hiPSC-derived 3D cardiac microtissue models with integrated immune cells and vasculature

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Appendix

Summary

The heart is one of the most important and complex organs with multiple layers and cell types. The dialogue between different cell types is critical for the structure and function of the heart. Moreover, the proper flow of blood through the whole organ is essential to keep all cells healthy and functional. Many 3D cardiac *in vitro* models incorporate multiple cardiac cell subsets to achieve complexity and physiological relevance. However, the lack of functional vasculature and immune components potentially limit their utility, especially in applications such as drug testing and disease modelling. Functional vasculature is important for selective nutrient, drug and cell transport to the other cell types. Immune cells play crucial roles in maintaining homeostasis in health and dysregulation of this innate and inflammatory cell type may trigger disease. Therefore, modelling these two critical components of the heart – fluidic flow and immune components- is important in using them to reveal underlying mechanisms in health and disease.

This thesis describes ways to integrate human induced pluripotent stem cells (hiPSC)-derived vascular and immune components in 3D cardiac microtissue platforms to study cellular crosstalk and its effect on cardiac response to chemical cues.

Chapter 1 provides a general introduction to the heart and its microenvironment. It delves in to importance of different components in the heart with a specific highlight on immune cells and vascular cells. It discusses different options to model the heart with a focus on *in vitro* alternatives.

In **Chapter 2** and **3**, current *in vitro* 3D cardiac models are reviewed with a focus on their applications, readouts and limitations. Implementation of future perspectives can increase the complexity, and possibly the relevance and value of these models.

Chapter 4 focuses on integration of hiPSC-derived macrophages (IPSDMs) into 3D cardiac microtissues consisting of cardiomyocytes, endothelial cells and cardiac fibroblasts. This chapter describes the optimization of culture conditions, such as culture plate type and medium components, and investigates their effect on different cellular subsets including IPSDMs, cardiomyocytes and endothelial cells. In summary, cardiac microtissues with IPSDMs can best be cultured in an ultra-low attachment plate since this ensures IPSDMs remain inside the tissues and localize near vessel structures. The number of IPSDMs in microtissues increases after addition of macrophage colony stimulating factor in medium in the early culture period. However, we found that overall IPSDM number throughout the culture period significantly decreases which might be due to the incompatibility of culture medium with IPSDMs. IPSDMs do not have a significant impact on cardiomyocyte contraction duration and vessel density parameters. Finally, cytokine profile of microtissues

with IPSDMs showed a mixture of pro- (IL-6 and IP-10) and anti- (IL-10 and IL-1RA) inflammatory cytokines, suggesting a heterogenous population of IPSDMs in the tissues. However, the higher concentration of anti- inflammatory cytokine (IL-1RA) might suggest that majority of IPSDMs acquire an M2-like macrophage phenotype while minor population is M1-like.

Chapter 5 describes a vascularized and perfusable hiPSC-derived 3D cardiac microtissue on a chip platform (VMToC). Here, prevascularized 3D cardiac microtissues were cultured together with an external vascular network which is formed by a self-organization of hiPSC-ECs and mural cells in a fibrin hydrogel. Contractile microtissues did not negatively impact the vascular formation, as evident with comparable vessel parameters in VMToCs and vessel-on-chip without microtissues. In short-term, preexisting microvascular networks in the microtissues and the external vascular network around them start interconnecting/ anastomosing and forming continuous lumenized vascular networks. These vascular networks can be perfused with beads where beads show oscillatory moves synchronized with the rhythmic beating of the tissues. Continuous bidirectional perfusion promotes an increase in the vessel density in and around the microtissues.

Chapter 6, further characterizes VMToCs functionally and establishes the utility of the platform. Vascularization did not significantly alter VMToC's sarcomere organization. VMToCs showed longer contraction duration and peak-to-peak time in their spontaneous and paced contraction, suggesting an enhanced endothelial cell-cardiomyocytes crosstalk. When this crosstalk was challenged by either nitric oxide synthase inhibitor or pro-inflammatory cytokine, VMToCs showed significant changes in their contraction parameters. These results indicate that the presence of vascular cells in VMToCs regulates their contractile dynamics and response to drugs.

Chapter 7 provides a step-by-step protocol to generate VMToCs. Supporting protocols describe functional characterization by bead perfusion assay and pacing; and structural characterization of VMToCs by fixing and immunofluorescent staining.

Chapter 8 summarizes 3D cardiac microtissue models that were developed in this thesis, highlighting their advantages. Limitations and future perspectives to improve these models are also discussed at the end of this chapter.