Combinatorial Effects of Matrix Elasticity and Cell Shape on Mesenchymal Stem Cell Differentiation

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Statement of Purpose: It has been shown that cells are able to sense and respond to their substrate microenvironment, affecting cell proliferation, migration, gene expression and stem cell differentiation[1]. In particular, lineage-specificity of human mesenchymal stem cells (hMSC) differentiation is dependent on substrate elasticity and size restrictions in cell shape[2,3]. Tuning these factors together will eliminate confounding between the two factors and may improve lineage-specific yields. Using a laser scanning lithography (LSL) technique we are able to surface-pattern restricted cell adhesive areas on poly(ethylene glycol)-diacrylate (PEGDA) hydrogels synthesized over a physiologically relevant range of stiffnesses and examine changes in hMSC differentiation with immunostaining for lineage-specific markers.

Methods:

Hydrogel preparation: PEGDA was synthesized by reacting 35 kDa PEG with acryloyl chloride in triethyl amine and dichloromethane overnight, purified with phase separation in a K_2CO_3 solution and precipitated in diethyl ether. Base hydrogels were prepared by photocrosslinking 35 kDa PEGDA using an acetophenone photoinitiator and 30 second UV exposure time. Polymer concentration was varied to form hydrogels of variable stiffness. Elastic moduli were determined via tensile testing.

Surface Patterning: A polymer solution with 35 µmol/mL acryloyl-PEG-RGDS, 1 µmol/mL eosin Y, 1.5% triethanolamine and 4 µL/mL NVP was prepared. A 5 mm diameter hydrogel sample was placed on 10 µL droplet of this polymer solution in a #1 cover glass chamber plate. A Zeiss 5Live confocal microscope was focused on the polymer-gel interface and scanned across the sample in programmed region-of-interest (ROI) geometries. Unrestricted patterns with ROIs spanning the entire gel surface were first tested as controls.

Cell Culture: hMSCs (Lonza) were seeded on patterned gels at 10,000 cells/cm^2 in MSC Basal Growth Media at passage numbers less than 6. Samples were taken through 3 cycles of osteogenic induction, adipogenic induction or control growth media changes over 12 days.

Immunostaining: Samples were fixed, permeablized, blocked and stained for lineage-specific markers: Primary antibodies were targeted against adiponectin for adipogenic differentiation and osteocalcin for osteogenic differentiation. Samples were counterstained with DAPI and imaged on a Zeiss 5Live confocal microscope.

Results:

Elastic moduli were found for 35 kDa hydrogels at various prepolymer concentrations (Fig 1). For most cell studies 3% and 10% were used with moduli of 5.6 and 19.9 kPa, respectively.

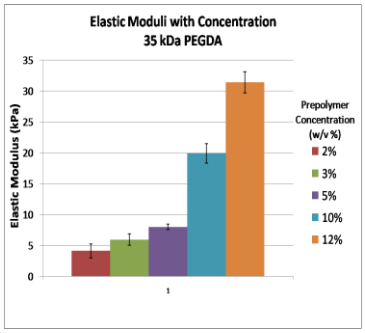

Figure 1: **Elastic Moduli of 35 kDa PEGDA Hydrogels**

In non-size restricted controls, staining differences were most evident with adiponectin and osteocalcin markers (Fig 2). Adiponectin staining demonstrated highest adipogenic differentiation on softer substrates in adipogenic media and elevated differentiation on softer substrates despite culture in osteogenic media. Osteocalcin staining showed a similar trend with highest osteogenic expression on stiffer substrates and elevated expression despite adipogenic media.

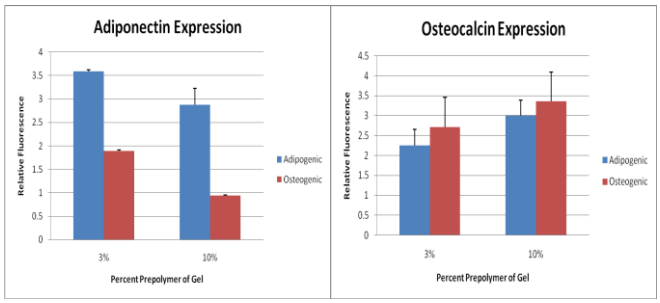

Fig 2: **Immunostaining Whole-surface Adhesive Hydrogels**

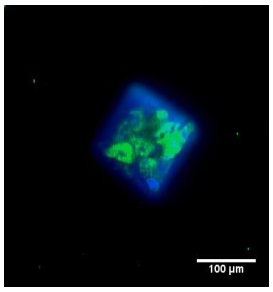

Figure 3: hMSCs on 100 µm Pattern
Adiponectin (Green) and DAPI (Blue).

Size-restricted adhesive islands were successfully patterned onto gel surfaces using the LSL method (Fig 3). Eosin Y autofluorescence in the DAPI channel outlined patterns.

Conclusions: PEGDA hydrogels were synthesized with varied stiffness over a physiologic range and exerted substrate-related effects on MSC lineage-specific differentiation. The LSL patterning method is successful at restricting cell shape and should provide a reliable platform to investigate combinatorial effects of stiffness and restricted cell shape on MSC differentiation. Furthermore, this system should be easily translated to 3-dimensional studies since cells can be easily encapsulated.

References:

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2. Engler, A.J., et al. *Cell*. 126: 677-689.
3. McBeath, R., et al. *Developmental Cell*. 6: 483-495.