A Microengineered Cardiac Ischemia on-a-Chip to Study Adaptive Myocardial Tissue Response to Hypoxia

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Introduction: Cardiovascular disease persists as the leading cause of death worldwide. The recent emergence of tissue engineering strategies has led to the creation of complex, *in vitro* models for experimental disease modeling applications. Despite significant advancements, there is still a great need for organotypic *in vitro* models that mimic the response of cardiac tissue during physiological insults. Herein, we describe development of co-cultured 3D stem-cell based human cardiac tissues, within a novel 3D heart on-a-chip model, and their response to varying levels of oxygen, namely hyperoxia (21% O_2), physioxia (5% O_2), and hypoxia (1% O_2) to mimic cardiac ischemia. Cardiomyocytes (CMs), differentiated from human induced pluripotent stem cells (hiPSC-CMs), and human cardiac fibroblasts (hCFs) were encapsulated within a 3D collagen-based hydrogel and housed within a microfluidic chip with surface topography to induce tissue-level anisotropy, representing mature morphology and highly aligned structure. Upon miming ischemic cardiac injury, we studied tissue fibrosis, targeted gene expression, and transcriptomic-level profiles of the cardiac tissues.

Materials and Methods: hiPSC-CMs and hCFs were mixed at a 4:1 ratio, encapsulated in a collagen hydrogel, then injected into microfluidic chips with innate microposts that serve as mesoscopic cues to form anisotropic structure. After 13 days of culture, the cardiac tissues were subjected to one of the three oxygen conditions for 24 hours (**Fig. 1A**). Following, the cardiac tissues were analyzed by immunofluorescent (IF) staining to assess for induction of fibrosis (**Fig 1B,C**). Furthermore, the expression of pertinent genes in both the hypoxia-response and contractile pathways were assessed, to study the effect of varying oxygen levels on the cardiac tissue (**Fig. 1D**). Lastly, unbiased transcriptomic analysis was performed to identify key biological pathways that are differentially regulated between the hypoxic and physioxic tissues.

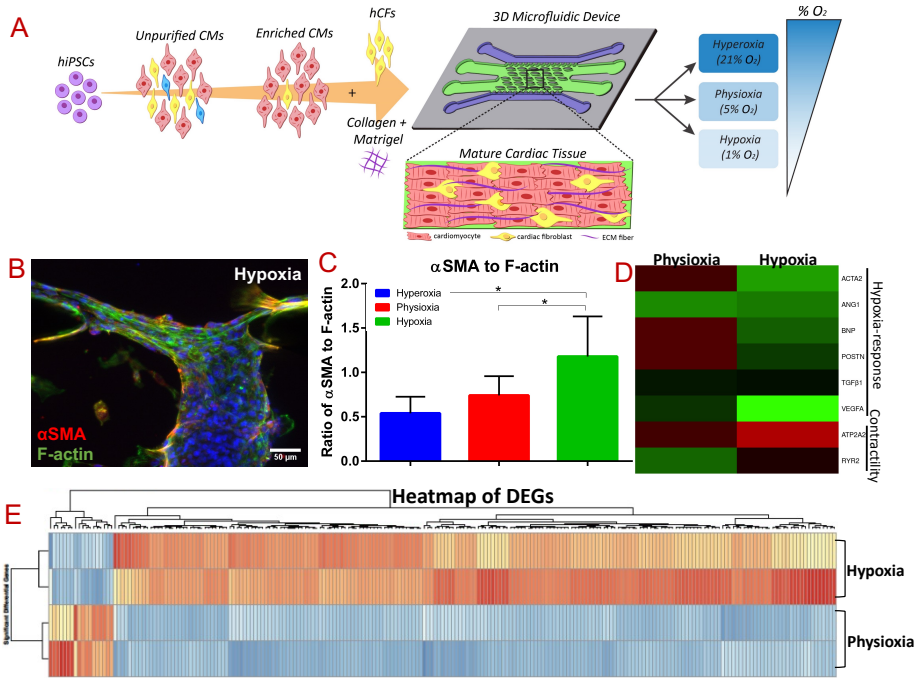

Fig 1. (A) Schematic of heart on a chip exposed to the different oxygen levels. **(B)** Fibrotic response of hypoxia-exposed cardiac tissues, with corresponding **(C)** quantification. **(D)** Expression of hypoxia-specific and contractility genes. **(E)** Transcriptomic response of tissues exposed to hypoxia and physioxia.

Results and Discussion: Fibrotic response was observed in the tissues exposed to 1% oxygen (hypoxia), compared to both physioxic and hyperoxic tissues. Additionally, a majority of the hypoxia-response genes assessed (i.e., ACTA2, VEGFA) were upregulated and the contractile-specific genes were downregulated in the hypoxic tissues (**Fig. 1D**). Notably, RNA-sequencing of the cardiac tissues revealed extensive differential regulation, dependent on oxygen environment (**Fig. 1E**), with pertinent pathways upregulated (i.e., HIF-signaling) and downregulated (i.e., oxidative phosphorylation) in hypoxic tissues, compared to tissues exposed to physioxia. Therefore, exposure of cardiac on-a-chip tissues to low oxygen revealed induction of fibrosis, dysregulation of contractile-specific genes, and differential regulation of key ischemic pathways. Overall, this study demonstrates the utility of a novel cardiac ischemia on-a-chip to assess the biological response of mature, anisotropic human cardiac tissue after exposure to extended hypoxic conditions.

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