Engineered 3D-Vessels-on-Chip to study effects of dynamic fluid flow on human induced pluripotent stem cell derived endothelial cells

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Addendum

Summery

To improve the predictive capability of pre-clinical models and reduce the use of animal models in drug discovery and disease modelling, advanced *in vitro* models are being developed. These microphysiological systems (MPS) or "Organs-on-Chip" (OoC) are being developed to include all aspects of the human physiology to improve the *in vitro* cellular response. OoCs combined with differentiated human induced pluripotent stem cells (hiPSC) allow the use of cells with patient specific genotypes and aid the development of personalized and precision medicine.

In this thesis, the development of tractable models of the vasculature is described. These models allow for the combination of hiPSC-derived vascular and tissue specific cells with haemodynamics to recapitulate essential stimuli of blood vessels.

In **Chapter 1,** the importance of blood vessels for organ function, drug transport, and the immune response are described. The endothelial cell (EC) phenotype is essential for proper organ function and drug response. Next, various cell sources used in biomedical research, including hiPSC-derived ECs, are summarized. Haemodynamics are essential for the vascular phenotype and to include these important modulators, 3D-VoCs were developed. Finally, common methodologies are described to engineer 3D-Vessels-on-Chips.

Chapter 2 describes a scalable methodology to generate hydrogel scaffolds that can be used for seeding of hiPSC-derived vascular cells. It uses a microfluidic technique called viscous finger patterning (VFP) and the protocol comprises simply of sequentially pipetting 2 fluids with different viscosities. These scaffolds are fully perfusable to apply realistic haemodynamic forces. The described method results in uniform lumens, important for perfusion studies.

Chapter 3 describes a fluidic perfusion platform using pressure controllers optimized for long term perfusion of 3D-OoCs. It also describes a simple distributing Fluidic Circuit Board (FCB) to increase throughput.

Chapter 4 describes a FCB specifically designed for the perfusion of the 3D-VoC described in **chapter 2**. The microfluidic circuit can be used with a range of luminal diameters, while maintaining the same mechanical stimulation, multiple samples can be perfused simultaneously. Using this system, the EC morphology changes in response to both circumferential stress and wall shear stress (WSS) are investigated.

Chapter 5 describes a methodology to engineer a perfusable model of the smallest capillaries of the vascular system. A specialized method is described to print a complex capillary bed into a hydrogel that can be seeded with vascular cells. The mechanical properties of this hydrogel can be tuned for

tissue specific conditions and allows to design complex capillary beds to generate complex flow patterns.

Chapter 6 is a general discussion of the findings presented in this thesis and summarizes the advantages of the methods and work still required to improve the presented models. Because the described methods do not rely on highly specialized materials and techniques, it brings OoC-technology closer to the end-user: the biomedical researcher. Scope for future work in the field is also proposed.