Abstract

Background and Significance:

The liver possesses a unique capability to return to a constant size within a short period after tissue loss. The regenerative process involves cytokines, growth factors, increases in portal blood flow, and a dynamic interplay between hepatocytes and non-parenchymal cells. Liver sinusoidal endothelial cells (LSECs) play a unique role by releasing paracrine-mediated growth factors. However, current model systems to elucidate factors, pathways and cell-types involved in liver regeneration are predicated on injurious insults in an animal liver and do not recapitulate the complex 3D human-specific interactions that occur as a human liver regenerates. While animal models are plentiful, there are differences in many ligand-dependent signaling pathways between rodents and humans, and even when there is homology, isolation of cells from whole tissues perturbs cell signaling pathways. To our knowledge, no current *in vitro* platform has identified the various factors involved in a flow-dependent regenerative system that includes both human endothelium and human hepatocytes.

Here we develop a microfluidic device (SHEAR) with a human-endothelium lined vascular channel and human hepatocytes. Under application of flow and cytokines, SHEAR devices elucidate flow- and cytokine-dependent regenerative signals. The model is responsive to fluid flow and is capable of incorporating cells from a variety of species. Through analysis of the secreted factors, we can delineate the effects of hemodynamic inputs such as shear stress and biochemical inputs such as cytokines on endothelium-derived paracrine factors. Specifically, we will probe whether flow can lead to an increased secretion of regeneration-associated factors. Once we narrow down candidates that can promote regeneration, we will assess whether these factors can lead to increased human hepatocyte cell cycling, a phenomenon that has been difficult to achieve.

Specific Aims:

To accomplish the goals of this project, we propose three specific aims: 1) Development of an integrated liver-endothelial platform that is responsive to flow; 2) Understanding the role of fluid flow on paracrine signaling between hepatocytes and endothelial cells; 3) Discovering mitogens using the vascularized platform as a testbed.

Methods:

For Aim 1, hepatocytes will be co-encapsulated with supporting stromal cells using a 3D cellular aggregation technique. These constructs will be embedded in a natural extracellular matrix (ECM) that is pre-patterned with endothelial channels. This device will be placed on a rocker platform that provides bidirectional oscillatory flow through the channel. For Aim 2, flow will be applied in the channel and the effects of shear stress on endothelial cells will be ascertained by assaying the secretome of the device. Specifically, we will assay for proteins that are involved in angiogenesis and regeneration. For Aim 3, the device will be utilized as testbed for discovering factors that can promote regeneration. By probing a large number of proteins and categorizing them through different clustering techniques, we will elucidate candidate factors that we will validate in various liver platforms.