The interaction of vascular cells and flow in vasculogenesis

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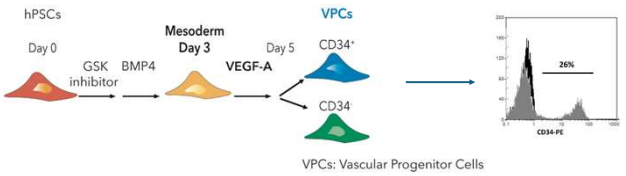
Introduction

Generation of new blood vessels occurs via two distinct processes. *Angiogenesis* involves the formation of new vessels from pre-existing ones, mainly by sprouting, whereas in *vasculogenesis* mesodermal cells differentiate to vascular progenitor cells (VPCs) and endothelial cells (ECs) to form a vascular plexus. Maturation and stabilisation of the nascent plexus relies on the recruitment of mural cells (MCs), a process called vascular myogenesis and deposition of extracellular matrix.
While angiogenesis is a well-studied process, vasculogenesis is not. Several factors are known to affect vasculogenesis *in vivo*, the most important of which are the presence of MCs and flow. Blood flow-derived forces control the growth and shape of both newly formed and established vascular network. Indeed, shear stress in laminar blood flow is essential for vessel development and along with circumferential and axial stress, define shape and wall thickness of the vasculature. Additionally, the interplay between ECs and MCs including growth factor secretion, cell-cell contact and extracellular matrix modulation is of great significance. In the newly formed vessels, recruited MCs exert a stabilising influence by inhibiting EC-migration and proliferation, through the release of distinct signaling molecules.

1. Differentiation of hPSCs to VPCs[1]

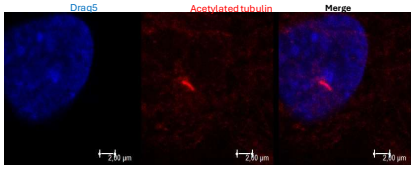


VPCs: Vascular Progenitor Cells

On the 5th day of the differentiation procedure up to 35% of the cells express CD34, a marker of VPCs and could be clearly segregated from CD34- cells.

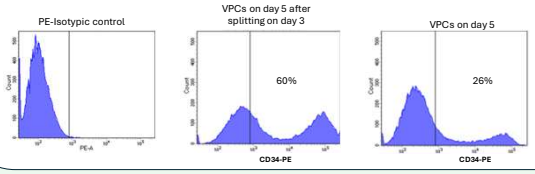
2. hiPSC-derived ECs can respond to flow

hPSC-derived ECs have primary cilia which allow them to respond to flow, as they act as calcium-dependent mechanosensors that sense blood flow.



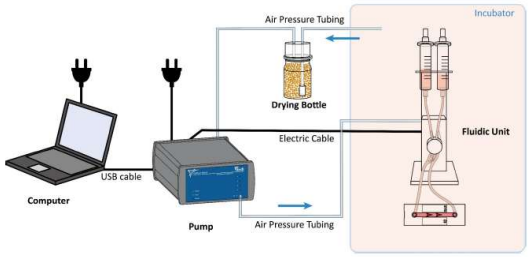
3. Replating of mesodermal cells increases the differentiation efficiency

Replating of mesodermal cells on microfluidic chambers is necessary to study the effect of flow.
Cell splitting on day 3 increases the differentiation efficiency up to 60%.

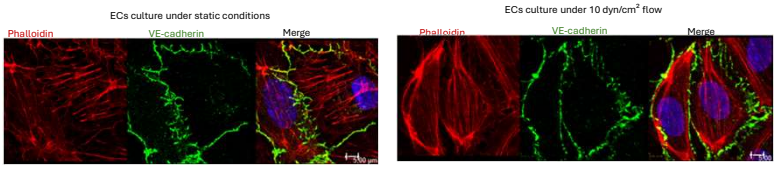


4. Culture of ECs Under Flow

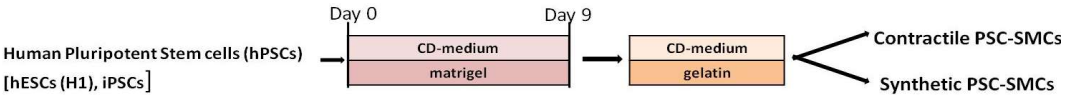
A microfluidic system to mimic flow conditions.



Shear stress influences cell morphology, cell structure, and organization. In contrast to static cell culture, cells start to orient in the direction of the flow, and a rearrangement of the cytoskeleton takes place with actin fibers aligning in the direction of the flow.



5. Differentiation of hPSCs to MCs[2]

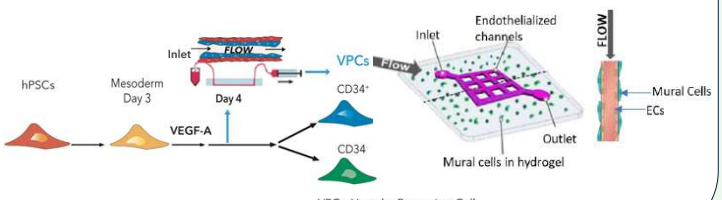


CD medium: ADSC basal medium, 2,5%FCS, Gln
CD(+) medium: CD medium plus bFGF, EGF, IGF

6. Effect of flow on vasculogenesis and vascular morphogenesis

The effect of flow on the differentiation of the MTP to VPCs

The combined effect of flow and MCs (synthetic SMCs) on the maturity of the final EC population on gel based microfluidic channels



VPCs: Vascular Progenitor Cells

Conclusions

1. We have established a model of vasculogenesis by differentiating hPSCs to VPCs and further to ECs
2. We confirmed that our hPSCs-ECs can respond to flow and we validate the best conditions for that
3. A microfluidic system has been installed
4. hPSCs differentiated successfully to sSMCs
5. To create an *in vitro* model of vascular morphogenesis we will address the role of MCs and flow in vasculogenesis using a microfluidic system

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[2] Markou M, Kouroupis D, Badounas F, Katsouras A, Kyrkou A, Fotsis T, Murphy C, Bagli E, 2020, Front Bioeng Biotechnol;8:278.