

***IN VITRO* 3D CARDIOVASCULAR ORGANOID GENERATION
FROM HUMAN PLURIPOTENT STEM CELL**

by

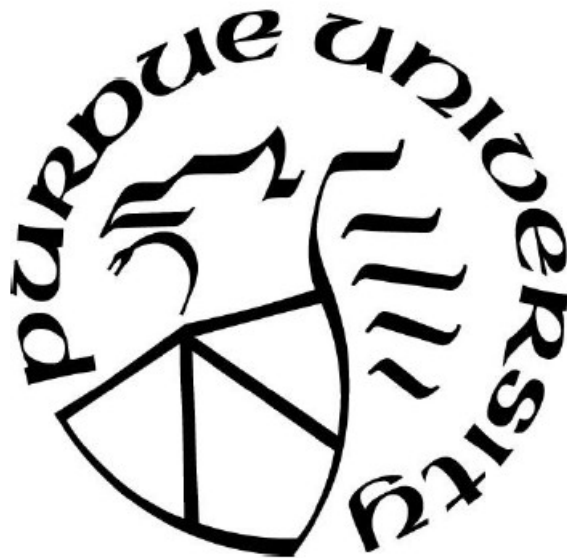
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ABSTRACT

Human cardiovascular disease (CVD) is the leading cause of death around the world for years. It costs millions of dollars and almost 700 thousand deaths annually in the USA alone. Organoids are emerging as an innovative platform for studying human biology and advancing health research. With their ability to replicate the complex 3D structure and multicellular interactions, organoids have advanced studies in all major organs as a reliable model. Recently, the Food and Drug Administration (FDA) announced a reduction in animal testing for drug development, which will be partially replaced by organoid-based toxicity testing. While many cardiovascular models have been developed, it remains a challenge to generate reproducible cardiovascular organoid systems that accurately mimic the native human heart. This thesis aims to establish a reliable and reproducible one-step cardiovascular organoid platform with tunable cell type composition, providing a versatile tool for disease modeling and drug testing.

The first section of this thesis focuses on creating a stable embryoid body (EB) system for 3D cardiovascular differentiation from human pluripotent stem cells (hPSCs). Unlike traditional 2D differentiation methods, the EB approach supports the formation of diverse 3D structures. The phenotype and crosstalk between different cardiovascular cells were studied in this 3D system under various differentiation conditions, highlighting its potential as an *in vitro* model for studying cardiovascular development and the self-organization of distinct cardiovascular lineages.

The second section of this thesis centers on generating cardiovascular organoids with adjustable endothelial cell composition by combining non-edited hPSCs with genome-edited, doxycycline (Dox)-inducible SOX17-overexpressing hPSCs. SOX17 is crucial during cardiogenesis and promotes endothelial cell differentiation. This

engineered hPSC line allowed us to construct a one-step 3D cardiovascular organoid with controlled endothelial specification, adjustable cellular composition, and a morphology resembling the human heart.

The final section explores the application of these cardiac organoids as a platform for disease modeling and drug testing. The resulting organoids successfully replicated key cardiotoxic effects of the FDA-approved chemotherapeutic agent doxorubicin, including reduced cell viability and impaired contractile function. When subjected to cryoinjury-induced myocardial infarction (MI), the organoids exhibited diminished beating, decreased cell viability, loss of α -actinin expression, and increased fibroblast formation — effects that were alleviated by Captopril treatment. Additionally, isoproterenol exposure led to increased peak Ca^{2+} transient amplitude and shortened APD_{50} , consistent with known β -adrenergic responses observed in cardiac injury models.

In conclusion, this thesis presents an advanced platform for cardiovascular disease modeling and drug testing. By integrating genome-edited and non-edited hPSCs, we established a method to modulate cell type composition while preserving essential intercellular interactions during cardiac organoid differentiation. The resulting cardiovascular organoids exhibit complex 3D architecture, including chamber-like and layered structures that closely resemble the native human heart. Furthermore, these cardiovascular organoids effectively model cardiovascular diseases and respond to pharmacological treatments, demonstrating their utility in preclinical research. Taken together, these findings represent a significant step forward in the controlled generation of organoids and the development of physiologically relevant models for cardiovascular research.