A Comparative Study of the 2D versus 3D Presentation of Matrix Stiffness on Stem Cell Fate

Murat Guvendiren, Sudhir Khetan, Jason A. Burdick
Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA

Statement of Purpose: Stem cells sense the elasticity of their surrounding matrix by pulling and pushing it, and in response generate biochemical activity through a process known as mechanotransduction.[1] Stem cell response to elasticity is well documented for 2D environments.[2] However, studies focusing on elasticity dependent stem cell behavior and fate decision are rather limited for 3D environments, particularly when cells are encapsulated in hydrogels. In this work, we used chemically crosslinked hyaluronic acid (HA) based hydrogels to study the effects of matrix stiffness on stem cell spreading, tractions and fate decisions in both 2D and 3D environments.

Methods: For 2D studies, 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 moduli were controlled by %DTT. For 3D studies, gels were prepared via light exposure of the MeHA solution in PBS buffer containing photoinitiator (I2959, 0.05 wt%) and cell suspension and moduli were controlled through wt% MeHA. Cell mediated degradation was incorporated in 3D hydrogels ("permissive") by functionalizing HA with both methacrylate and maleimide groups (MeMaHA) and using Michael-type reactions between MeMaHA maleimides and thiols on bifunctional MMP-degradable peptides. Permissive hydrogels were then incubated with I2959 and exposed to light, introducing kinetic chains that impede proteolytic degradation ("restrictive"). Human mesenchymal stem cells (hMSCs) were seeded ($6.5x10^3$ cells/cm^2) or encapsulated ($15x10^6$ cells/mL) and cultured in a mixed differentiation media containing osteogenic and adipogenic induction factors. Atomic Force Microscopy and Dynamic Mechanical Analysis were used to determine the modulus (E) of the 2D hydrogels and 3D hydrogels, respectively. For traction force microscopy (TFM) studies, 2D substrates and 3D gels were embedded with fluorescent beads and bead displacements were measured from fluorescent images of the beads before and after cell removal. Cells were fixed and stained with alkaline phosphatase (ALP) for osteogenesis, and with oil red o (ORO) for lipid droplets.

Results: For 2D presentation, hMSC spreading increased with increasing stiffness after 1 day of culture (Fig 1a); cells were rounded with much lower average cell areas ($\tilde{A}$=400μm^2) and tractions (1kPa) on ~3 kPa substrates and spread to a greater extent ($\tilde{A}$=1500μm^2) with much higher tractions on ~30 kPa substrates (Fig 1b-c). Moreover, hMSCs on 3kPa gels differentiated towards an adipogenic lineage whereas on 30kPa gels an osteogenic lineage was preferred (Fig 1d-e). Surprisingly, when encapsulated in restrictive MeHA hydrogels of various stiffness (E=4-92kPa) hMSCs remained rounded and adipogenic lineage was favored for all conditions (Fig 2a-b), regardless of the modulus. In gels permissive to degradation (4.5kPa), cells were able to spread and increase their tractions, leading to primarily osteogenic differentiation when compared to inhibitory gels (Fig 2d).

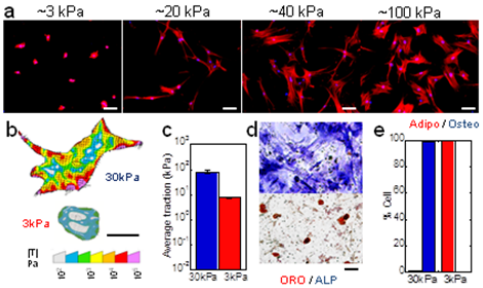

Figure 1. (a) Fluorescent images of hMSCs after 1 day of culture on 2D MeHA gels with varied moduli. (b-c) Colored representation of traction magnitude and average traction values for cells cultured on 30kPa and 3 kPa gels. (d) Images and (e) quantification of hMSCs stained for ALP and ORO after 14 days of culture in mixed differentiation media on 30kPa and 3 kPa gels. Scale bars: (a,d) 100, (b) 25 μm.

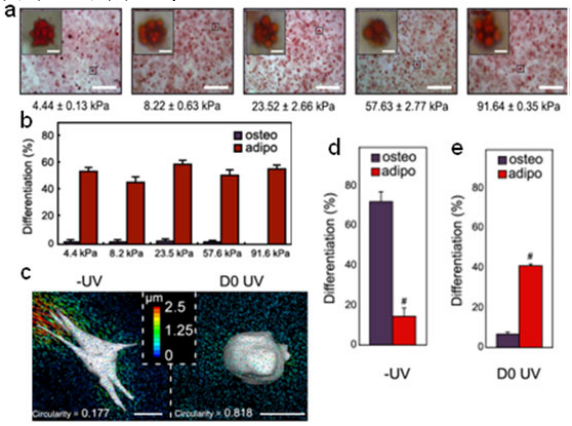

Figure 2. (a) Images and (b) quantification of hMSCs stained for ALP and ORO after 7 days of culture in mixed differentiation media encapsulated in MeHA gels of various stiffness. (c) TFM images and (d-e) differentiation quantification of cells for permissive (-UV) and inhibitory (D0 UV) gels. Scale bars: (a) 100, 5 (inset), (c) 10 μm.

Conclusions: On 2D substrates, cellular spreading, tractions, and osteogenesis increased with stiffness. This is consistent with observations on a range of materials for anchorage dependent cells. However, when encapsulated in 3D covalently crosslinked networks, hMSCs remained rounded and exhibited low tractions and primarily adipogenesis, regardless of mechanics. When the same networks were designed to support cellular degradation, cells were able to spread into the hydrogel and to exert force on the environment. In this case, cells favoured osteogenic differentiation. Thus, degradation plays an important role in the ability of cells to interact with their surroundings in 3D covalently crosslinked networks.

References: [1]Fletcher D.A. & Mullins D. *Nature*, 2010, 463:485. [2]Guvendiren M. & Burdick J.A. *Nature Communications*., 2012, 3:792.