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Bioacoustic-enabled patterning of human iPSC-derived cardiomyocytes into 3D cardiac tissue

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ABSTRACT

The creation of physiologically-relevant human cardiac tissue with defined cell structure and function is essential for a wide variety of therapeutic, diagnostic, and drug screening applications. Here we report a new scalable method using Faraday waves to enable rapid aggregation of human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) into predefined 3D constructs. At packing densities that approximate native myocardium (10^8-10^9 cells/ml), these hiPSC-CM-derived 3D tissues demonstrate significantly improved cell viability, metabolic activity, and intercellular connection when compared to constructs with random cell distribution. Moreover, the patterned hiPSC-CMs within the constructs exhibit significantly greater levels of contractile stress, beat frequency, and contraction-relaxation rates, suggesting their improved maturation. Our results demonstrate a novel application of Faraday waves to create stem cell-derived 3D cardiac tissue that resembles the cellular architecture of a native heart tissue for diverse basic research and clinical applications.

1. Introduction

The generation of three-dimensional (3D) functional tissues that can restore the structure and/or function of damaged myocardium is a central goal in cardiac regenerative medicine [1,2]. In addition, the creation of high-fidelity *in vitro* tissue models may improve our understanding of various biological processes including heart development, myocardial damage, and disease [2–5]. The creation of 3D tissue constructs that mimic native myocardium requires an

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appropriate selection of cell source and biomaterial that resembles the native tissue structure and support cell viability, function, electromechanical integration with host tissue, and vascularization [6].

The intricate structure of native myocardium is characterized by closely-packed cardiomyocytes (CMs) (*e.g.*, 10^{8-9} cells/cm^3 in adult rat myocardium) [7] that contract synchronously via the orchestrated propagation of an electrical signal generated by specialized pacemaker cells. The challenge in restoring the structure and function of damaged heart tissue is due, at least in part, to the complexity of recreating the natural myocardial architecture. In particular, the ability to create 3D cardiac tissues with high packing density of functional CMs that are organized in a pre-defined pattern is critical to mimic the native tissue in maintaining CM viability, intercellular connections, and contractile function [8–10].

To date, majority of technologies that enable control over spatial cell arrangement in 3D tissue constructs are based on assembly of cell-encapsulating microscale hydrogels [11–14], seeding cells in scaffolds with defined architecture [15–17], and additive manufacturing/3D bioprinting techniques [15,18,19]. While these techniques have been successfully used in a wide range of applications including bone and skin regeneration [20–22], gene delivery [23,24], and cell differentiation [21,25], they are not yet able to achieve spatially-controlled, physiologically-relevant cell packing densities, comparable to that in the native cardiac tissue [7] for cardiovascular applications.

Using the Faraday wave principle to induce patterning in liquid medium, we recently demonstrated rapid and dynamic aggregation of microscale objects (*e.g.*, cell spheroids) into diverse sets of geometric configurations at the air-liquid interface [26,27]. In this study, by applying Faraday waves to the fibrin prepolymer, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) were rapidly patterned (<10 s) into ordered, closely-packed, symmetric 3D constructs. Within the high cell density regions, hiPSC-CMs demonstrated significantly improved viability, intercellular connections, and contractile function (force and motion), in comparison to constructs with random cell distribution. Our results support the feasibility of creating 3D cardiac tissue constructs that approximate native human tissues in their cell density, structure, and function for diverse basic research and clinical applications.

2. Results

2.1. Faraday wave patterning of hiPSC-CMs

Experimental setup designed for this study is shown in Fig. 1A and SI Fig. 1. Altering hydrodynamic drag force fields tuned by Faraday wave frequency generated a variety of particle aggregation patterns (SI Fig. 2). Numerical modeling of hydrodynamic drag exerted on the particles demonstrated that regions with minimum force potential overlapped with the nodal pattern of the Faraday waves (Fig. 1B), resulting in an aggregation pattern consisting of circles and squares (top-down view, Fig. 1C, SI Movie 1). The force potential field penetrated inside the liquid layer and decayed exponentially (cross-sectional view Fig. 1D–E). Particles within the force potential field were driven away from the regions with the higher to lower force potential and closely packed together into a multilayer structure. The maximum force potential was on the surface of substrate at $z = 0$ (Fig. 1D). For this reason, we waited for 1 min for CMs to settle down at the bottom of container, before starting the wave-patterning. Difference in force potential decreased by an increase in the thickness of the liquid medium (Fig. 1D–F). Using Equation (1) in Methods, we predicted the difference in max and min force potential (green curve in Fig. 1B) as a function of liquid bath thickness. This modeling showed that for three different Faraday wavelengths ($\lambda = 7$, 13.33, and 20 mm), the force potential decreased exponentially as the liquid thickness increased (Fig. 1E).

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Our modeling was experimentally validated by formation of more loosely packed patterns of plastic beads at deeper liquid baths (1.5–6 mm, Fig. 1F, i-iii). Based on this data, a fibrin prepolymer solution at the thickness of 1.5 mm was used to yield a compact hiPSC-CM aggregation with minimal cell dispersion (Fig. 1G–L). hiPSC-CMs aggregated at the nodal patterns of the Faraday waves, where the lowest force potentials existed. Further *in vitro* culture of the patterned scaffolds gave rise to formation of self-organized, closely-packed 3D constructs (Fig. 1K and L).

As control, random patterning with no waves generated cell clusters that showed spotty distribution within the dish (Fig. 2A–D). Application of Faraday waves (*i.e.*, ordered patterning) resulted in formation of multiple (6–7) stacking layers of closely-packed cells ("band") (Fig. 2E–G) and a small number of unpatterned cells in the "gap" areas (Fig. 2H). Following 5 days of culture, aggregated cells established an elongated morphology and formed packed, continuous tissue constructs in ordered patterns (Fig. 2I–L). As quantified by optical microscopy at *day 0*, a significantly greater cell packing density was obtained within the cell bands (8000 cells/mm^2) in comparison to those in random patterns and in the gap areas ($n = 10$, Fig. 2 M).

To quantify hiPSC-CM survival after encapsulation in 3D fibrin hydrogels, we performed an Alamar Blue™ reduction assay and showed that highly-packed cells exhibited a significant increase in cell metabolic activity from day 0 (right after patterning) to day 5 of culture ($n = 5$/group/time point, Fig. 2 N). This was in contrast to randomly distributed cells in 3D fibrin gels (random pattern) showing significantly decreased cell metabolism.

2.2. Scanning electron microscopy of patterned constructs

Scanning electron microscopy of hiPSC-CMs in random and ordered pattern groups after 5 days in culture provided a detailed view of cells within the bands versus randomly dispersed cells (SI Fig. 3). hiPSC-CMs within the bands were closely-packed while cells in the gap areas were sparse. This is further illustrated by cells at the periphery of the band that were oriented indiscriminately to both perpendicular and parallel directions to the band axis (as indicated by yellow and green arrows in panel E).

2.3. Immunohistochemical analysis of patterned constructs

Cells encapsulated in random, cell band, and gap areas within 3D fibrin gels were immunostained for multiple cardiac markers at day 5 of culture (Table 1, Fig. 3). Confocal microscopy and 3D reconstruction demonstrated the continuous presence of interconnected hiPSC-CMs at a vertical height of ~300 μm for the band area within fibrin hydrogel (SI Fig. 4 and SI Movie 2–3). While cells in the random patterns exhibited disorganized α-actinin expression and sarcomeric pattern (Fig. 3B–C), a majority of the hiPSC-CMs in the bands showed elongated shape, strong α-actinin expression (Fig. 3D–F), and organized sarcomeric structure (white arrows, Fig. 3F and SI Fig. 5). α-actinin$^+$ cells in the gap areas were sparse and disorganized similar to those in the random patterns (Fig. 3G–I). This was further quantified for both total cell density and the α-actinin expressing cell density at day 5 in culture ($n = 10$, Fig. 3J). Moreover, CMs within the band regions showed significantly higher levels of α-actinin expression compared with the other two groups ($n = 10$, Fig. 3K).

Supplementary data related to this article can be found online at