Shrink Wrapping Cells in a Defined Extracellular Matrix to Modulate the Chemo-Mechanical Microenvironment

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Statement of Purpose: Cell-matrix interactions are important for the physical integration of cells into tissues and the function of insoluble, mechanosensitive signaling networks. Studying these interactions *in vitro* can be difficult because the extracellular matrix (ECM) proteins that absorb to *in vitro* cell culture surfaces do not fully recapitulate the ECM-dense basement membranes to which cells such as cardiomyocytes adhere to *in vivo*. Towards addressing this limitation, we have developed a surface-initiated assembly process to engineer ECM proteins into nanostructured, microscale sheets that can be shrink wrapped around single cells and small cell ensembles to provide a functional and instructive matrix niche. Unlike current cell encapsulation technology using alginate, fibrin or other hydrogels, our engineered ECM is similar in density and thickness to native basal lamina and can be tailored in structure and composition using the proteins fibronectin (FN), laminin (LAM), fibrinogen (FIB), and/or collagen type IV (Col IV). A range of cells including C2C12 myoblasts, bovine corneal endothelial cells, and chick cardiomyocytes survive the shrink wrapping process with high viability. Further, we demonstrate that the engineered ECM modulates integrin expression and adhesion in both 2D and 3D microenvironments.

Methods: The engineered ECM was fabricated using a surface-initiated assembly technique[1]. Briefly, ECM proteins were adsorbed onto a polydimethylsiloxane stamp with raised 75 µm x 75 µm squares. They were then patterned on a poly(N-isopropylacrylamide) (PIPAAm) surface using conventional microcontact printing methods. Embryonic chick cardiomyocytes, C2C12 myoblast, or bovine corneal endothelial cells were seeded onto FN, LAM, FIB, or Col IV squares and allowed to adhere for at least 2 hours. To prevent premature dissolution of the thermally sensitive PIPAAm, all solutions and substrates were heated to 40 °C. After the cells adhered, the non-adherent cells were washed away and the solution temperature was allowed to cool below the lower critical solution temperature (32°C) of the PIPAAm leading to dissolution of the thermally sensitive layer and subsequent wrapping of the assembled ECM around the cells (Figure 1A).

Results: To demonstrate the ability to modulate the chemical composition of the engineered ECM microenvironment, we first demonstrated the ability to engineer freestanding 75 µm squares composed of

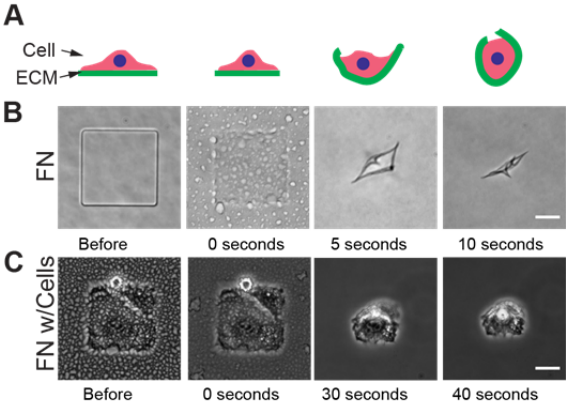

Figure 1: (A) Schematic of the "shrink-wrapping" encapsulation of a cell in an ECM scaffold. (B) Example of a square FN ECM scaffold by itself during the thermal release process. The initial square shape results in spontaneous folding over along one of the diagonal axes. (C) Example of a square FN ECM scaffold with adhered C2C12 myoblasts. As the underlying PIPAAm dissolves, the cell contracts and the FN ECM scaffold partially folds around them. Scale Bars are 25 µm.

the ECM proteins FN, LAM, FIB, or Col IV. Each protein was able to properly fold over itself upon the dissolution of PIPAAm in a manner depicted in Figure 1B. We then repeated the release after first seeding C2C12 myoblasts, bovine corneal endothelial cells, and chick cardiomyocytes and found that each cell type was capable of being encapsulated by the releasing ECM protein (Figure 1C). Importantly, each cell type maintained >95%viability following the encapsulation process. Finally, we found that the shrink wrapped ECM promoted the formation of focal adhesions and that these adhesions were maintained throughout the shrink wrapping process and persisted afterwards.

Conclusion: We have developed a method to engineer ECM protein sheets that can be used to shrink wrap single cells and small cell ensembles. We found that the FN, LAM, FIB, and Col IV were all capable of shrink-wrapping multiple cell types while maintaining high cell viability. Further by modulating the specific ECM proteins used, it was possible to tailor the chemo-mechanical properties of the cell microenvironment by maintaining focal adhesion complexes. Future work will investigate whether the shrink-wrapped ECM promotes long term viability and proliferation in 2D and 3D environments.