Hepatic organoids co-populated with hepatocytes and cholangiocytes: Towards engineered liver grafts with biliary drainage

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The only treatment method for end stage liver disease is orthotopic liver transplantation which is limited by the severe shortage of high quality donor organs available for transplantation. Tissue engineering aims to create transplantable liver grafts that can serve as substitutes for donor livers. Current approaches in liver tissue engineering have yet to address the biliary component of the organ and hence fail to deliver a three-dimensional model that correctly mimics the native liver architecture. Biliary component of the liver architecture is composed of cholangiocytes that line the biliary ductules and are responsible for transporting and modifying the bile produced by hepatocytes. The major challenge in recreating the biliary drainage in an engineered construct has been the integration of bile canaliculi with the biliary ductular network and allowing the clearance of bile from the hepatocytes to the host duodenum.

In this work, we aim to create such a hepatic organoid by co-culturing rat hepatocytes with cholangiocytes and stromal cells and evaluate the viability, function and microarchitecture for cellular rearrangement. The organoids are subsequently embedded in extracellular matrix hydrogel to create a higher order ductular network resembling hepatic lobules. We tested viability using Presto Blue assay for up to 7 days, we evaluated hepatic function and polarity by measuring albumin and urea synthesis and formation of bile canaliculi. The organoids were examined morphologically via histology, scanning and transmission electron microscopy and immunofluorescence analysis.

Our results indicate that within the organoids, hepatocytes maintain viability and function as detected by Presto Blue and functional assays, viable hepatocytes become polarized by forming bile canaliculi with presence of tight junctions. Morphologically, hepatocytes form the core of the organoid, while cholangiocytes line the outside forming as monolayer shell and form tubular structures extending outward. When the organoids are embedded within the hydrogel, cholangiocytes continue to grow outward forming ductules interconnecting different organoids which may allow fluid exchange. Our current work is focused on strategies that enable long term culture and evaluation of functional biliary ductile formation. The three-dimensional constructs prepared as a result of this work will be utilized to repopulate whole liver scaffolds for engineering transplantable liver grafts.