Creating Liver-Mimicking Tissue Constructs in a 3D Biomimetic Hydrogel

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Statement of Purpose: Liver diseases are the 4th most frequent cause of death in middle-aged adults in the US[1]. Although liver transplants are an important source for treatment, there is a large shortage of liver transplants available for the number of patients needing them. Thus, scientists and engineers have turned to liver tissue engineering. A vascular network is critical for survival and function of the liver; however, creating vascularized liver tissue engineered constructs remains a challenge. When investigating formation of microvasculature in scaffolds, we have been using human umbilical vein endothelial cells (HUVECs) and pericyte precursor cells (10T½) in our well established angiogenic model, yet these cells do not naturally exist in the liver. It is therefore important to engineer vascularized constructs with cells endogenous and compatible with the target organ to best mimic the structure and function of that tissue *in vivo*. To create microvasculature endogenous to liver tissue, endothelial and pericyte cells specific to the liver were used in this work. This was tested in a 3D poly(ethylene glycol)-based proteolytically degradable and cell adhesive hydrogel. Results indicate that encapsulated hepatic vascular cells can form tubules in the biomimetic hydrogel. A parenchymal hepatic cell was also added in tri-culture to evaluate effect on tubule formation and function of the parenchymal cell.

Methods: The collagenase-sensitive peptide GGGPQGIWGQGK (PQ) was synthesized and conjugated onto a heterobifunctional acrylate-PEG polymer via NHS ester chemistry, rendering the polymer (PEG-PQ-PEG) biodegradable. The cell adhesive peptide RGDS was conjugated onto PEG in a similar fashion. By mixing 5% (w/v) PEG-PQ-PEG, 3.5mM PEG-RGDS and cells in a photoinitiator solution, the cell laden polymer solution was photopolymerized under white light. Cell laden hydrogels incubated in media for 7 days. Hepatic sinusoidal endothelial cells (SECs) and hepatic stellate cells (HSCs) hydrogels were compared to HUVECs and 10T½ controls. Tubulogenesis was analyzed using confocal microscopy, with CD 31 (green) staining for endothelial cells, α-smooth muscle actin (αSMA) (red) for pericytes and DAPI (blue) for cell nuclei. Parenchymal hepatic cells (HepG2) were added in tri-culture and stained with FOXA2/HNF3β (purple). Albumin synthesis by HepG2 cells was measured using an albumin ELISA assay. Tubule parameters (vessel radius, vessel length and vessel volume) were quantified using vessel image analysis software RAVE.

Results: When HUVECS and 10T½ were encapsulated with HepG2 cells in tri-culture, we observed that tubule formation was maintained, as seen in Fig. 1A. Tubule quantification indicated that vessel radius, vessel length and vessel volume were not statistically different between tubules formed with or without HepG2. Additionally, the presence of tubules did not hinder the synthesis of albumin by HepG2. SECs are morphologically unique endothelial cells due to the presence of fenestrations in their cytoplasm and lack of basal lamina. We characterized some morphological features of these cells, as well as their matrix metalloproteinase secretion and marker expression. When the hepatic vascular cells were encapsulated and co-cultured in 3D in our biomimetic hydrogel, we observed that SECs were able to degrade our hydrogel and migrated within the hydrogel to form cell-cell junctions and create tubules, while HSCs helped stabilize the tubules (Fig. 1B). Preliminary tubule quantification of the tubules formed by the hepatic vascular cells does not show a difference when compared to the tubules formed in our well established HUVECs and 10T½ angiogenic model. Additionally, the hepatic vascular cells could undergo tubulogenesis in the presence of hepatocellular carcinoma (HepG2) cells.

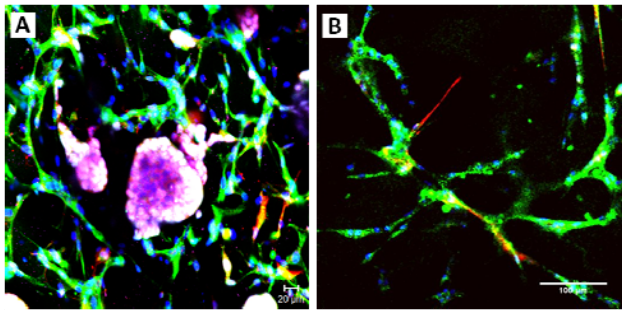

Figure 1. 3D biomimetic hydrogel encapsulations show that A) tri-culture of HUVECs, pericyte precursor cells and HepG2 cells maintain tubule formation in presence of hepatic parenchymal cells, and B) co-culture of hepatic sinusoidal endothelial cells and hepatic stellate cells can form tubule networks. (Blue = DAPI, Green = CD31, Red = αSMA, Purple = FOXA2/HNF3β)

Conclusions: In this work, we observed that tubule formation can be maintained and is not altered when a hepatic parenchymal cell was added in a tri-culture model with HUVECs and 10T½. Synthesis of albumin by HepG2 was also not affected by the presence of tubules. This indicates that tubule formation and functionality of parenchymal hepatic cells could be maintained within our biomimetic hydrogel. We also observed that endothelial cells and pericytes endogenous to the liver were able to form tubules in 3D in our biomimetic hydrogel. A hepatic parenchymal cell can be added in tri-culture with the hepatic vascular cells. We are currently further investigating the effect of hepatic vascular cells on hepatic parenchymal cell viability and function. We hope that by using SEC and HSC, vascular cells specific to the liver, that they may be more active in maintaining differentiation and functionality of hepatocytes in vitro, bringing us a step closer to engineering liver-mimicking tissue constructs.

References: Bhatia, S.N. Sci. Transl. Med. 2014; 6, 245sr2.