Synthetic extracellular matrix for investigating 3D vascular network formation

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Statement of Purpose: A major limitation for many tissue engineering strategies is poor nutrient supply due to a lack of a functional vasculature (1-2). To overcome these limitations, there is a need for improved understanding of the biological signals that induce blood vessel formation and strategies to promote revascularization of engineered tissues. Here, we describe a strategy for tailoring poly(ethylene glycol) (PEG) hydrogels to promote and optimize vascular network formation using human endothelial cells (EC) cultured in 3D in vitro environments.

Methods: Poly(ethylene glycol) (PEG) hydrogels were formed using "thiol-ene" photopolymerization to couple thiol-containing peptides with multiarm PEG molecules functionalized with terminal norbornene groups (3). For 3D cell culture, we formed hydrogels with matrix metalloproteinase (MMP)-degradable crosslinks to allow proteolytic remodeling and pendant RGD-containing peptides to promote adhesion (Fig. 1). We investigated network formation and sprouting for human umbilical vein endothelial cells ("HUVECs") or induced pluripotent stem cell-derived endothelial cells ("iPSC-ECs", Cellular Dynamics iCell® ECs, Madison, WI) encapsulated in PEG hydrogels with varying RGD concentrations to tune adhesion and MMP-crosslinking density to change mechanical properties. HUVECs and iPSC-ECs were encapsulated in PEG hydrogels at different cell densities: (1) 5-40 million cells/mL to monitor network assembly or (2) 40 million cells/mL to form high density clusters, which were then surrounded by a second hydrogel layer to investigate sprouting.

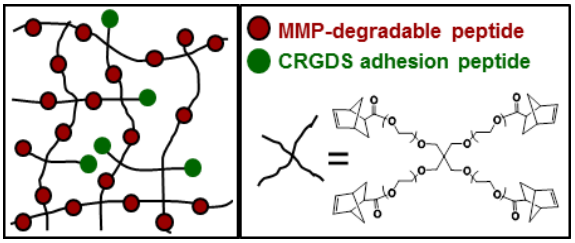

Figure 1. Poly(ethylene) glycol hydrogels formed through thiol-ene photopolymerization.

Results: ECs assembled into organized networks when encapsulated as dispersed cell suspensions in PEG hydrogels while tube-like structures consistent with sprouting were observed when high density clusters were surrounded by cell-free PEG matrices (Fig. 2). The extent of network formation was dependent on adhesion and crosslinking density (stiffness) and the stability of the resulting vascular structures was dependent on matrix properties, culture media, and the presence of support cells.

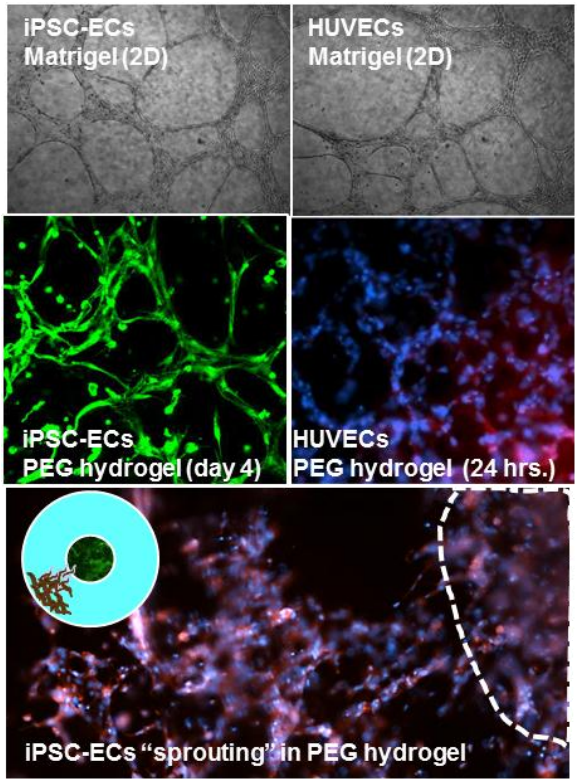

Fig. 2. Endothelial cell in 2D and 3D culture.

Conclusions: HUVECs and iPSC-ECs formed networks in PEG hydrogels, which may provide a valuable tool for systematically investigating and manipulating blood vessel formation due to strict control over biochemical and biophysical properties provided by the synthetic 3D culture platform. The PEG hydrogels described here are both versatile and biocompatible, making them suitable for tissue modeling and clinical applications.

References:

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