Harnessing Cell-Traction Mediated Nanopatterning of Microenvironments by Controlling Matrix Compliance

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Statement of Purpose: Functional complexity is a critical requirement for tissue engineering scaffolds, as such materials must function as local micro-environments that provide programming cues to host or transplanted cells. One approach to this goal is the design of complex, synthetic analogs of the natural extracellular matrix (ECM). Both the specific composition of receptor-binding adhesion epitopes presented by such materials and the nanoscale heterogeneity in their presentation, have demonstrated effects on cell behavior. In a seemingly unrelated observation, a variety of cell types, including mesenchymal stem cells (MSCs), change their behavior in response to the mechanics of their micro-environment. While mechanisms for cell responses to specific molecular adhesion epitopes are well established, the biophysical means through which cells sense and ultimately respond to the mechanical properties of the ECM are incompletely understood. We hypothesized that matrix mechanics affect cell fate by controlling bond formation between integrin adhesion receptors and adhesion ligands presented by the matrix.

Methods: A homogenous, clonally derived murine MSC line, D1 (American Type Cell Culture) was used for these studies. 3D, cell encapsulating hydrogels were formed using a variety of alginate polymers and crosslinking molecules to control mechanical properties. Hydrogel mechanics were assessed with rheology and compression testing. A FRET-based technique (Kong 2006) to non-invasively measure bonds between a homogenous MSC encapsulated into 3D gels and biomimmetic adhesion peptides (G_4RGDASSKY) attached to alginate. A second FRET technique (Kong 2005) was used to monitor cell-traction mediated clustering of RGD peptides presented by different alginate polymers. Intracellular localization of α_5-integrins was assessed by generating an MSC line that stably express chimeric EGFP-α_5-integrins. A novel biochemical method based on peptide-ELISA was used to assess the specific integrin receptors MSC used to bind biotinylated RGD peptides presented by either 2D or 3D alginate gels. MSC lineage specification was assessed with RT-PCR, Western Blot and histology.

Results. Strikingly, the number of RGD-integrin bonds in 3D indeed depends on matrix compliance, in a biphasic manner (Figure 1). This is in contrast to the monotonic relationship between cell spreading and matrix compliance typically seen for 2D substrates, and was independent of the specific type of alginate polymer or crosslinking molecule. Further analysis revealed that enhanced binding to RGD correlated not with microscale changes in MSC morphology but rather with their ability to exert traction forces to reorganize RGD peptides on the nanoscale. This suggests that stem cells can nanopattern their microenvironment in a mechanically regulated fashion. Consistent with that hypothesis, bond formation, along with matrix reorganization, was decoupled from ECM mechanics with pharmacologic cell traction inhibitors.

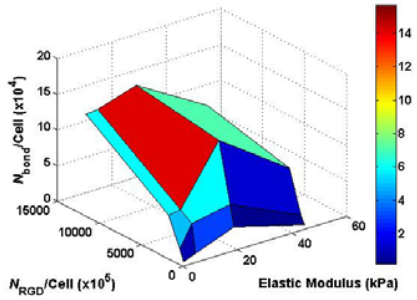

Figure 1. Cell-RGD bond formation is controlled by matrix compliance in a biphasic manner

We speculated that this mechanical nanopatterning by stem cells would increase the structural complexity of the ECM and therefore engender the micro-environment with functional complexity. Thus, we examined the effects of ECM elastic modulus on the specific receptors MSC used to bind RGD and on their lineage specification. Consistent with previous work, 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. MSCs' ability to form α_5-integrin-RGD bonds had important consequences on their fate, as their differentiation toward an osteoblast-like phenotype correlated with the α_5-integrin-RGD bond formation, whereas differentiation toward adipocyte-like cells was optimal in softer matrices.

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. These changes are in part due to mechnically-dependent nanoscale remodeling of the micro-environment by cells. Combined with the dimensionality of the micro-environment, this traction-mediated process allows MSCs to use α_5-integrins to bind RGD presented without synergy sites, and to alter their lineage specification. Moreover, mechanically-mediated changes in α_5-integrin-RGD bond formation correlate with stem cell osteogenesis, implying that phenotypic responses of cells to matrix stiffness are due at least in part to changes in the molecular integrin-ligand interface. These studies highlight a role for stem cells, not only in terms of their ability to respond to complex biomaterials, but also in terms of their ability to transform simple template materials into information-rich, structurally complex materials *in-situ*.

References: Kong HJ. Proc Natl Acad Sci USA 2005; 102(12): 4300-5. Kong HJ. Proc Natl Acad Sci USA 2006; 103(49): 18534-9.