Chapter VIII

Summary

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Acknowledgements

Curriculum Vitae

List of Publications

Summary

In the last decades, microvascular diseases are a leading cause of mortality worldwide. Chronic activation of the endothelial cells by cardiovascular risk factors can inflict the loss of pericytes that plays a critical role in microvascular stabilization. To diagnose and treat microvascular diseases, we aim to explore the association of circulating plasma factors with microvascular integrity. As current human 2D models with cultured endothelial cells lack sufficient complexity to assess the function of microvascular endothelial-pericyte interactions, research on microvascular loss largely depends on animal models. To mimic the microarchitecture and functions of the human blood vessel in a more efficient way for drug discovery, the organs-on-chips are proposed to be an advanced 3D culture system as introduced in **Chapter I**. The scope of this thesis is to develop a human microvessel-on-a-chip that experiences mechanical fluid flow, biological and environmental sensing, which allow for analysis of organ-level microvessel physiology and pathophysiology. Furthermore, this device is high-throughput and can be used as a diagnostic and drug screening tool for drug development.

In **Chapter II**, we reviewed organ-on-a-chip efforts published over the last two years in light of user friendliness, compatibility, assay ability and product readiness. This review is written to introduce organs-on-chips to scientists from various research field. Elegant platforms from a single chip to microtiter plate format with various integrated sensors were reported. Also, functional assays for angiogenesis, calcium imaging of neurons and neuro-muscular contractility were notified. The system is gaining its momentum in compatibility with standard analysis techniques such as sequencing, fluorescent activated cell sorting and mass spectrometry. Organs-on-Chips is rapidly shifting from academic proof-of-concept studies to real-world solutions.

The development of models that effectively recapitulate the human blood vessel is important for studying the pathogenesis and therapeutic approaches for microvascular diseases. In order to do this, in **Chapter III**, we engineer a perfusable human microvessel-on-a-chip with human umbilical vein endothelial cells (HUVECs) that is partly surrounded with extracellular matrix (ECM). The perfusion of VEGF, histamine and TNFα leads to increase in vessel permeability, reconstituting the microvascular leakage seen *in vivo*. To screen microvascular destabilization factors in blood, we prepared plasma samples from whole blood and treated with hirudin, corn trypsin inhibitor (CTI) and compstatin. Healthy volunteer plasma samples spiked with

VEGF, histamine and TNFα leads to vascular leakage in the microvessel-on-a-chip. These findings strengthens the idea that our assay can be used for analysing human microvascular pathophysiology and possible carrying out preclinical drug evaluations.

As an example, in **Chapter IV** we show that a perfusable, endothelialized microvasculature-on-a-chip featuring a collagen hydrogel that minimally mimics the ECM of host tissue allows the modeling of Ebola-induced vascular phenotypes and provides an *in vitro* platform for drug studies. Luminal infusion of Ebola virus-like particles (VLPs) leads to albumin leakage from the engineered vessels. Mechanistically, we demonstrate that this process involves the Rho/ROCK pathway. The Ebola glycoprotein ($GP_{1,2}$) plays a key role in this process. Finally, we demonstrate the applicability of this platform for studying the efficacy of potential drugs by measuring the potency of the recently developed experimental drug FX06 and melatonin, in phenotypic rescue.

Besides imaging analyses of phenotypic changes in the microvessels, metabolomics may deliver a more informative readout of endothelial function. As an exploratory study, in **Chapter V** we assessed whether the 3D microvessels display a less inflammatory phenotype compared to 2D endothelial cell cultures. In order to do this, we use an optimized targeted liquid chromatography–tandem mass spectrometry measurements of a panel of pro- and anti-inflammatory bioactive lipids to generate expression profiles both in TNFα treated microvessels as well as in 2D endothelial cell cultures. We demonstrate that bioactive lipid profiles can be readily detected from 3D microvessels-on-a-chip and display a more dynamic, less inflammatory response to TNFα, that resembles more the human situation, compared to classical 2D endothelial cell cultures.

In vivo, the microvessels experience physical stress associated with laminar blood flow and it plays a central role in maintaining vascular integrity and homeostasis. **Chapter VI** reports the microvessels-on-a-chip with integrated multi-channel microfluidic pumping and *in situ* oxygen monitoring system. This system is used to study the effect of flow on the cytoskeleton remodeling and the expression of flow responsive genes in the microvessels-on-a-chip. Our results demonstrate that the generated unidirectional flow changes the orientation of angle of endothelial cells and increase the expression of flow responsive genes *in vitro* compared to bidirectional flow.

In conclusion, as illustrated in this thesis, the microvessels-on-chips may serve as a unique tool for microvascular destabilization studies as well as for the development of novel therapeutic strategies to combat microvascular complications. In the future, with the developed method to perfuse plasma samples in the microvessels, destabilizing profile of patients can be developed in order to predict ongoing microvascular rarefraction and risk of complications.