Biomaterial Systems to Assess the Influence of Cell-Matrix Interactions on Hematopoietic Stem Cells

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Statement of Purpose: Hematopoietic stem cells (HSCs) are adult stem cells that give rise to the blood and immune cells in the body. These cells reside in a local microenvironment known as the stem cell *niche,* which consists of stromal cells, the extracellular matrix (ECM), and other soluble and growth factors. It has become clear that the HSC niche provides critical spatial and temporal extrinsic signals to modulate HSC biology: quiescence, self-renewal, differentiation. To elucidate the underlying mechanisms of HSC biology, which still remain largely unknown, it would be important to decouple the extrinsic variables in a controlled manner. In this project, we aim to assess the cell-matrix interactions on HSC biophysical properties and early fate decisions (proliferation, motility, differentiation) by using flexible collagen hydrogel systems with varying mechanical properties.

Methods: Type I collagen gels were fabricated by mixing collagen stock solution (8.25-9.03mg/ml) with HSC media (Stemline™ II Hematopoietic Stem Cell Expansion Medium supplemented with Flt-3 and SCF[1]). For 2D gels, the mixture was placed on a 10-mm microwell glass bottom dish and incubated at 37°C for at least two hours before adding cells. For 3D gels, the mixture was made with an appropriate amount of HSC suspension at the start of culture. For stiffer 2D substrates, collagen-coated polyacrylamide (PA) gels were used. In brief, acrylamide/bis-acrylamide mixture in desired concentrations was placed on a pre-aminosilanized glass bottom dish according to previously published protocol[2]. A circular coverslip was then placed on top and the dish was kept upside down at 37°C for at least 30 minutes for complete polymerization. Type I collagen (100µg/ml) was coated on the gel surface via UV cross-linking with sulfo-SANPAH. The mechanical properties of the fabricated collagen gels were characterized via rheology, and polyacrylamide gels via AFM.

The HSC ($Lin^-cKit^+Sca1^+$) population was harvested from 4-8 week-old female C57BL/6 femurs and tibias via FACS. HSCs were then seeded at 7,000-8,000 cells per sample onto 2D or inside 3D gels and cultured at 37°C for 24h, at which point the cells were fixed and immunostained to visualize the cytoskeletal organization (Alexafluor 488-phalloidin), nucleus (DAPI), and focal adhesions (Alexafluor 568-vinculin) with a 63x multiphoton confocal microscope. Alternatively, cell viability was determined using Live/Dead assay.

Results: Two type I collagen hydrogels (1.45, 2.9 mg/ml; moduli: 14.8 ± 6.1, 44.2 ± 10.7 Pa) and three PA gels (moduli: 3.23 ± 0.75, 8.95 ± 1.40, 4000 ± 1500 Pa) were created. HSCs cultured within or on top of the collagen hydrogels or on the PA gels for 24h showed differences in cell morphology (Fig. 1). Cells on 2D substrates exhibited more spread out, amorphous shape with a tail-like region rich in F-actin while cells within 3D constructs maintained the initial rounded morphology with small cytoskeletal extensions.

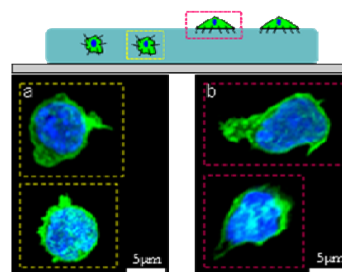

Fig. 1. Cell morphology at 24h.

Using Image J, cell spread area on 2D substrates was calculated and it was found to be positively correlated with the substrate stiffness, showing the maximum cell spread area on the stiffest substrate (Fig. 2a). Cell viability was also found to be significantly influenced by substrate stiffness (Fig. 2b) suggesting that HSC-matrix interactions might be engineered to modulate HSC behavior.

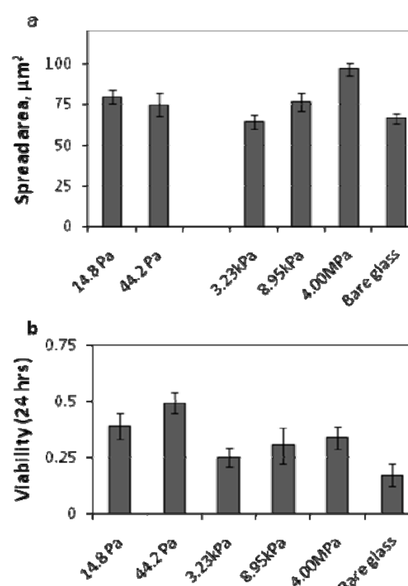

Fig. 2. Cell spread area and viability with increasing substrate elasticity.

Conclusions: Differences in cell morphology, spread area, and viability were observed in HSCs cultured on top of or within collagen hydrogels. Ongoing work is investigating the influence of matrix chemistry, integrin blocking, myosin inhibitors, and variations in ligand density on HSC behavior. Further experiments will incorporate cell division tracing assays, colony forming and competitive repopulation assays to assess the frequency of HSC differentiation, self-renewal, quiescence in these defined matrix environments.

References:

1. Lutolf MP *et al.* Int. Bio. 2009;1:59-69.
2. Pelham RJ, Wang YL. PNAS. 1997;94:13661-13665.

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