Microfluidic Hepatocyte Bioreactor
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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