Independently Tunable Matrix Elasticity and Cell Shape for Directed Mesenchymal Stem Cell Differentiation

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Statement of Purpose: Human mesenchymal stem cells (hMSC) differentiation is influenced by substrate elasticity and cell shape[1,2]. Tuning these parameters 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: 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 and elastic moduli were determined via tensile testing. For experiments with unpatterned hydrogels, 3.5 mM acryloyl-PEG-RGDS was incorporated in the hydrogel bulk to permit cell adhesion.
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 PEGDA hydrogel sample was placed on 10 µL droplet of this polymer solution on a #1 cover glass surface. A Zeiss 5Live confocal microscope was focused on the polymer-gel interface and scanned across the sample in programmed region-of-interest (ROI) geometries.
Cell Culture: hMSCs (Lonza) were seeded on patterned and unpatterned hydrogels in MSC Growth Media at passage numbers less than 6. Samples were cultured in osteogenic induction, adipogenic induction, or growth media for 12 days.
Immunostaining: Samples were fixed, permeablized, and blocked. Cells on hydrogel patterns were stained with DAPI and phalloidin to evaluate cell spreading across patterns. Cells on unpatterned hydrogels were stained for lineage-specific markers: Primary antibodies were targeted against adiponectin for adipogenic differentiation and RUNX2 for osteogenic differentiation. Samples were counterstained with DAPI and imaged on a Zeiss 5Live confocal microscope.

Results: On unpatterned hydrogels, staining for the osteogenic marker RUNX2 indicated preferential osteogenesis in cells on 40 kPa PEGDA matrices relative to 3 kPa matrices (Fig. 1). Staining also demonstrated a matrix stiffness-related effect on adipogenesis with adiponectin expression higher on 3 kPa matrices relative to 25 kPa matrices.
PEG-RGDS adhesive islands were successfully patterned onto gel surfaces using the LSL method and cell adhesion and spreading was restricted to patterned areas (Fig. 2). Patterns were visualized through eosin Y autofluorescence and addition of a small amount of fluorescently tagged PEG-RGDS. The LSL-method allows alteration of pattern size and shape (Fig. 3).

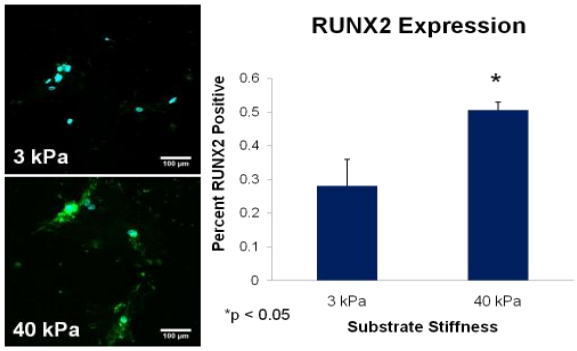

Fig 1: **Stiffer Matrices Favor Osteogenesis**
RUNX2 (Green), DAPI (Cyan)

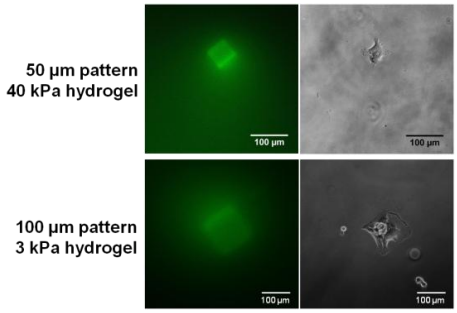

Fig 2: **Cell Spreading on PEGDA Surfaces Limited to Patterns**

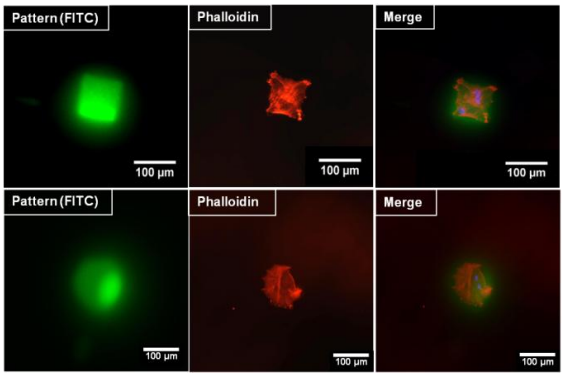

Fig 3: **Cells Adopt Pattern Shape on Different Pattern Geometries**

Conclusions: PEGDA hydrogels exerted substrate-related effects on hMSC differentiation. The LSL patterning method is successful at restricting cell spreading to user-defined geometries and should provide a reliable platform to investigate combinatorial effects of substrate stiffness and cell shape on MSC differentiation.

References:

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