A Micro fabricated Platform for Investigating Multicellular Organization in 3-D Microenvironments

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Understanding how complex intrinsic and external cues are integrated to regulate cell behavior is crucial to the success of cell-based therapies in the treatment of human disease. Systematic and quantitative investigation of these microenvironment signals was first enabled by precise cell positioning using 2-D micropatterning tools [1]. However, cellular signaling is often altered in adherent tissue culture where structural cues are lacking (including tumor, stem, and differentiated cells), in contrast to 3-D culture systems that more closely resemble in vivo cell behavior [2]. Our goal was to develop new micropatterning tools capable of micronscale cell patterning and organization within a 3-D hydrogel with tissue-like properties. We developed a technique for the rapid formation of reproducible, high-resolution, 3-D cellular structures within a photo-crosslinkable hydrogel using dielectrophoretic forces (Figure 1) [3]. We demonstrate parallel formation of ~20,000 cell clusters of precise size and shape within a 1 x 2 cm² slab of tissue (Figure 2a), with high cell viability and differentiated cell function maintained over 2 weeks in culture. By modulating cell-cell interactions in clusters of various size (independent of hydrogel geometry, chemistry, or volumetric seeding density; Figure 2b), we present the first evidence that 3-D microscale tissue organization regulates chondrocyte behavior (Figure 2c) [3]. This dielectrophoretic cell patterning (DCP) technology enables further investigation of the role of tissue architecture in many other multicellular processes from embryogenesis to regeneration to tumorigenesis.

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This project utilizes microfluidic systems to study how groups of liver cells acquire emergent tissue properties. Hepatocytes (the parenchymal cells of the liver) respond to many cues in their microenvironment: neighboring cells, growth factors, extracellular matrix, dissolved oxygen, and their interactions. One tissue property of interest is the compartmentalization of gene expression in multicellular domains along the liver sinusoid. This process, often described as “zonation,” underlies much of liver physiology and regional susceptibility to toxins. We have previously shown oxygen gradients can be used to compartmentalize mixed populations of hepatocytes in a large-scale reactor [1]. Here, we present a microdevice that enables one to explore the crosstalk between two inputs (oxygen gradients and soluble growth factors) in a systematic fashion. The device consists of a two-layer PDMS microfluidic network with an on-chip dilution tree bound to a glass slide with an array of microreactors. Hepatocyte zonation is induced in each microreactor through local oxygen concentration, which is modulated through gas channels separated from the bioreactor by a 100-µm PDMS layer as shown in Figure 1. The local oxygen concentration in the microchannels is quantified in Figure 2. Primary rat hepatocytes are seeded into microreactors together with 3T3 fibroblasts, which act to stabilize the hepatocyte phenotype as described previously. This device will be useful to further explore liver tissue biology in vitro including the dynamics of zonation, mechanisms of oxygen sensing, and the role of growth factors in zonal response.

**Figure 1:** A.) Schematic and B.) Picture of the microfluidic network. Two inlets (yellow and blue) feed a dilution gradient generator to yield a titration, which feeds into 8 discreet bioreactors. Gas channels (dyed red and blue) run perpendicular to the bioreactors and each connected to a separate gas cylinder with a premixed oxygen concentration (21%, 10%, and 1%). The gas channels are separated from the PDMS microchannels through a thin PDMS membrane. C.) Magnification of the red box in A showing two bioreactors and the gas channels. The arrows indicate how the gas and liquid flow in the channels.

**Figure 2:** Oxygen concentration along the length of the bioreactor as a function of distance and flow rate. This data was acquired through a ruthenium modified substrate which fluoresces under 450nm light and is quenched by oxygen. The data was calibrated and the intensities are directly related to the local oxygen concentration through the Stern-Vollmer logarithm.

**REFERENCES**

Micromechanical Control of Cell-Cell Interaction

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Cellular behavior within tissues is driven by environmental cues that vary temporally and spatially with granularity on the order of individual cells. Local cell-cell interactions via secreted and contact-mediated signals play a critical role in these pathways. In order to study these dynamic small-scale processes, we have developed a micromechanical platform to control microscale cell organization so that cell patterns can be reconfigured dynamically. This tool has been employed to deconstruct the mechanisms by which liver-specific function is maintained in hepatocytes upon co-cultivation with stromal support cells. Specifically, we examine the relative roles of cell contact and short-range soluble signals, duration of contact, and the possibility of bi-directional signaling.

The device consists of two silicon parts that can be locked together either to allow cell-cell contact across the two parts or to separate the cells by a uniform gap of approximately 80 µm (Figs. 1 and 2). Switching between these two states is actuated simply by pushing the parts manually using tweezers; no micromanipulation machinery is necessary. Micron-scale precision is possible due to a 10:1 mechanical transmission ratio and microfabricated snap locks, both of which are monolithically incorporated into the silicon structure. The entire device is fabricated in a simple single-mask process using through-wafer deep reactive ion etching. To provide a surface compatible with cell culture, the surface is coated with a layer of polystyrene and plasma-treated, providing a standard tissue-culture surface.

Figure 1: Hepatocytes separated from stromal cells by 80-micron gaps, which prevent contact between the two cell types.

Figure 2: Hepatocytes and stromal cells cultured with no separation. The system can be switched back and forth between the states shown in Figures 1 and 2.