Engineering human cardiac organoids at scale to accelerate in vitro cardiovascular research

Oxford supervisors: Prof Molly Stevens[1], Prof Paul Riley[1], Prof Georg Holländer[2]
Please note that a Novo Nordisk mentor will also be assigned to this project.

Departments: 1. Department of Physiology, Anatomy and Genetics
2. Department of Paediatrics

Project outline

Background: Organoids are emerging 3D *in vitro* model systems which harness stem cell biology to recapitulate the complex multicellularity, 3D structures, and functions of many human tissues. Organoids can be used to study tissue development, model diseases, and develop therapies at a fraction of the price of and without the interspecies complications introduced by animal models. However, labour intensive production processes and limited reproducibility have hindered widespread adoption of organoid models for high-throughput studies such as genetic perturbations and drug discovery. We (Stevens) recently reported and patented a scaffold-based strategy for high-throughput production of organoids[1, 2].

Here, we seek to extend our technique to the high-throughput production of human cardiac organoids (cardioids), which would circumvent the high-labour and high-cost of current cardioid production protocols. Our project would build on several recent advances in cardioid engineering[3-5] and draw on our expertise in cardiac biology[6-8] (Riley) and tissue development[9-11] (Holländer). If successful, our project would deliver a high-throughput strategy to reproducibly and inexpensively produce cardioids, thus accelerating large scale studies of cardiac biology, disease modelling, and therapeutic development. The high disease burden of cardiovascular diseases – which remain the leading cause of death worldwide[12] – underscores the potential impact of such a tool for cardiovascular research.

Hypothesis: Human cardioids which recapitulate the physiology of human heart tissue can be produced at scale

Aims & Description of Work: In the proposed project, the Fellow would work closely with Professors Molly Stevens, Paul Riley, and Georg Holländer to generate, characterise, and apply human cardioids at scale. This project includes 3 synergistic work packages (WPs):

WP1: Adapt a scaffold-based tissue engineering strategy for high-throughput production of cardioids
The Fellow would use melt electrospinning writing to generate and tune arrays of scaffolds, as previously reported and patented[1, 2]. These scaffolds would be seeded with human pluripotent stem cells, followed by directed differentiation of cells into cardioids containing all 3 cardiac cell lineages (cardiomyocyte, endothelial layer, epicardium) and replicating native cardiac structures (e.g. vessel networks, heart chambers). Acoustic cell patterning techniques – previously shown to organise engineered muscle tissue – may also be incorporated to improve cardioid tissue organisation[13].

WP2: Characterise the phenotype of our cardioids
The Fellow would characterise and benchmark our human cardioids against existing cardioid models[3-5] using state-of-the-art techniques focussed on markers previously reported to influence cardioid phenotype. These methods will include: immunohistochemical analysis; evaluation of morphological features like muscle fibre alignment; cardioid beating; spatial transcriptomics[9, 10]; and in-house Raman microscopy methods, which enable the real-time label-free quantification of cellular and extracellular composition of engineered tissues[14-16].

WP3: Demonstrate the utility of our cardioids to study tissue regeneration
To validate the utility of our technique, the Fellow would cryoinjure the human cardioids and evaluate the regenerative response. Expected outcomes[3] – which can be comprehensively interrogated via immunohistochemistry, spatial transcriptomics, and Raman microscopy – include fibrosis, and, after several days, accumulation of the extracellular matrix proteins fibronectin and collagen.

Expected Outcomes: The proposed project would deliver a method for high-throughput production of cardioids to the research community, which could accelerate large scale studies of cardiac biology and development of therapies for cardiovascular diseases. This research would prepare the Fellow for an independent career in translational biomedical research through a scientific training in tissue engineering and cardiac biology; and professional development via Novo Nordisk symposium/workshops, possible exposure to the process of spinning IP out of university labs, and the opportunity to present at conferences and publish in academic journals.

References: **1)** DOI: 10.1002/adma.202300305; **2)** Patent: 2206768.0/GB/PRV; **3)** DOI: 10.1016/j.cell.2021.04.034; **4)** DOI: 10.1016/j.cell.2023.10.030; **5)** DOI: 10.1038/s41587-023-01718-7; **6)** DOI: 10.1172/JCI97192; **7)** DOI: 10.1038/nature10188; **8)** DOI: 10.1038/nature14483; **9)** DOI: 10.1101/gr.171645.113; **10)** DOI: 10.1038/s41467-023-39722-9; **11)** DOI: 10.1126/sciadv.abm9844; **12)** DOI: 10.1016/j.jacc.2020.11.010; **13)** DOI: 10.1002/adma.201802649; **14)** DOI: 10.1038/ncomms14843; **15)** DOI: 10.1016/j.crmeth.2023.100440; **16)** DOI: 10.1021/acscentsci.6b00222

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Prof Molly Stevens

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