Matrix Compliance and Cell Function: Understanding Differences Between 2D and 3D Cultures

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Statement of Purpose: It is widely accepted that the intrinsic mechanical properties of the extracellular matrix (ECM) govern cell fate in two-dimensional (2D) cultures. However, the impact of ECM mechanics on cell fate in three-dimensional (3D) cultures remains unclear, due in part to the fact that substrate mechanical properties, adhesion ligand density, and proteolytic sensitivity are intimately linked in native biopolymers systems. To decouple these effects, many research groups (ours included) have explored the use of synthetic hydrogels, based on the argument that their bulk moduli can be tuned independent of changes in biological recognition motifs. However, altering cross-link density to change bulk mechanical properties simultaneously alters the micro- and nanostructure of most hydrogels, which in turn profoundly influences cell shape. Macromolecular diffusive transport is also significantly slowed, which thereby impacts the delivery of soluble chemical cues to cells. Further complicating interpretations is the fact that the bulk mechanical properties of many systems change significantly with time, due to passive hydrolysis, cell-mediated proteolysis, and/or new ECM synthesis and deposition. Despite these inherent complications asociated with 3D cultures, we posit that the ability of the ECM to resist cell-generated tractional forces influences cell decision-making processes in3D via mechanisms similar to those used by cells in 2D.

Methods: To better understand how the ECM's intrinsic mechanical properties influence cell fate in 3D, new methods are necessary to measure microscale mechanics of ECM-mimetic biomaterials. Ideally, these measurements should be non-destructive so that they can performed on live cultures during complex morphogenetic processes. We have developed two complementary methods that enable us to achieve these goals. The first method utilizes a laser based microrheology (MR) system to both characterize the microstructure of pores within hydrogels and to investigate mechanical properties. The second method combines confocal reflection microscopy (CRM) with spatial and temportal image correlation spectroscopy (STICS) to track the speed with which individual matrix filaments are displaced due to cell-generated forces. We have beun applying these two methods to a 3D model of capillary morphogenesis (Fig. 1). In this model system, endothelial cells (ECs) are grown on microcarrier beads within a 3D fibrin-based microenvironment. A monolayer of stromal cells a plated on the top surface of the gel, or distributed throughout the gel, to provide a source of crucial pro-angiogenic factors. Over several days in culture, the encapsulated ECs begin to differentiate and execute a branching morphogenesis process similar to angiogenesis *in vivo*.

Results: Passive trajectories and mean square displacements (MSDs) of microspheres (1, 2 or 4.7 μm in diameter) reveal viscous diffusion within pores and confinement by the rigid solid protein boundaries.

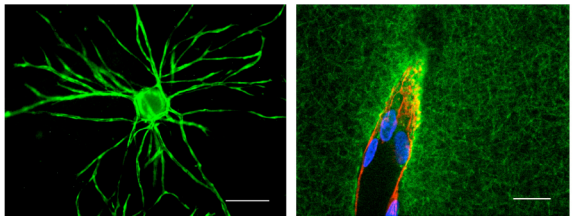

Figure 1: ECs encapsulated within a 3D fibrin matrix undergo capillary morphogenesis, invading the matrix to form multicellular structures with hollow lumens.

Active microrheological measurement of viscoelastic ECM near budding capillaries at 2.5 mg/ml fibrin revealed complex shear moduli that ranged from stiff (>20 Pa) to very soft (<10 Pa), in contrast to standard macrorheological measurements that report homogeneous material properties.

Complementary confocal reflection imaging and STICS revealed that endothelial cells generate measurable displacements of individual ECM fibers. The magnitude of these displacements was found to depend on ECM density, as the fibers in 10 mg/ml fibrin gels are displaced significantly less than those in 2.5 mg/ml gels. In experiments involving ECs expressing constitutively-active V14-RhoA, the rate of fiber displacement was significantly elevated relative to cultures containing wild-type ECs. Conversely, expression of dominant-negative N19-RhoA in ECs significantly reduced fiber displacement compared to wild-type controls (Fig. 2).

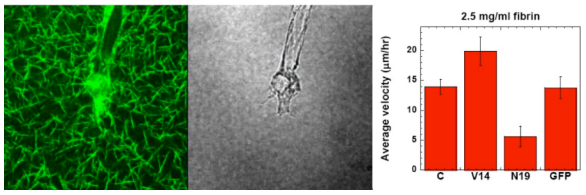

Figure 2: Using confocal reflection microscopy and STICS during capillary morphogenesis in 3D fibrin gels enables quantification of ECM displacement due to cell-generated tractional forces.

Conclusions: Passive and active MR allow for both the viscous and the elastic components of the local cellular microenvironment to be characterized in a non-destructive manner. CRM/STICS approaches enable the cell-mediated displacements of individual matrix fibers to be quantified. Together, these matrix platform-independent methods provide new insights into the mechanical role of the ECM during complex morphogenetic processes. While our understanding of the mechanical role of the cell-ECM interface in 3D cultures is still incomplete, using these tools to characterize this interface is already revealing new insights that distinguish cellular responses in 2D vs. 3D cultures. These insights will be discussed more extensively in this presentation.