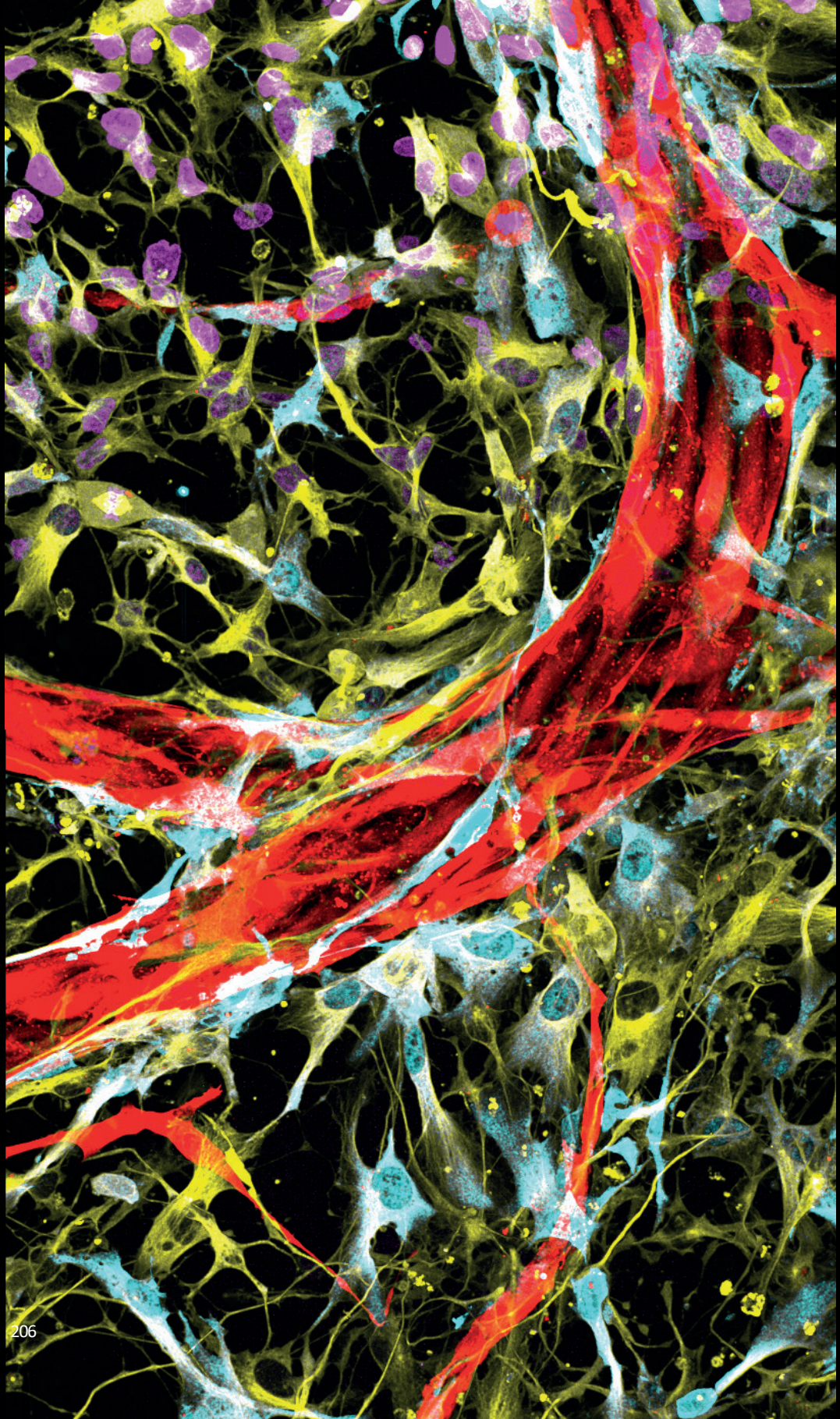
Modeling vascular disease using self-assembling human induced pluripotent stem cell derivatives in 3D vessels-on-chip

Nahon, D.M.



Appendix

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Nederlandse samenvatting
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Summary

A properly functioning vascular system is crucial for the overall health of our body. It transports the necessary oxygen, nutrients, and immune cells to all organs while removing harmful waste products. Blood vessels consist of a single layer of cells called endothelial cells (ECs) and are supported by smooth muscle cells or pericytes. The exact composition and functionality of these vessels vary from one organ to another, and they play a significant role in various medical conditions.

Vascular diseases impose a significant burden on society, mainly due to the lack of effective treatment methods. One major reason for this is the inadequacy of current preclinical modeling systems. Traditional models, such as mice or cultured cells in plastic petri dishes, often fail to accurately replicate human physiology. While mice come close in terms of complexity, ethical concerns and important human-specific aspects in many complex diseases remain significant challenges. Cultured cells in petri dishes may have a human origin but lack the complexity of a fully functional mature organ, including its 3D and multi-cellular structure.

Organs-on-Chip (OoC) is an innovative technology that uses microscopic culture environments to more realistically mimic organ functions. This is achieved by replicating one or more physiological stimuli, such as blood flow, tissue stretching, or 3D organ structure. While various cell sources can be used in these systems, human induced pluripotent stem cells (hiPSCs) are particularly attractive for future disease modeling and drug development. These hiPSCs can be generated in the lab by reprogramming normal human cells from any individual and have the ability to differentiate into nearly all cell types. Because these cells retain the genetic background of the donor, they are highly suitable for researching hereditary diseases. In this thesis, we differentiate hiPSCs from patients with neurovascular disorders into vascular cells, with the aim of using them in the development of so-called (blood) Vessels-on-Chip (VoC) models.

In chapters 1 and 2 of this thesis, we introduce OoC and hiPSCs, and we describe the current limitations in the field of OoC. Specifically, in Chapter 2, we emphasize that the (industrial) adoption of OoCs in drug development and disease modeling would be accelerated if researchers paid more attention to comparing experimental results with physiological human data when designing and interpreting experiments. We discuss which OoC data already align well with human data and highlight emerging technological developments that can contribute to a more accurate replication and measurement of other organ functions.

In Chapter 3, we utilize hiPSCs from a patient with a hereditary form of cerebral amyloid angiopathy (also known as D-CAA or HCHWA-D). This disease is characterized by the accumulation of amyloid-ß protein specifically in the brain's blood vessels, which can lead to cerebral hemorrhages. We correct the hereditary DNA mutation in the hiPSCs of this patient to enable further studies into the underlying disease mechanisms.

Similar to Chapter 3, in Chapter 4, we correct the causal DNA mutation in hiPSCs

from a patient suffering from Retinal Vasculopathy with Cerebral Leukoencephalopathy and Systemic Manifestations (RVCL-S). This rare disease causes unstable small blood vessels and results in symptoms in various organs, including the brain. To investigate the specific role of ECs in this disease, we differentiate the hiPSCs from the patient (‘diseased’ cells) and the hiPSCs with the corrected DNA (‘healthy’ cells) into ECs. Functionally, the differentiated ECs did not show significant differences. Further research with more complex models is needed to better understand the differences between these ECs.

In Chapter 5, we once again compare 'healthy' and 'diseased' ECs, this time differentiated from hiPSCs from a patient with the vascular disease Hereditary Hemorrhagic Telangiectasia (HHT). This condition leads to abnormal formation of small blood vessels, resulting in severe blood loss. While standard two-dimensional analyses of the ECs did not show significant differences, we identified differences after integrating the cells into a more complex VoC model. The ECs, together with supporting pericytes, formed a complex 3D microvascular network in the microfluidic chips. The 'healthy' ECs formed larger and more stable blood vessels with more direct interactions with the supporting pericytes than the 'diseased' ECs. This lack of stability and interaction aligns with patient data, highlighting the value of such complex VoC models.

As many of the diseases discussed in this thesis specifically affect blood vessels in the brain, we expand the VoC model further in Chapter 6 by adding brain-specific astrocytes. By, for the first time in the research field, adding astrocytes that are also differentiated from hiPSCs, future research into the influence of astrocytes on the formation of microvascular networks becomes possible. We successfully integrated these astrocytes into the VoC model and observed direct interactions between the cells that resemble what we observe in the human body. Additionally, we demonstrated that mimicking blood flow or adding specific components in the chip can enhance the development of these astrocyte containing VoC models.

Ultimately, in Chapter 7, we discuss the results of this thesis and its future perspectives. We emphasize that the generated patient-specific hiPSCs serve as essential tools for further research into these diseases. Moreover, the results underscore the potential of VoC models for understanding and studying vascular diseases. Finally, the research also highlights the need to further refine current models by integrating additional factors (such as immune cell integration or more precise regulation in mimicking blood flow) to more accurately replicate the disease's characteristics. Additionally, improvements in standardization, reproducibility, and enhanced continuous monitoring of conditions in cellular VoC models are necessary.