

Integration of Microfabricated Channels and Self-assembled Microvasculature to Support Engineered Hepatic Tissue

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Statement of Purpose: Transport of oxygen and nutrients to cells within engineered tissues remains one of the most significant challenges in tissue engineering. Co-cultures of endothelial cells and pericytes have been used to form microvascular networks in bioactive scaffolds previously, and we now seek to grow these networks in the presence of parenchymal cells in a system that allows network perfusion.

Methods: Bioactive poly(ethylene glycol) (PEG) derivatives were synthesized by reacting acrylate-PEG-succinimidyl carboxymethyl (PEG-SCM, 3.4 kDa) with functional peptides. The cell-adhesive RGDS peptide was reacted with PEG-SCM at a 1:1 molar ratio to form acrylate-PEG-RGDS. 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). Human hepatocellular carcinoma cells (HepG2 cells), human umbilical endothelial cells (HUVECs), and 10T $\frac{1}{2}$ cells (pericyte precursors) were maintained in standard culture. A hydrogel precursor solution was prepared with 25 mg/ml PEG-PQ, 14 mg/ml PEG-RGDS and 3 mg/ml Irgacure 2959. HUVECs were added to the hydrogel precursor at 2.4×10^7 cells/mL, 10T $\frac{1}{2}$ cells at 6×10^6 cells/mL, and HepG2 cells at 1×10^6 cells/mL. A hydrogel slab was formed within a PDMS microfluidic device by injecting 200 μ L of precursor-cell suspension into the device, applying a photomask to define counter-current channels alongside the hydrogel, and photopolymerizing (Cuchiara 2012). Figure 1a depicts the layout of the microfluidic device. The remaining precursor-cell suspension was used to prepare static hydrogel controls.

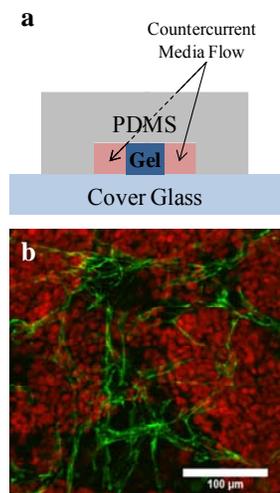


Figure 1. (a) Cross-section of microfluidic device. (b) Confocal image of tri-culture hydrogel, exhibiting vascular (green) and hepatic (red) structures.

Media flow was initiated through the channels at a rate of 10 μ L/min and continued for 96 hours. After completion of flow, the hydrogel was immunostained, using PECAM

(CD31) as a marker for HUVECs, α -smooth muscle actin for pericytes, and FoxA2/HNF3 β for HepG2 cells. Viability was assessed by calcein AM/ethidium homodimer staining. HepG2 monoculture and HUVEC-10T $\frac{1}{2}$ co-culture experiments were also included as controls. Separate experiments were performed to evaluate microvascular perfusion for the cell networks in the hydrogels. 70 kDa fluorescent dextran was added to the flow media after 4 days of culture, and fluorescent images were captured and analyzed for vascularized and non-vascularized conditions.

Results: Confocal images of the statically cultured hydrogels exhibit the structures spontaneously formed by cells. HepG2 cells aggregate to form clusters, while HUVECs and 10T $\frac{1}{2}$ cells, in both co- and tri-culture gels, form microvasculature-like networks. Figure 1b depicts the spontaneous organization of the HepG2-HUVEC-10T $\frac{1}{2}$ tri-culture in a cell-degradable and cell-adhesive PEG hydrogel. Quantitative image analysis was performed and demonstrated that the vessel densities in co-culture (HUVEC-10T $\frac{1}{2}$) and tri-culture (HepG2-HUVEC-10T $\frac{1}{2}$) hydrogels were not significantly different ($p < 0.05$), demonstrating that the presence of hepatocytes did not inhibit tubule formation. When cultured in the microfluidic device, cell viability could be maintained across significantly longer distances than in static culture ($p < 0.05$). Imaging of fluorescent dextran added to the perfused media indicates that the cellular tubules within the hydrogel construct anastomose to the microchannels and can be effectively perfused.

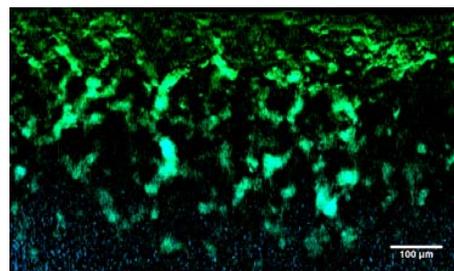


Figure 2. When 70 kDa fluorescent dextran was added to the media in the microfluidic channels (bottom of image), cellular tubules in the hydrogel construct were also perfused.

Conclusions: Successful anastomosis between microfabricated channels and cell-formed microvascular networks demonstrates the role that such a combination may play in the scaling up of engineered tissues. The improvements in mass transport achieved within the PEG scaffolds in this work indicates that cell-formed microvascular networks are a promising tool for maintaining the viability of engineered tissues upon implantation.

References:

Cuchiara MP, Adv Funct Mater. 2012; 22: 4511-4518.