 kPa) substrates, soft substrates were equilibrated in PBS containing I2959 photoinitiator (0.05 wt%) for 30 min and exposed to ultraviolet light (10 mW cm^{-2}) for 2 min. Hydrogels with patterned mechanics were also fabricated by exposing the soft hydrogels to UV light through a photomask with transparent circles on a dark background, with diameters equal to 1000, 200, 50 and 25 microns (Fig.1). Atomic force microscopy was used to determine the modulus (E) of the substrates. Freshly isolated HSCs were seeded ($6.5x10^3$ cells/cm^2) and cultured on substrates for 14 days. Cells were fixed and co-stained for α-smooth muscle actin (α-SMA) and peroxisome proliferator-activated receptor-γ (PPAR-γ).

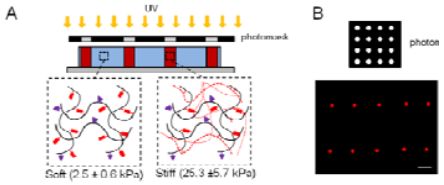

Figure 1. (A) Schematic for the process to fabricate patterned stiffness. (B) Fluorescent images of hydrogel surfaces (red from methacrylated rhodamine indicating secondary crosslinking) for corresponding masks. Scale bars: 200 μm.

Results: When cultured on soft hydrogels, HSCs retained their quiescent rounded morphology with the majority of the cells (70%) expressing high levels of PPARγ (marker for quiescent HSCs) for 14 days. On stiff substrates, cell shape changed significantly with intense cellular spreading correlating with a loss of vitamin A-storing lipid droplets during the first 4 days of culture, and the majority of the cells (85%) staining positive for α-SMA at day 14, indicating myofibroblastic differentiation.

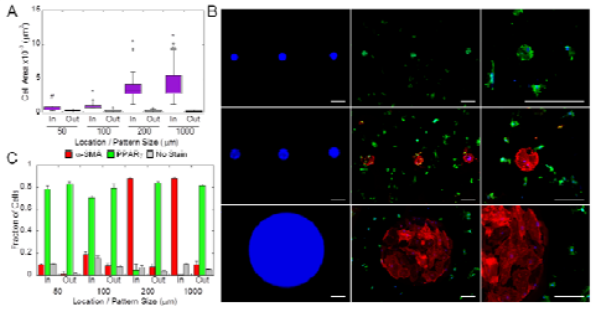

Figure 2. (B) Cell area of HSCs cultured on soft substrates with stiff circular patterns with a range of diameters from 50 to 1000 microns. "Out" for soft and "In" for stiff regions. (B) Fluorescent images of patterns (blue indicating stiff region) and HSCs (green for PPAR-γ and red for α-SMA) cultured for 14 days. (C) Quantification of staining in (B) for HSCs cultured on hydrogels with patterned stiffness for 14 days. In (A), # P < 0.01 and * P < 0.0001 compared to Out (for 3 samples, n=50 cells). All scale bars = 200 μm.

In the case of patterned mechanics, cell area increased significantly for HSCs that were in the stiffer regions (In, Fig. 2A) and HSCs mainly differentiated into myofibroblastic HSCs (indicated by mature α-SMA fibers, red, Fig.2B and C) when compared to HSCs that were on the soft regions (Out) where HSCs mainly stained positive for PPARγ (green, Fig 2B) at day 14. However, in the case of 100 and 50 μm patterns, cell area values were much lower in the stiff regions and the majority of the HSCs stained positive for PPARγ for these substrates independent of cell location, suggesting that cell spreading was necessary for myofibroblast differentiation, even on a stiff substrate.

Conclusions: Myofibroblastic differentiation of HSCs was observed for HSCs cultured on stiff substrates whereas on soft substrates the majority of the HSCs remained quiescent. This behaviour is directly correlated to cellular spreading such that on stiff substrates HSC morphology changes significantly with intense cellular spreading, whereas cells maintain their quiescent rounded morphology on soft substrates. Our results show that HSC cell spreading can be controlled by patterning stiffness where cells show significantly low spreading for circular stiff regions at diameters ≤ 100μm. This study shows that cell spreading plays an important role and is necessary for myofibroblastic differentiation of HSCs, even on a stiff substrate.

References: [1] Olsen A.L. et al. *American Journal of Physiology-Gastrointestinal and Liver Physiology,* 2011, 301:G110. [2]Guvendiren M. et al. *Journal of the Mechanical Behavior of Biomedical Materials*, in revision.