Modeling Stem Cell-Derived Human Myocardium in a Microengineered Tissue Platform

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Introduction: Despite significant advances in cardiovascular research, cardiovascular diseases still dominant as the universal leading attributor to mortality. Modeling of the adult myocardium remains an arduous task due to its highly complex, anisotropic structure, composition, and state of maturity. Currently implemented models incorporate human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs) for cardiac-related study, however the immature state and isotropic structure of these cells in 2D culture limits the translation of such *in vitro* studies to therapeutic and clinical applications. Therefore, there remains a need for a platform that models the complex structure of the human adult myocardium, with enhanced tissue function and maturity, to enable cardiac biology and pharmacological studies. In our previous work, we presented the design of a precisely-engineered 3D microfluidic model with innate microposts to model the anisotropic myocardium, using neonatal rat-derived cardiac cells. In this work, we describe development of a physiologically relevant cardiac tissue model, composed of human pluripotent stem cell-derived cardiomyocytes (hPSC-CMs), consisting of highly organized co-culture of cardiac cells (i.e. CMs, CFs), with enhanced function and relevant expression of structural genes.

Materials and Methods: The 3D microfluidic model is comprised of microposts to induce alignment of hydrogel-encapsulated cardiac tissues within the system. To generate the cardiac tissue, hPSCs were differentiated into CMs, and combined with human cardiac fibroblasts (CFs), then encapsulated in Collagen 1: Matrigel hydrogel. The cell-hydrogel mixture was loaded into microfluidic devices (**Fig. 1A**) with and without microposts, as well as on 2D monoculture, and cultured the cells for 14 days. The effect of 3D culture within the devices, and effect of micropost on tissue function, cardiac-specific structure, spontaneous contractility, and differential gene expression were assessed.

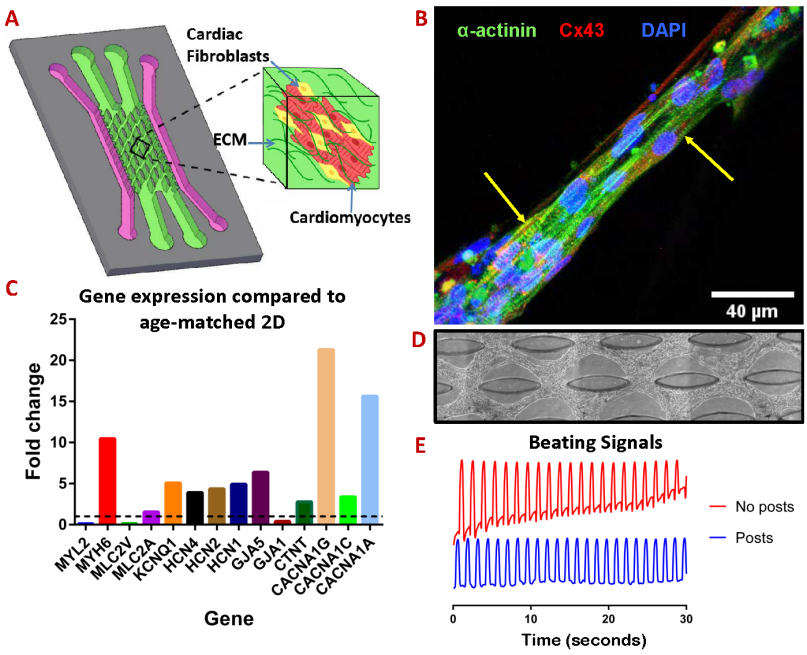

Figure 1. A) Schematic of microengineered cardiac platform with arrays of microposts. B) Cardiac marker expression assessed via immunofluorescence. C) Gene expression of tissues in devices with microposts. D) Phase contrast of tissue surrounding microposts within platform. E) Representative spontaneous beating signals on Day 14 of device culture.

Results and Discussion:
The experimentally-optimized micropost design induced consistent spontaneous beating (**Fig. 1E**) at extended culture period (i.e. 14 days) as compared to the device counterpart lacking the microposts. To confirm proper expression of cardiac markers, the tissues were stained for sarcomeric α-actinin (green) and connexin 43 (red). Fluorescent microscopy revealed highly aligned, striated sarcomeres and abundant expression of cardiac gap junctions (**Fig. 1B**) within the anisotropic tissue (**Fig. 1D**). Compared to age-matched 2D monolayers, the tissue within the microfluidic device with precisely-spaced microposts exhibited an upregulation of 12 out of 15 cardiac-specific function genes, as analyzed using qPCR (**Fig. 1C**). These findings demonstrated the development of a highly organized 3D cardiac tissue model within a microfluidic platform, exhibiting a mature state both on the gene and protein level.

Conclusions: This biomimetic platform exhibits the capability of physiologically relevant 3D human cardiac tissue with optimized cardiac-specific structures and functionalities, and enhanced expression of functional and structural genes. The ongoing work involves further analysis of the hPSC-CM derived tissue and potential capabilities in disease modeling applications.

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