Hydrogels to Support 3D Microvessel Formation by Blood-derived Endothelial Progenitor Cells

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Introduction: The development of stable, robust microvessels remains an important obstacle for tissue engineering functional organs. Endothelial progenitor cells (EPCs) derived from umbilical cord blood are a promising cell source for vascular tissue engineering[1] as they are readily obtainable and can outperform mature endothelial cells (ECs) in network formation studies in vitro[2] and in vivo[3]. However, scaffolds used to evaluate EPC microvessel formation employ biologically-derived matrices, limited in their amenability to mechanical and biochemical properties. Poly(ethylene glycol) (PEG) hydrogel systems are FDA approved, biocompatible, and can undergo modifications to recapitulate the biochemical and biophysical microenvironment of vascular niches.[4]

The aim of this study was to investigate the use of PEG hydrogel systems to support EPC microvessel formation for use in tissue engineering and regenerative medicine applications.

Materials and Methods: Late outgrowth endothelial colony forming units (ECFU) were isolated from the mononuclear cell fraction of umbilical cord blood[5] and characterized as EPCs based upon the expression of endothelial markers CD31, VE-cadherin, eNOS, vWF; hematopoietic progenitor cell marker CD34, and lack of monocytes markers CD45, CD19, CD14.
RGDS was conjugated to acryl-PEG-succinimidyl valerate (SVA) through amine chemistry and grafted to the surface of diacrylate PEG (PEGDA) using 2,2-dimethoxy-2-phenylacetophenone under UV light. EPCs were plated atop PEG hydrogels or tissue culture glass in coculture with vascular smooth muscle cells (SMCs) at a 1:4 ratio with a total cell number of $8x10^4$ cells/cm^2 in EBM2 media containing 10% FBS. As an endothelial cell control, human umbilical vein-derived endothelial cells (HUVECs) were cocultured with SMCs utilizing identical conditions as EPC cocultures. EPCs and HUVECs were transduced with GFP or Td.tomato lentivirus prior to coculture.
PEG hydrogels were rendered cell-degradable by incorporation of the matrix metalloproteinase (MMP) sensitive peptide, GGGPQGIWGQGK (PQ). SMCs and EPCs or HUVECs were combined at a 1:1 ratio using a total seeding density of $1x10^4$ cells/µl and encapsulated with 3.5 mM of RGDS and 5 wt% PEG-PQ, crosslinked using Eosin Y as the photoinitiator under white light to create 380 µm-thick hydrogels.

Results: PEGDA hydrogels containing surface-immobilized RGDS can support EPC microvessel formation to the same extent as tissue-culture glass based upon the lack of significant differences in the network morphology parameters of total tubule length, branch points, and average segment diameter.
We were able to extend the 2D EPC microvessel system into the MMP-sensitive 3D PEG hydrogel system. The 3D EPC-generated microvessels exhibited a distinct morphology from the HUVEC microvessels with a greater amount of connectivity, shown by a significant increase in branch points, and decreased average segment diameter, and average segment length (**Figure 1**). The 3D EPC microvessels were stable for at least 30 days in vitro and contained patent lumen, deposition of basement membrane proteins collagen IV and laminin adjacent to microvascular structures, and pericyte-like wrapping of SMCs positive for PDGFR-β and α-SMA.

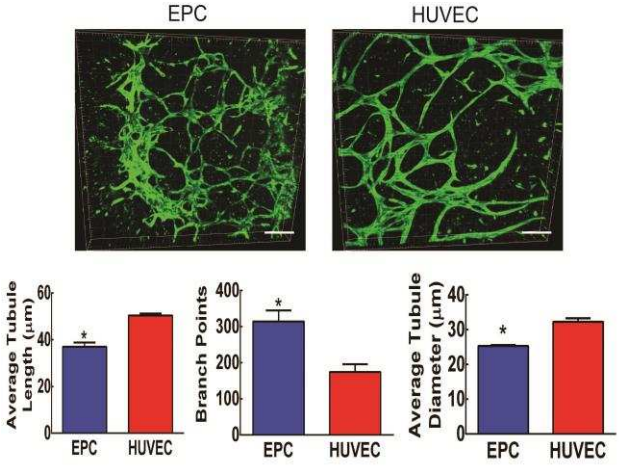

Figure 1: Representative images of 3D EPC and HUVEC microvessels within PEG hydrogel systems after 14 days of coculture with SMCs. Scale bar equals 200 um. *indicates $p < 0.05$ in comparison to HUVEC conditions.

Conclusions: This study demonstrates the ability of EPCs to form stable, robust microvessels recapitulating aspects of physiological angiogenesis within a tunable and clinically translatable 3D PEG hydrogel system. Future work will focus on customizing PEG hydrogel biochemical and biomechanical properties to regulate progenitor cell-driven microvessel formation.

References: Jain RK, et al. Nat. Biotechnol., 2005. (2) Au P, et al. Blood 2008. (3) Peters EB, et al. Tissue Eng. Part A., 2013. (4) Culver JC, et al. Adv Mater, 2012. (5) Ingram DA, et al. Blood, 2004.