Formation of engineered 3D vascular networks using human induced pluripotent stem cell (iPSC)-derived endothelial cells

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Abstract

Vascular biology plays a critical role in many aspects of normal and disease-relevant physiology, including: barrier function, inflammation, cell migration (wound healing and metastasis), thrombosis and hemostasis, atherosclerosis, as well as angiogenesis. The development of reliable vascular biology tools, such as endothelial cell (ECs), is a critical need for the generation of relevant in vitro human disease models for drug and therapeutic research, as well as enabling regenerative medicine. To that end, we have developed human induced pluripotent stem cell (iPSC)-derived endothelial cells (iCell Endothelial Cells) that exhibit vascular endothelial markers including PECAM1 (CD31), VEGF-receptor 2 (KDR/Fk1), Endoglin (CD105), VE-cadherin (CDH5/CD144), von Willebrand factor (vWF), and ZO-1, a tight junction protein. Additionally, the cells express matrix metalloproteinases (MMP-2, MMP-3 and MMP-9) which are essential to penetrate extracellular matrix for vascularization and angiogenesis. Functionally, the iPSC-derived ECs form tube structures in Matrigel, take up low density lipoprotein (LDL), and exhibit thrombin-dependent barrier function, all of which are consistent with a mature EC phenotype. We also investigated the formation of engineered 3D vascular networks using these iPSC-derived ECs encapsulated in synthetic extracellular matrices (synthetic ECM) formed by crosslinking 8-arm polyethylene glycol molecules with a matrix metalloproteinase (MMP)-degradable peptide and also included a fibronectin-mimicking RGDDS sequence for adhesion. When encapsulated in synthetic ECM, iPSC-derived ECs form vascular networks that were stable for days and can be further stabilized for weeks by surrounding a cell-free support layer. Sprouting from the iPSC-derived EC dense patches into a cell-free synthetic ECM layer was also observed, demonstrating the potential for investigating angiogenesis. Comparing iPSC-derived ECs to HUVECs, iPSC-derived ECs present an increased expression of vascular related morphogenesis genes, which is consistent with the increased vascular network structures observed for cells in synthetic ECM. These data demonstrate that the iPSC-derived ECs provide a stable, well-defined source of human ECs that are well-suited for investigating vascular biology and are also useful for addressing current challenges facing vascular therapeutic development and tissue engineering applications.

Engineered 3D Matrices to Support Vascular Networks

Engineered scaffolds afford the potential to provide a defined matrix architecture with molecular cues for cell culture enabling robust and reproducible tissue formations suitable for diverse applications. Here we have designed PEG hydrogel scaffolds that have been decorated with RGD peptide functionality to encourage adhesion and cell-homing and attachment along with a matrix metalloproteinase degradable peptide sequence to encourage vascularization. The type of controlled scaffold building chemistry applied here can also be envisioned to include soluble cues which are noncovalently bound and create a high local concentration of soluble ligand to guide scaffold colonization or cause signaling. Additionally, a heterogeneously functionalized or layered scaffold could be generated to create a spatially defined coculture situation.

Vascularization and Angiogenesis Studies

Our functionalized PEG hydrogel scaffolds were employed to create defined vascular networks. Initially, the iPSC derived endothelial cells were shown to form complex vascular 3D networks when cultured on the RGD/MMP degradable peptide functionalized scaffold (shown at right). These cultures were stable over several weeks in culture. In addition, the scaffold was capable of supporting co-cultures with pericytes (iPSC-derived mesenchymal stem cells (MSCs) lower right) to generate a more physiological vascular network. We also investigated iPSC-endothelial cell sprouting angiogenesis by taking advantage of photopolymerization to rapidly form a high density cell cluster within a synthetic ECM droplet. The synthetic ECM droplet was then surrounded by a cell-free hydrogel layer and cultured to observe iPSC-endothelial cells migration out of the high density locus. This type of assay could be quantified and employed as a screening tool for the identification of pro- and anti-angiogenic molecules.

Summary

There is great potential for application in drug discovery and regenerative medicine with the advent of iPSC technology. Working with stem cell derived tissues offers the promise to generate autologous transplantable organs produced from patient derived donor tissue. iCell Endothelial Cells provide a novel human relevant platform for disease modeling, drug discovery applications and also in support of vascularized transplant tissues laying the foundation of supporting architecture for target synthetic organs. The iPSC-ECs investigated here stably and reproducibly display morphological, phenotypic and functional characteristics common to HUVECs and HAECs. The endothelial cells were deployed into novel defined PEG hydrogels that displayed both adhesion signaling and protease degradable peptides to promote vascular structure formation via an angiogenic mechanism. The resulting structures were stably viable for weeks in culture. Co-culture with astellate cell type further stabilized the network. The work presented illustrates the synergistic potential for bioengineered scaffolding combined with stem cells technology to advance vascular biology tools.

iCell Endothelial Cells Morphology and Functional Characterization

The iPSC-derived endothelial cells (iCell Endothelial Cells) display morphological and functional characteristics typical of endothelial cells. Shown here are a phase contrast image displaying the cellular architecture and immunohistochemical staining for expression of von Willebrand Factor. Functionally, the cells were observed to take up acetylated LDL and also form 3D vascular networks when plated atop a Matrigel plug. The iCell Endothelial Cells also form a consistent barrier as evidenced by the outlining of the cells by the staining for ZO-1 protein found at the tight junctions. The barrier could be physically assessed by measuring impedance across the culture on the ACEA xCELLigence platform. The barrier can be disrupted in a dose dependent fashion by the addition of thrombin and can recover over time from non destructive doses as a function of wound healing.

iCell Endothelial Cells Purity and Expression of Vascular Markers

iCell Endothelial Cells express vascular markers similarly to HUVECs and HAECs, with a greater similarity to HAECs for key angiogenesis related genes. A comparison of mRNA expression of key endothelial markers and angiogenesis related genes is presented here. For each gene, array probe data from the highest and lowest reporting probes is shown. Shown below, these cells provide a consistent source of high purity human cells that can be cultured for at least 5 passages maintaining that high degree of purity.