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Three-dimensional filamentous human diseased cardiac tissue model

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ABSTRACT

A human *in vitro* cardiac tissue model would be a significant advancement for understanding, studying, and developing new strategies for treating cardiac arrhythmias and related cardiovascular diseases. We developed an *in vitro* model of three-dimensional (3D) human cardiac tissue by populating synthetic filamentous matrices with cardiomyocytes derived from healthy wild-type volunteer (WT) and patient-specific long QT syndrome type 3 (LQT3) induced pluripotent stem cells (iPS-CMs) to mimic the condensed and aligned human ventricular myocardium. Using such a highly controllable cardiac model, we studied the contractility malfunctions associated with the electrophysiological consequences of LQT3 and their response to a panel of drugs. By varying the stiffness of filamentous matrices, LQT3 iPS-CMs exhibited different level of contractility abnormality and susceptibility to drug-induced cardiotoxicity.

1. Introduction

Effective treatments for numerous cardiovascular diseases are significant unmet needs, and one significant challenge is the lack of an established *in vitro* model of human cardiac tissue that would be useful for developing and testing potential therapies. Human induced pluripotent stem (iPS) cell technology allows the recapitulation of human disease models *in vitro*, which can be used to both study disease mechanisms and ultimately design and screen personalized therapeutics prior to large animal or clinical trials. Patient-specific iPS cell-derived cardiomyocytes (iPS-CMs) have been shown to provide valuable models of congenital cardiomyopathies caused by mutations in genes coding for ion channels or ion channel-associated proteins, such as long QT syndrome (LQTS) [1,2]. LQT3, with a mutation in the SCN5a gene, enhances late Na^+ channel currents (I_{Na+}) that fail to inactivate completely and conduct increased inward currents. This prolonged depolarization results in delayed repolarization, a prolonged QT interval, and increases the risk of fatal arrhythmia.

Although genetics play a critical role in the onset of cardiac disease, non-genetic stress – for example, due to forces and paracrine factors normally experienced in the heart, or changes in those cues in the setting of drugs or disease – also makes an important contribution to cardiac pathology. Therefore, developing a "patient-/disease-specific" three-dimensional (3D) cardiac model of LQT3 and other cardiac diseases would be a significant advancement for understanding the disease mechanism *in vitro*. Native myocardial tissues are organized into parallel cardiac muscle fibers with aligned intracellular contractile myofibrils and gap junction complexes between contacting CMs, which are important to form the integrated electrical and mechanical properties of human heart. Previous studies on the disease mechanisms of LQT3 and drug screening for treatment were highly dependent on two-dimensional (2D) cell culture, which does not represent the cellular environment within the myocardium [3]. The interplay between gene expression and environmental intricacies causes large functional variations of cells [4–7]. For example, an "*in vivo-like*" microenvironment with aligned CMs has been shown to facilitate mesenchymal stem cells acquisition of cardiac-specific contractile cytoskeleton proteins, transcription factors, gap junction protein distribution, and electrophysiological properties [8].

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The substrate stiffness contributed to the maturation of neonatal CMs [9] and regulated sarcomere structure and calcium transient of adult CMs [10].

Previous strategies for generating aligned 3D cardiac tissue relied on the implementation of mechanical loading by pairs of cantilever posts to stretch 3D matrices (collagen I and fibrinogen) mixed with CMs [11,12]. By loading fibrin-based hydrogels mixed with CMs onto microfabricated polydimethylsiloxane (PDMS) molds, others have created a 3D cardiac tissue with aligned structure [13]; however, these animal-derived extracellular matrix (ECM) scaffolds suffer from vial-to-vial variability, inhomogeneous structure, inconsistent reproducibility and lack of precise control over essential scaffold parameters. Others have also successfully electrospun 3D scaffolds with aligned nanofibers using synthetic polymers to structurally mimic the orientation of ECM in the myocardium, which helped CMs self-organize into an anisotropic structure [14]. However, the electrospinning method results in nanoscale and uncontrollable porosity and cannot allow immediate cell infiltration into the matrix to create a true 3D cellular structure. In order to systematically study human stem cell behavior in a 3D environment, more advanced fabrication methods are needed to produce scaffolds with accurately defined micro and nanoscale features. Two-photon initiated polymerization (TPIP), a laser writing technique based on the phenomenon of two-photon absorption (TPA), can be confined to cure photoresists only near the laser focal volume, enabling fabrication of arbitrary 3D structures with spatial resolution approaching 100 nm [15–17].

With the TPIP technique, we created a bioinspired cardiac tissue model with a cohort of 3D filamentous matrices that precisely regulated the structural alignment of CMs and adjusted the cellular mechanical environment. The filamentous matrices consisted of synthetic parallel fibers with tunable fiber diameter and spacing. The CMs differentiated from LQT3 iPS cells were verified to faithfully recapitulate the electrophysiological abnormality of delayed repolarization. By seeding LQT3 iPS-CMs on such a highly controllable filamentous matrix, we generated a disease-specific 3D cardiac tissue and studied the contractility malfunctions associated with the electrophysiological consequences of LQT3 syndrome. By the comparison with 2D cell culture, we highlighted the different response of our 3D tissue model to a panel of drugs associated with cardiotoxicity.

2. Materials and methods

2.1. Fabrication of filamentous matrices

The filamentous matrices were fabricated via the TPIP system (Fig. 1A) based on a femtosecond laser beam irradiated vertically to the photoresist, a UV-curable organic–inorganic hybrid polymer (ORMOCER®, Micro resist technology) [18]. The photoresist was spin-coated onto glass plates (25 mm in length, 3 mm in width, and 1 mm in thickness) at 4000 RPM for 100 s, pre-baked on a hotplate at 80 °C for 2 min, and cured by UV light illumination for 30 min. Two glass plates were assembled with two 0.5 mm-thick spacers at the ends and subsequently hard baked at 140 °C for 1.5 h. The assembled glass scaffold was filled by uncured photoresist and placed on PC-controllable X–Y–Z motorized stages (Aerotech, ANT95-XY-MP and ANT95-50-L-Z-RH) with high precise positioning. Single fibers were fabricated along the laser beam path with a high-repetition rate femtosecond laser irradiation (Movie S1). The femtosecond laser (pulse duration: ~400 fs, repetition frequency: 1 MHz, wavelength: 1045 nm, FCPA μJewel D-400, IMRA America, Inc.) was frequency-doubled to the wavelength of ~522 nm by lithium triborate (LBO) second harmonic nonlinear crystal (Newlight photonics) and focused onto the glass plate/photoresist interface with a 5× microscope objective (M Plan Apo, N.A. = 0.14, Mitutoyo). The fibers with different diameters could be fabricated by changing the laser power and exposure time. The power of the laser beam emitted downstream of the objective lens was measured by a power meter and controlled by a half-wave plate and a polarizing beam splitter. The exposure time was set by a PC-controllable mechanical shutter. Fibers with diameters of 5 μm and 10 μm were created with powers of 2.6 mW for 0.7 s exposure and 6.4 mW for 1 s exposure respectively. Different fiber spacing within the matrices was controlled by the X–Y–Z stage with high positioning precision operated by a PC. After finishing the polymerization process, the samples were placed on a hotplate at 120 °C for 30 min for post-baking. After cooling in the air for 10 min, the matrices were immersed in the developer (ORMODEV®, Micro resist technology) for 1 h to remove uncured photoresist. To reduce the tendency of adherent between of fibers, the matrices were immersed into the 60 mg/mL aso-lectin (Sigma Aldrich) solution for 30 s, and then rinsed five times with 2-propanol (Sigma Aldrich) and deionized water by succession. To sterilize the sample, suspended filamentous matrices were immersed in 70% ethanol before usage.

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.10.052.

2.2. Cardiac differentiation from iPS cells

The diseased iPS cell line (LQT3) and a healthy cell line counterpart (WT) were obtained from Dr. Conklin's laboratory at the Gladstone Institute of Cardiovascular Research. A small molecule WNT-mediated protocol was used to derive iPS-CMs

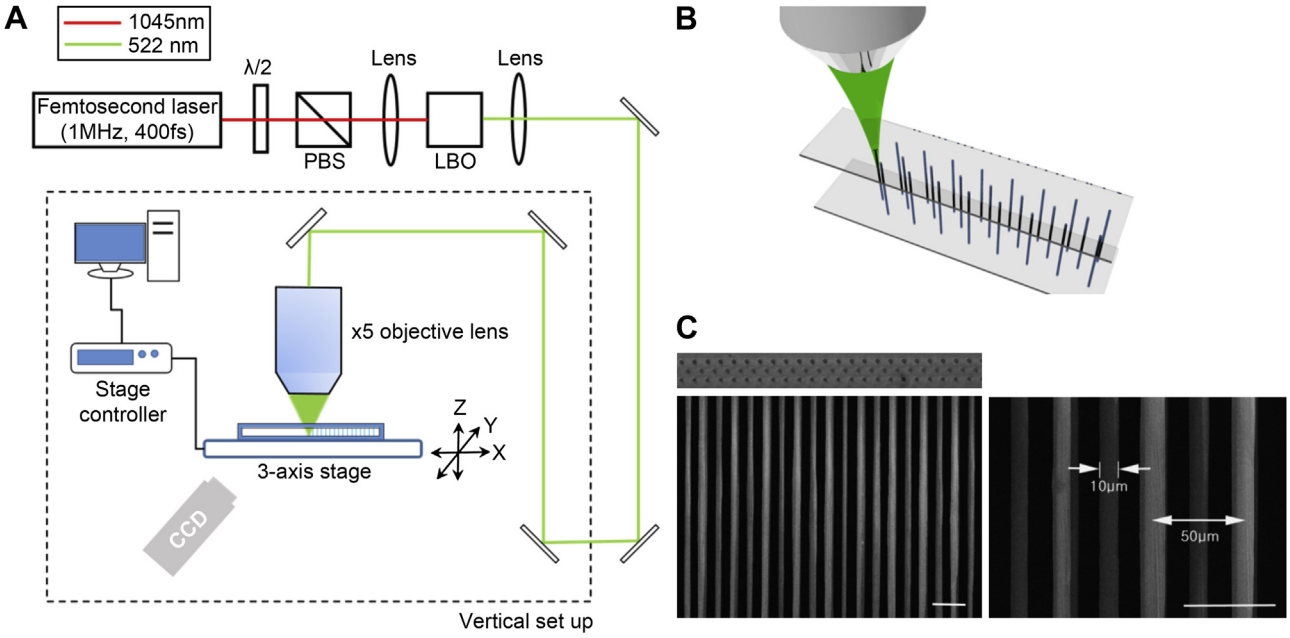

Fig. 1. (A) Schematic of TPIP system to fabricate the filamentous matrices; (B) Schematic of TPIP fabrication process to polymerize fibers with highly defined diameter and spacing; (C) SEM images of a fabricated F/10–50 filamentous matrix. (Scale bar: 50 μm).