Harnessing Cellular Manipulation of the Cell-Matrix Interface to Control Stem Cell Fate

Nathaniel Huebsch[1,2], Praveen Arany[1], Angelo Mao[1], Dmitry Shvartsman, Omar A. Ali, Jose Rivera-Feliciano[1], David J. Mooney[1,3].

1. Harvard University School of Engineering and Applied Sciences. 2. Harvard-MIT Division of Health Sciences and Technology. 3. Wyss Institute for Biologically Inspired Engineering

Statement of Purpose: Cell therapies hold great clinical promise, but control of transplanted cell fate remains a significant challenge. Material-based transplantation systems offer a promising means to control cell fate, and because cell-matrix interactions are central to eukaryote biology, synthetic analogs of the natural extracellular matrix (ECM) have been designed to exploit those interactions to manipulate cell fate, typically via presentation of integrin binding ligands (e.g. RGD). Interestingly, *in-vitro* studies with synthetic ECM analogs have demonstrated that in 2D cultures, cell fate can be manipulated both by ligand presentation and also by the mechanical properties of the substrate. However, the extent to which ECM rigidity affects cell phenotype in more physiologically relevant 3D culture systems, and the underlying mechanisms, are uncertain. In this work, we first describe changes in mesenchymal stem cell (MSC) phenotype in response to 3D matrix rigidity, and subsequently identify a novel mechanosensing mechanism that appears to be underlie these observations.

Methods: Both primary human MSC, as well as a clonally derived murine MSC line, D1 (American Type Cell Culture) were used for these studies. 3D, cell encapsulating hydrogels were formed using a variety of alginate polymers and crosslinking molecules to control mechanical properties (Rowley 1999). Hydrogel mechanics were assessed with rheology and compression testing. A FRET-based technique (Kong 2006) was used to non-invasively measure bonds between a homogenous MSC encapsulated into 3D gels and biomimetic adhesion peptides (G_4RGDASSKY) attached to alginate. Stem cell commitment to either osteogenic (bone) or adipogenic (fat) lineage, as well as cellular deposition of endogenous matrix, were assessed using histology and Western Analyses. Specific integrin receptors used by MSC to bind RGD in 2D or 3D matrices were identified using a novel ELISA method based on biotinylated RGD.

Results. MSC fate changed markedly as the mechanics of 3D matrices were varied, with adipogenesis predominating in softer (2.5-5 kPa) gels and osteogenesis being optimal at intermediate (11-20 kPa) rigidity. However, in contrast to previous work done in 2D model systems, changes in cell morphology (which were largely absent in our 3D gels) did not appear to underlie this behavior. Strikingly, however, the number of molecular RGD-integrin bonds formed in 3D gels was dependent on matrix elasticity, and optimal integrin ligation correlated with osteogenesis, occurring optimally in 20 kPa gels (Fig. 1). This was independent of the specific type of alginate polymer or crosslinking molecule. Further studies revealed that matrix-mechanics dependent changes in cell fate persisted even in the presence of endogenous ECM deposition by cells.

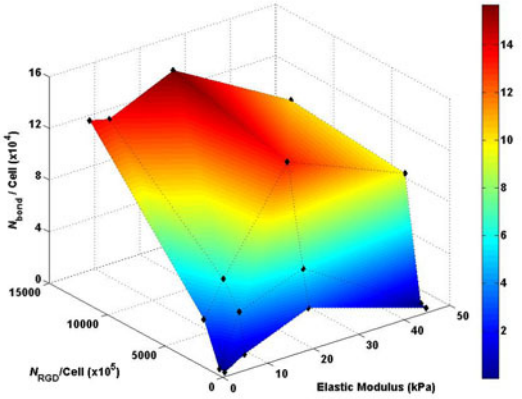

Figure 1. FRET-based measurements reveal a role for 3D matrix rigidity in determining integrin-RGD binding

Quantitative measurements of the specific receptors used by MSC to bind RGD revealed that the α_5-integrin could not act as an RGD receptor in 2D – however, in 3D matrices, this receptor bound RGD in a mechanically-dependent manner. Differentiation studies performed in the presence of function-blocking antibodies revealed a functional role for α_5-integrins in MSC fate, as blocking these receptors switched MSC from osteogenesis to adipogenesis even in 20 kPa gels.

Conclusions. This work demonstrates that the bonds between integrins and adhesion ligands are sensitive to the interplay between cell-traction forces and the compliance of the material presenting the ligand. This suggests that cells interpret a change in the physical properties of their environment as though it were a change in adhesion ligand presentation. From a biomaterials processing standpoint, it also suggests that cells do not simply react to inputs provided by materials, but can themselves be harnessed as tools to process simple, scalable materials into functionally complex structures that feedback to manipulate their fate.

References: Kong HJ. Proc Natl Acad Sci USA 2006; 103(49): 18534-9. Rowley J Biomaterials 1999; 20(1): 45-53.