Perfusable Biomimetic Scaffolds within Microfluidic Devices

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Statement of Purpose: We aim to use oxygen-sensing microparticles to aid the design of biomimetic scaffolds within microfluidic devices that support the formation of perfusable microvessels for use in creating large-scaled functional tissue engineered constructs.

Tissue engineered constructs have been restricted in size due to insufficient diffusivity of oxygen and nutrients to the interior of the implant. Our lab is developing perfusable microvascular networks *in vitro* through the design of microfluidic hydrogels integrated with self-assembling, pro-vasculogenic co-cultures, which allow for an anastomotic interface for integration with native vasculature. The ECM-mimicking hydrogel contains polyethylene glycol (PEG) modified to be cell-adhesive and proteolytically-degradable by tethering integrin-binding RGDS peptides to PEG chains and incorporating MMP-sensitive GGPQG↓IWGQGK peptides (abbreviated PQ) into the polymer backbone. This system has been shown to support the self-assembly of a 4:1 co-culture of human umbilical vein endothelial cells (HUVEC) and pericyte precursors (10T1/2) into microvessel networks.[1,2]

Since oxygen delivery plays a critical role in vasculogenesis, precise spatiotemporal monitoring of molecular oxygen within our system would facilitate optimal microfluidic device design. Based off work by Acosta *et al*., we synthesized PEG-coated ruthenium (Ru)-based oxygen sensors that operate via reversible luminescence quenching by oxygen without needing to consume it. We hypothesized that these particles could be encapsulated in the presence of cells without negatively affecting their viability or local oxygen diffusion.

Methods: Fabrication of the multilayer microfluidic devices is described in detail elsewhere.[2] Briefly, optically clear polydimethylsiloxane (PDMS) microchannel housings were fabricated via standard soft lithography. Next, a PEG-acrylate hydrogel precursor solution containing 4% acryl-PEG-PQ-PEG-acryl, 3.5 mM acryl-PEG-RGDS, and a 4:1 mixture of HUVEC and 10T1/2 cells was injected into the PDMS housing and photocrosslinked into a confined geometry using a photomask (Fig. 1a). Media and buffer were perfused at physiologic microvascular shear stresses (15 dyne/cm^2) on opposite sides of the hydrogel. The resulting networks were fixed and immunostained at 96 h and imaged throughout the hydrogel using a Zeiss® 510 inverted confocal microscope.

Oxygen-sensing microparticles were synthesized by binding $Ru(Ph_2phen_3)Cl_2$ to silica microparticles, then coating them with PEG diacrylate (PEGDA) via an oil-in-water emulsion. The resulting microparticles were characterized with a Zeiss® Axiovert 135 inverted microscope and TECAN® fluorescence plate reader to determine size and excitation/emission spectra, respectively, and their cytotoxicity was measured using a Live/Dead assay (Invitrogen). We performed preliminary work on incorporating oxygen-sensing microparticles within our system by encapsulating microparticles with live cells at a 1:10 ratio within a PEGDA hydrogel, and measuring microparticle fluorescence every hour throughout a 48 h culture (Olympus® VivaView). We developed a MATLAB® algorithm to extract normalized fluorescence intensity from the images, then converted these measurements to oxygen concentration using the Stern-Volmer relationship, assuming 20% oxygen at t=0.

Results: Perfusable microvessels formed within our microfabricated PEG-based hydrogel system up to at least 96 h (Fig. 1b). Immunofluorescence micrographs indicate robust tubule formation nearest to the media channels, but show decreasing tubule number and length with increasing distance from the nutrient source.[2]

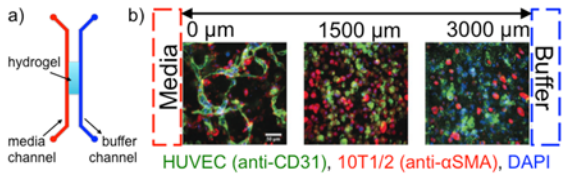

Fig. 1 a) Microfluidic device schematic. b) Microvascular network morphology after 96 h at 0, 1500, and 3000 µm from the perfused media microchannel.

PEGDA-coated oxygen-sensing microparticles were synthesized with diameters ranging from 20-35µm (Fig 2, inset) and exhibited excitation/emission maxima at 555/620 nm. A Live/Dead assay of cells exposed to PEGDA-coated microparticles showed no decrease in cell viability compared to a media-only control. Furthermore, microparticles encapsulated amongst cells within a PEGDA hydrogel detected a decrease in oxygen concentration over the first 20 h in culture, stabilizing at 5-10% oxygen for the remainder of the study (Fig. 2**)**. As expected, these changes were not seen in the acellular control, which lacked an oxygen-consuming species.

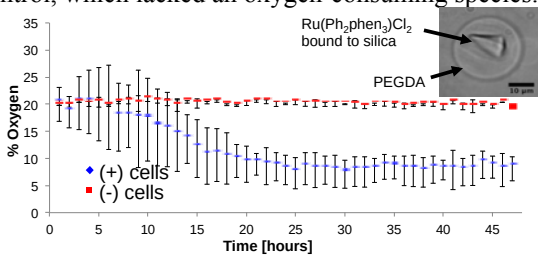

Fig. 2 Changes in % oxygen detected by microparticles encapsulated in the presence or absence of cells. The inset shows a PEGDA-coated microparticle.

Conclusions: Our work demonstrates self-assembly of perfusable microvessels within a microfluidic device; however, tubule number and length decrease with increasing distance from a media channel. To aid in the design of our microchannels, we synthesized biocompatible oxygen-sensing microparticles, and demonstrated their ability to incorporate into our microfabricated hydrogels. Further optimization of the microparticle synthesis will result in a more homogenous population, and thus improve sensing capabilities.

References: [1]Moon JJ, *et al*. *Biomaterials* **2010**; [2]Cuchiara MP, *et al*. *Adv Funct Mater* **2012**; [3]Acosta, M.A., *et al*. *Biomaterials* **2009**