Combinatorial ECM arrays reveal the effects of biomechanics in liver progenitor differentiation

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Introduction: Recent findings suggest that biomechanical signals within the liver microenvironment can regulate the differentiation of mature hepatocytes **[1]**. However, the role of ECM biomechanics in liver progenitor differentiation remains primarily unexplored, despites its potential importance in the processes of liver morphogenesis and regeneration. To examine these mechanisms, we created high-throughput cellular arrays with the capacity to reiterate combinatorial ECM cues and characterize the corresponding phenotypic expression. Moreover, we combined these arrays with substrates of modular stiffness and integrated them with traction force microscopy (TFM) to assess the associated traction stress. This strategy provides a novel avenue to examine cell differentiation and elucidate the role of combinatorial ECM cues in cellular fate.

Materials and Methods: Cellular arrays were formed on polyacrylamide (PA) gels of modular elastic modulus and controlled presentation of ECM proteins. Using solutions of known acrylamide to bis-acrylamide ratios we made 4kPa and 30kPa gels by UV induced cross-linking, containing far-red fluorescent beads (1um) for TFM studies. ECM proteins were arrayed by micro-contact printing using a nanospot arrayer **[2]**. Bipotential mouse embryonic liver (BMEL) cells were cultured on top of the ECM arrays for 3 days in the presence of TGFβ1.

Results and Discussion: We fabricated high throughput BMEL cell arrays on soft (4kPa) and stiff (30kPa) substrates presenting all single and pairwise combinations of 5 ECM molecules (Fig. 1A). We found that the ECM composition along with the substrate stiffness coordinately control cholangiocytic differentiation (e.g. OPN+ cell fraction). Most notably, collagen IV (C4) and fibronectin (FN) exhibited distinct effects (Fig.1B). BMEL cells on FN expressed stiffness-mediated cholangiocytic differentiation, indicated by the higher OPN+ fraction on stiff substrates (Fig.1B), while the cells on C4 exhibited cholangiocytic differentiation indifferent of substrate stiffness. Moreover, BMEL cells on FN developed higher traction stress on stiff substrates (Fig.1B), while cells on C4 exhibited significant traction on both substrates. These data are suggestive of a mechanism by which liver progenitor differentiation is regulated by traction stress at the cell-ECM interface.

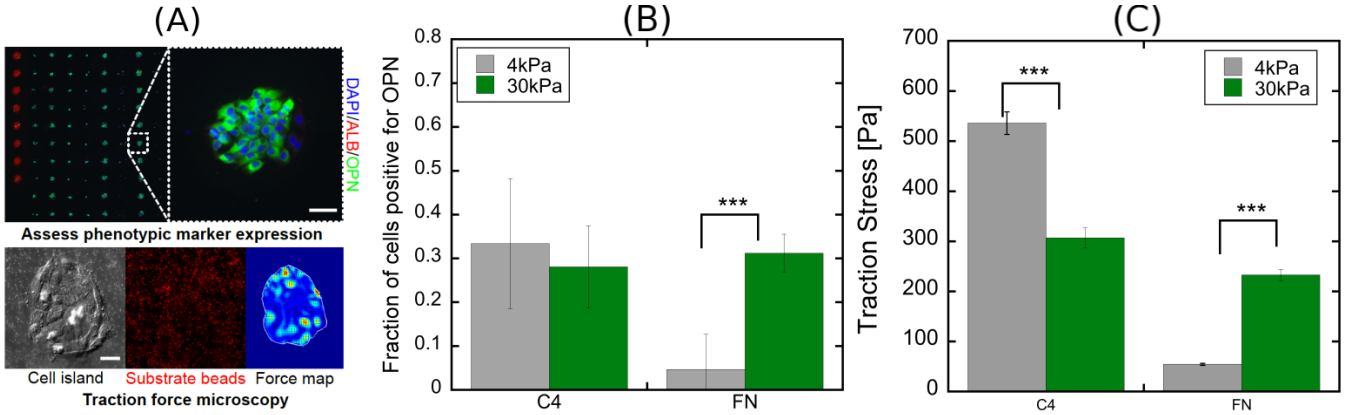

Figure 1: (A) Successful fabrication of cell arrays with BMEL domains of a diameter of ~150um. The BMEL arrays are immunostained for hepatocytic (albumin: ALB) and cholangiocytic (osteopontin: OPN) markers for high-throughput analysis. Lower raw demonstrates bead embedded gels for TFM with the corresponding phase contrast and heat maps. OPN expression (B) and traction stress (C) in response to stiffness and ECM composition.

Conclusions: We integrated microarray fabrication with TFM in order to reveal the role of cell biomechanics in the differentiation of liver progenitor cells. We showed that cells integrate combinatorial ECM cues using traction stress before they commit to their differentiated phenotype. Future experiments will assess the intracellular pathways that direct this differentiation response. Overall, this strategy provides new insight into liver progenitor fate decisions that could aid the investigation of liver disease mechanisms and the development of cell-based therapies.

References:

[1] Wells, R.G., Hepatology, 2008. **47**(4): p. 1394-400.
[2] Kaylan K.B. et al., Scientific Reports, 2016, 6, 23490.