Fabrication of multi-layer hydrogel microenvironments to control the spatial presentation of biochemical cues

Melissa Pope[1], Hao Zhang[2], John Oakey[2], and Kristi Anseth[1,3].
[1]University of Colorado, [2]University of Wyoming, and [3]Howard Hughes Medical Institute.

Statement of Purpose: *In vitro* cell culture platforms that recapitulate the biochemical (adhesion ligands; growth factors) and biomechanical (stiffness) cues presented in the *in vivo* stem cell niche are highly useful for the culture and study of stem cells. However, one key design element that is often missing from current biomaterial niches is the spatial organization of ligands at the sub-cellular level. Muscle stem cells (satellite cells), for example, lie sandwiched between the basal lamina and sarcolemma. This niche creates an asymmetric 3D environment that presents distinct cues on the cell's basal versus apical side. This asymmetric arrangement of niche signals is shared by other tissue-specific stem cell niches (e.g., epithelial; testes) and is thought to be critical for stem cell polarity and asymmetric self-renewal [1-3]. To recapitulate this geometry *in vitro*, we are exploiting microfluidic approaches to create multi-layer poly(ethylene glycol) (PEG) hydrogel microenvironments that present basal-lamina derived peptides in one layer and sarcolemma-derived peptides in the other. Stem cells are then entrapped at the interface between the two hydrogel layers, and thus experience a polarity in biochemical cues.

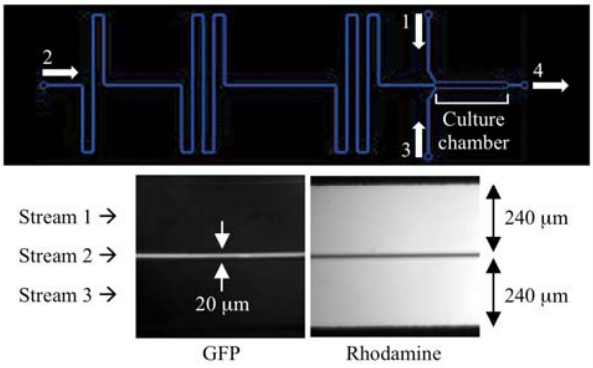

Figure 1. Fabrication method. (Top) Microfluidic device used to create multi-layer hydrogels. (Bottom) Fluorescence images of the filled cell culture chamber. Streams 1 and 3 contained rhodamine-labeled PEG and stream 2 contained a suspension of GFP-labeled beads.

Methods: PDMS molds were attached to glass slides functionalized with a silane to facilitate hydrogel attachment. Holes were punched through the PDMS at the inlets and outlet. PEG solutions were pipetted onto the inlets and pulled through the device using silicon tubing inserted into the outlet and a 3mL syringe. The following hydrogel formulation was used: 3 mM (6 wt%) PEG-tetra-norbornene, 5 mM di-cysteine crosslinker and 1 wt% photoinitiator LAP [4]. Once fluid flow was initiated and the culture chamber filled, PEG monomers were crosslinked using 15 mW/cm^2 of 365 nm light.

Results: We have designed and tested a microfluidic device (Figure 1) that creates three parallel flowing streams in a cell culture chamber 5 mm in length. Upon polymerization, the cells (stream 2) become encapsulated at the interface between two PEG layers (streams 1 and 3), each of which has a distinct biochemical composition. Figure 2B shows MDCK cells (blue) cultured between an unlabeled hydrogel and an Alexa Fluor 594 labeled hydrogel. The localization of the fluorescence signal to the basal, but not the apical, side of the cell is visually striking. To optimize the spatial segregation of stream components, devices with varied widths (5 – 20 μm) of stream 2 were tested. For each stream 2 width, the mean pixel intensity at the cells' basal side and the mean pixel intensity at the cell's apical side were determined from confocal images using MATLAB. For MDCK cells, a stream 2 width of 7.5 μm yielded the largest fold change in fluorescence intensity across cell bodies (Figure 2C). Work is currently underway to characterize cellular responses to culture in these asymmetric synthetic hydrogel microenvironments. Future work will include studies of muscle stem cell self-renewal.

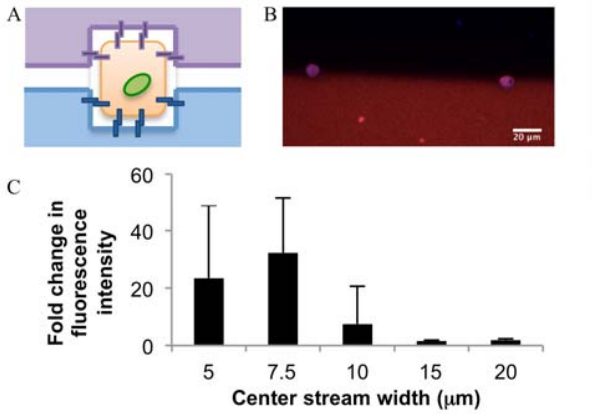

Figure 2. Cells reside at the interface between two distinct hydrogel layers. (A) Schematic. Each hydrogel layer engages distinct ligands on the cell surface. (B) MDCK cells (blue) cultured at the interface between a fluorescently labeled hydrogel (red) and a non-labeled hydrogel. (C) Quantification of fluorescence intensity (fold change = intensity at cell's basal side ÷ intensity at cell's apical side). Mean + STD, N ≥ 5 cells.

Conclusions: Using unique microfluidic devices, we fabricated multi-layer hydrogel niches that recapitulate the asymmetric spatial presentation of biochemical ligands present in the *in vivo* stem cell microenvironment.

References:

1. Kuang *et al*. Cell Stem Cell. 2008;2:22-31.
2. Fuchs E. J Cell Biol. 2008;180:273-84.
3. Fuchs *et al*. Cell. 2004;116:769-78.
4. Fairbanks *et al*. Advanced Materials. 2009;21:5005-10.