A Biomimetic PEG Hydrogel to Evaluate the Effects of a Vasculogenic Co-culture on Hepatocyte Bioactivity

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Statement of Purpose: Hepatocyte function is highly dependent on physical and chemical cues from non-parenchymal cells present in hepatic tissue. These cell types include fibroblasts, specialized macrophages, and vascular cells (endothelial cells and pericytes). Co-culture with various non-parenchymal cell types has repeatedly been shown to improve hepatocyte bioactivity in 2D culture; however, few studies have looked at these effects in 3D. In this work, a biomimetic poly(ethylene glycol) (PEG) hydrogel was used to probe the effects of a vasculogenic co-culture on primary hepatocytes in an engineered tissue. The results exhibit the beneficial effects of vascular cells on hepatocyte function, while building towards an ultimate goal of engineering hepatic tissue with a functional, perfusable vasculature.

Methods: Bioactive poly(ethylene glycol) (PEG) derivatives were synthesized by reacting acrylate-PEG-succinimidyl carboxymethyl (PEG-SCM, 3.4kDa) with functional peptides. The cell-adhesive RGDS peptide was reacted with PEG-SCM at a 1:1 molar ratio to form acrylate-PEG-RGDS, and the MMP-sensitive peptide GGGPQGIWGQGK (PQ) was reacted with PEG-SCM at a 1:2 ratio to form acrylate-PEG-GGGPQGIWGQGK-PEG-acrylate (PEG-PQ). Primary rat hepatocytes, human umbilical vein endothelial cells (HUVECs), and 10T½ cells (pericyte precursors) were maintained in standard culture until encapsulation. Hydrogel precursor solutions were prepared with 50mg/mL PEG-PQ and 14 mg/mL PEG-RGDS in HBS with TEOA (15 µL/mL), eosin Y photoinitiator (10 µM), and NVP (3.5 µL/mL). HUVECs were added to the hydrogel precursor solution at a concentration of $2.4x10^7$ cells/mL, 10T½ cells at $6x10^6$ cells/mL, and hepatocytes at $3x10^6$ cells/mL. Hepatocyte mono-culture and HUVEC-10T½ co-culture precursors were also prepared. Hydrogels were formed by placing 5 µL of precursor-cell suspension between a methacrylated coverslip and a glass slide and then photopolymerizing. Hydrogels were transferred to a 24-well plate and cultured in standard culture medium for 3 weeks. 7-ethoxy-4-trifluoromethylcoumarin (EFC) was added to the media and its degradation by cytochrome P450 (CYP) enzymes was measured fluorescently (ex. 385 nm, em. 502 nm) every 3-4 days. Culture medium was also assayed for albumin synthesized by hepatocytes cells. CYP and albumin assays of culture medium were also performed on hepatocytes cultured with the non-parenchymal cells in 2D. After culture, the hydrogels were fixed and immunostained, using PECAM (CD-31) as a marker for HUVECs, α-smooth muscle actin for differentiated 10T½ cells, and FoxA2/HNF3β for hepatocytes. Gels were imaged using a confocal microscope.

Results: 2D cell culture and 3D PEG hydrogel encapsulation demonstrated that hepatocytes exhibited enhanced CYP activity when tri-cultured with HUVECs and 10T½ cells. Three weeks after encapsulation, hepatocytes encapsulated with the HUVECs and 10T½ cells had CYP activity more than 2.5-fold greater than hepatocytes encapsulated alone ($p<0.05$). ELISA results indicate that hepatocyte albumin synthesis was also enhanced, both in 2D and 3D, when tri-cultured with HUVECs and 10T½ cells. In three weeks of culture, encapsulated hepatocytes produced more than twice as much albumin in tri-culture with HUVECs and 10T½ cells than in mono-culture ($p<0.05$). CYP and albumin data for the encapsulated hepatocytes are summarized in the left panel of Figure 1. Images taken with a confocal microscope allowed for analysis of the cellular structures formed during 3 weeks of culture in the hydrogels. The right panel of Figure 1 depicts the spontaneous organization of the hepatocyte-HUVEC-10T½ tri-culture in a cell-degradable and cell-adhesive PEG hydrogel; microvasculature-like structures were formed by the vascular cells and hepatocytes were dispersed throughout the engineered tissue. The results demonstrate that the presence of vascular cells enhanced hepatocyte function, and that the presence of hepatocytes did not prevent the vascular cells from forming microvasculature-like networks.

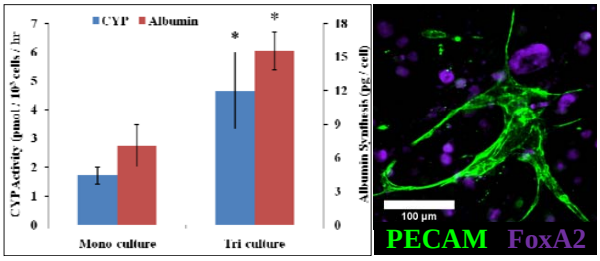

Figure 1. Left: CYP activity and total albumin synthesis of encapsulated hepatocytes at 3 weeks in culture (* $p<0.05$ for tri-culture vs. mono-culture control). Right: Confocal image of tri-culture hydrogel, exhibiting vascular (green) and hepatic (violet) structures.

Conclusions: The biomimetic PEG hydrogel supported primary hepatocyte viability and bioactivity over several weeks, and the co-encapsulation with HUVEC and 10T½ cells enhanced hepatocyte function. Enhancement of CYP activity and albumin synthesis became more prevalent as culture time increased, suggesting that the inclusion of the non-parenchymal cells prolonged the functionality of hepatocytes in the engineered tissues. Consequently, this cellular hydrogel system is promising for the engineering of vascularized hepatic tissue, as functional hepatic and vascular structures can form spontaneously and simultaneously. Future studies will address the functionality of the vascular structures formed by the HUVEC-10T½ co-culture in the engineered hepatic tissues; specifically, whether or not the vascular networks formed are perfusable and capable of supporting mass transport to hepatocytes throughout the constructs.