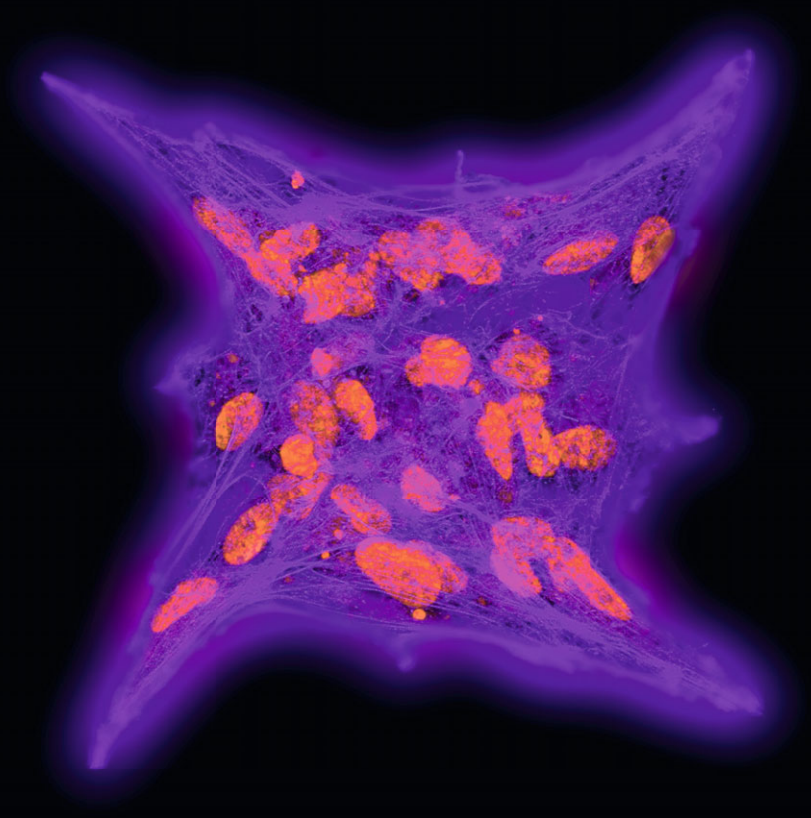
Maskless photolithography for rapid Organ-on-a-Chip prototyping and microvascular engineering

Kasi, D.G.

Contents



Appendix

Summary

Central to this thesis was the development and implementation of facile and interdisciplinary bioengineering methods that would support the creation of human stem cell-based Organ-on-a-Chip (OoC) and other types of (microvascular) *in vitro* models. These methods are based on a commercially available system for maskless photolithography (i.e. Alvéole PRIMO) that uses a "digital micromirror device" to spatially modulate and project UV light. The system is connected to a conventional inverted microscope with an automated stage and the resulting benchtop setup can project high-resolution, arbitrary 2D patterns (also known as digital photomasks) via the microscope optics. The methods presented in this thesis enable rapid prototyping and fabrication of OoC devices, microvascular models and cell-adhesive micropatterns that are compatible with differentiated derivatives of human induced pluripotent stem cells (hiPSCs).

As described in **Chapter 1**, the need for such methods is driven by translational difficulties in biomedical research associated with limitations of animal models that include their inability to capture certain aspects of human physiology and anatomy, or precisely predict human responses to drugs. Moreover, since ethical concerns and regulatory pressure have increased, there is an ongoing search for alternatives to animal models. When the engineering methods are combined with patient-derived hiPSCs (with or without gene-editing technology), they provide powerful tools for model development, as they allow an holistic approach to OoC prototyping, (vascular) disease modeling, and drug development.

In **Chapter 2**, I first used the PRIMO system to develop a cleanroom-free workflow for rapid prototyping of OoC devices in regular biochemistry labs that can be used with conventional photoresists and glass coverslips with a one-day turnaround. This approach was demonstrated as suitable for facile in-house fabrication of polydimethylsiloxane (PDMS)-based microfluidic chips that are compatible with cell culture (i.e. OoCs). I subsequently used this workflow in **Chapter 3** to produce microfluidic chips containing a central compartment and to perform hydrogel patterning on-chip. Together, this allowed me to engineer a Vessel-on-Chip (VoC) containing perfusable human microvasculature, perform compartmentalization for co-cultures and fabricate advanced functional structures such as microscopic valves. The integral approach of **Chapters 2 and 3**, therefore, has applications for both rapid prototyping and fabrication of OoCs and different types of microvasculature.

Next, I employed the PRIMO system in **Chapter 4** for different methods of photopatterning that form the foundation for a "reference-free traction force microscopy" contraction assay to study contractility of single cells with predefined and controllable morphology. Simple *in vitro* models like this are compatible with high-throughput screening and are useful to study drug responses and contractile dysfunction, as is present in various cardiovascular diseases. The photopatterning methods developed are applicable to multiple research areas where controlled micropatterning of cells is required.

In **Chapter 5**, we made the first steps towards developing a disease model by generating and characterizing hiPSCs from a patient suffering from retinal vasculopathy with cerebral leukoencephalopathy and systemic manifestations (RVCL-S), a rare autosomal dominant small vessel disease caused by C-terminal truncating mutations in the *TREX1* gene. First, genetic correction of the mutation was performed to generate isogenic healthy control hiPSCs. To investigate the role of endothelial cells (ECs) in this disease, we then derived ECs from the isogenic hiPSC pair. Despite having these isogenic pairs, it proved challenging to find a disease phenotype or differences that could be quantified robustly using 2D assays. This illustrated one of the major limitations to these models at the present time, namely what are the ideal circumstances that can reveal disease phenotypes. Further research using more complex models is needed to better understand the differences between healthy and RVCL-S ECs.

Finally, in **Chapter 6,** I discuss the results presented in this thesis, its limitations, suggestions for improvement, and future perspectives.