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Modeling vascular inflammation with immune cell-vessel crosstalk in hiPSC-derived 3D vessels-on-chip

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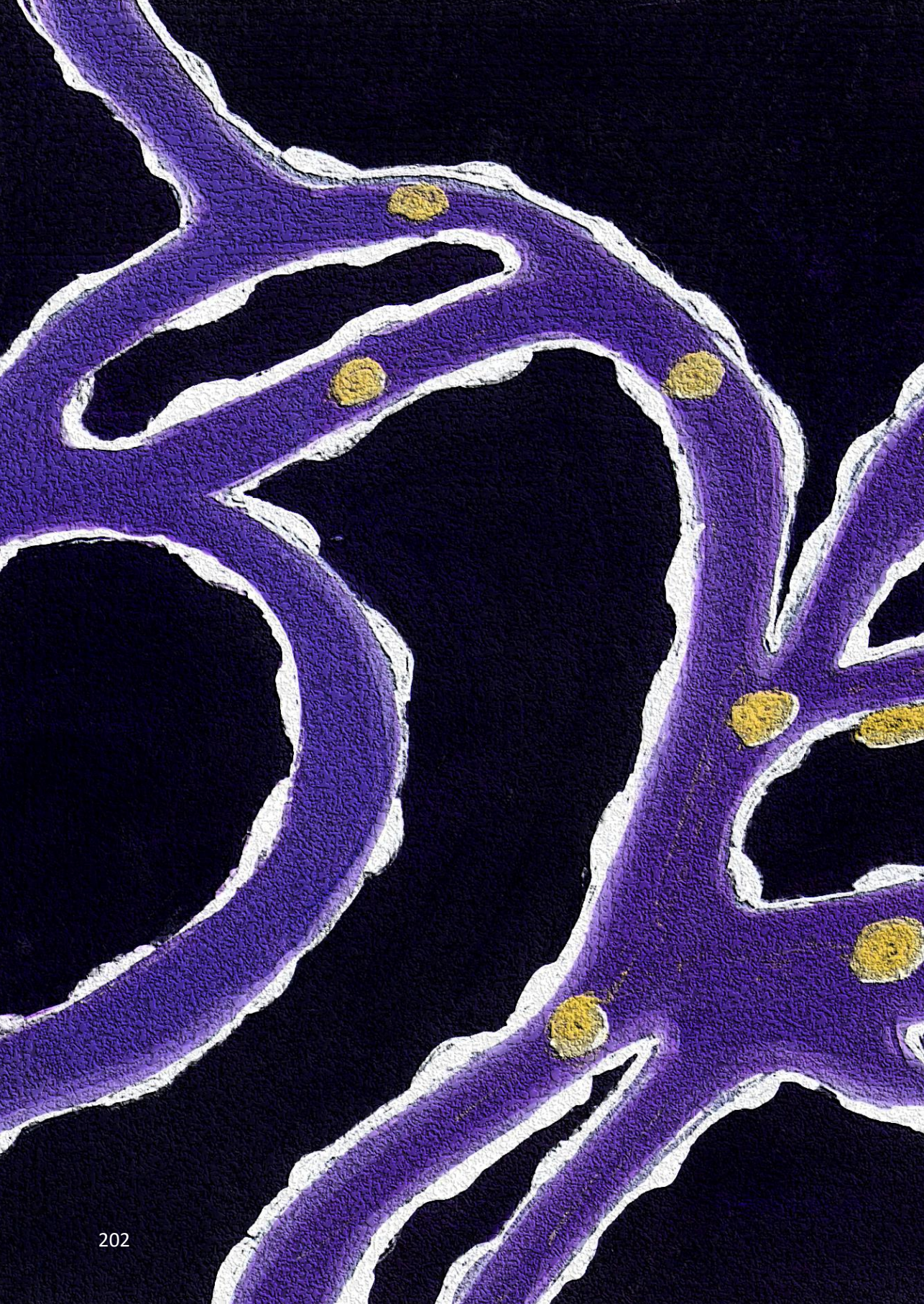

Appendix

Summary

Nederlandse samenvatting

Curriculum Vitae

List of publications

Acknowledgments

Summary

The vascular system plays a fundamental role in development, tissue homeostasis, and host defense. It is composed of endothelial cells (ECs) and mural cells, including vascular smooth muscle cells (vSMCs) and pericytes, which work together to maintain vascular integrity and regulate hemodynamics. A critical function of the vasculature is to coordinate inflammatory responses, particularly through the recruitment and transmigration of leukocytes. While this process is essential for immune defense and repair, its dysregulation can lead to chronic inflammation and contribute to various vascular pathologies. To better understand these complex mechanisms, *in vitro* models that mimic the cellular composition and mechanical forces of the human vasculature are needed. Recent advances in human induced pluripotent stem cell (hiPSC) technology, combined with organ-on-chip (OoC) systems, have enabled the development of vessel-on-chip (VoC) platforms. These allow controlled investigation of endothelial barrier function, immune cell transmigration, and vascular inflammation under dynamic flow conditions. The goal of this thesis was to enhance the physiological relevance of existing VoC models by integrating hiPSC-derived vascular and immune cells, and to apply these systems to study vascular inflammation in both healthy and disease contexts.

Chapter 1 introduces the biology of vascular development, the role of endothelial and mural cells, and how inflammation affects the vascular barrier. It describes how immune cells like monocytes and macrophages contribute to inflammatory responses and how their interaction with vascular cells is crucial for homeostasis and disease progression in Hereditary hemorrhagic telangiectasia type 1 (HHT1). The chapter also outlines the rationale for using hiPSC-derived cells and VoC systems to model vascular inflammation and highlights the limitations of conventional models.

Chapter 2 presents the development of a single-lumen VoC model composed of hiPSC-derived endothelial and mural cells. Using controlled fluid flow, this model enabled the formation of a perfusable vessel structure ideal for studying barrier integrity and cell-cell interactions. This model provides a foundation for studying leukocyte interactions with the vascular wall under flow.

Building on this, Chapter 3 investigated inflammatory responses using the single-lumen VoC upon TNFα stimulation. hiPSC-derived monocytes were perfused under physiological shear stress to assess transendothelial migration (TEM). Mural cells significantly influenced the cytokine environment and enhanced leukocyte transmigration. The chapter also assessed hiPSC-monocytes derived from HHT1 patients, revealing impaired adhesion, which supports their potential role in disease pathophysiology.

Chapter 4 used a self-assembling VoC model to mimic vasculogenesis and network remodeling *in vitro*. Here, the model was applied to study HHT1-specific vascular defects

under inflammatory conditions. Endothelial cells from *ENG*-mutant hiPSCs showed delayed network formation and exaggerated responses to TNF-α, particularly in their interaction with mural cells. These results likely mirror the inflammatory exacerbation seen in HHT1 patients, validating the model's relevance to human pathology.

In Chapter 5, the model's complexity was extended by incorporating hiPSC-derived macrophages to mimic perivascular immune environments. These cells were characterized for their phenotype and function in the 3D microvascular context. The study demonstrated their ability to integrate into the network and modulate the local inflammatory response.

Chapter 6 offered a broader perspective, reviewing strategies for integrating functional vasculature into organoids. It discusses how functional vascularization supports organoid maturation and how perfusable networks can be used to build more physiologically relevant human tissue models.

Finally, Chapter 7 synthesized the key findings and presents the future perspectives of this thesis. This chapter critically compares the strengths and limitations of the two VoC systems used in this thesis. The single-lumen VoC offers reproducibility and control over flow dynamics, making it suitable for studying barrier function and immune cell transmigration. In contrast, the self-assembling VoC better mimics in vivo vascular morphogenesis but presents challenges in standardization. The chapter also discusses future directions, including incorporating shear stress into the self-assembling VoC, using patient-specific immune cells, and employing single-cell transcriptomic approaches to further dissect cell-specific roles in inflammation. Finally, the broader potential of VoC platforms for drug discovery, disease modeling, and bridging in vitro systems with clinical research is outlined, with an emphasis on the need for standardization, functional validation, and integration with organoid models to improve physiological relevance.