Biomaterials 67 (2015) 52–64

Contents lists available at ScienceDirect

Biomaterials

journal homepage: www.elsevier.com/locate/biomaterials

Combinatorial polymer matrices enhance in vitro maturation of human induced pluripotent stem cell-derived cardiomyocytes

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ARTICLE INFO

Article history:
Received 27 June 2015
Accepted 1 July 2015
Available online 14 July 2015

Keywords:
iPSC
Cardiomyocytes
Maturation
Combinatorial polymer
Myosin light chain-2*v*
Troponin I

ABSTRACT

Cardiomyocytes derived from human induced pluripotent stem cells (iPSC-CMs) hold great promise for modeling human heart diseases. However, iPSC-CMs studied to date resemble immature embryonic myocytes and therefore do not adequately recapitulate native adult cardiomyocyte phenotypes. Since extracellular matrix plays an essential role in heart development and maturation *in vivo*, we sought to develop a synthetic culture matrix that could enhance functional maturation of iPSC-CMs *in vitro*. In this study, we employed a library of combinatorial polymers comprising of three functional subunits – poly-ε-caprolacton (PCL), polyethylene glycol (PEG), and carboxylated PCL (cPCL) – as synthetic substrates for culturing human iPSC-CMs. Of these, iPSC-CMs cultured on 4%PEG-96%PCL (each % indicates the corresponding molar ratio) exhibit the greatest contractility and mitochondrial function. These functional enhancements are associated with increased expression of cardiac myosin light chain-2v, cardiac troponin I and integrin alpha-7. Importantly, iPSC-CMs cultured on 4%PEG-96%PCL demonstrate troponin I (TnI) isoform switch from the fetal slow skeletal TnI (ssTnI) to the postnatal cardiac TnI (cTnI), the first report of such transition *in vitro*. Finally, culturing iPSC-CMs on 4%PEG-96%PCL also significantly increased expression of genes encoding intermediate filaments known to transduce integrin-mediated mechanical signals to the myofilaments. In summary, our study demonstrates that synthetic culture matrices engineered from combinatorial polymers can be utilized to promote *in vitro* maturation of human iPSC-CMs through the engagement of critical matrix-integrin interactions.

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1. Introduction

Heart disease is the leading cause of death in developed countries, accounting for over 36% of all deaths in the United States [1], yet the mechanistic study of human heart disease beginning at the cellular level has been limited by the lack of suitable human cardiomyocyte models. However, thanks to recent revolutionary advances in cellular reprogramming, and directed differentiation of human induced pluripotent stem cells (iPSCs) into cardiomyocytes (iPSC-CMs), a number of *in vitro* models of healthy and diseased human cardiac tissues have been developed [2,3]. Despite these advances, an important concern regarding the use of iPSC-CMs is their functional immaturity relative to primary cardiomyocytes. For instance, early studies indicated that cardiomyocytes generated *in vitro* from pluripotent stem cells exhibit fetal phenotypes with

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http://dx.doi.org/10.1016/j.biomaterials.2015.07.004
0142-9612/Published by Elsevier Ltd.

respect to their physiological performance [4,5]. Several recent studies have addressed maturation of iPSC-CMs or embryonic stem cell-derived cardiomyocytes (ESC-CMs) [6–10], but the majority were limited to calcium handling and electrophysiological evaluation. Thus, considerable unmet needs remain for the adequate study of *in vitro* maturation of iPSC-CMs, particularly at the cell and molecular levels, and factors that modulate it. One such factor influencing cell maturation, including cardiomyocytes, is the tissue microenvironment. In particular, cell-substratum interaction is essential for proper development and maintenance of tissue architecture and function. In many complex organisms, the extracellular matrix (ECM) plays a critical role in cardiomyocyte development, but the full mechanism of its impact remains unknown due to the ECM's heterogeneity in both composition and structural orientation. Yet, despite considerable progress being made to engineer niches that control cellular responses through purpose-specific biomaterial designs (e.g., surface patterning, biomolecule addition) that would encompass some of the native ECM properties, the direct effects of characteristic biochemical and biophysical properties of unmodified materials alone have largely been underexplored. To address the need, we employed a library of copolymer scaffolds with varying physicochemical properties as culture substrates [11]. The copolymer library contained different mole percentages of three components: hydrophilic poly(ethylene glycol) (PEG), hydrophobic poly(ε-caprolacton) (PCL), and negatively-charged carboxylated-PCL (cPCL). Each copolymer subunit was selected for the specific properties it contributed to the resulting copolymer: PCL is a semi-crystalline, biodegradable, and hydrophobic, as well as being FDA-approved in medical devices [12]; PEG is a biocompatible, hydrophilic, and repellent polymer that reduces protein adsorption and cell attachment through steric exclusion [13,14]; and cPCL facilitates cell attachment to the scaffold surface by providing a negative charge, effectively counteracting the PEG's repellant effects [14]. These combinatorial polymers were electrospun to make fiber mesh scaffolds that mimic ECM fiber structure and orientation, and subsequently used as test culture substrates.

Human iPSCs were differentiated into human iPSC-CMs through a directed differentiation protocol [15]. After 15–30 days of culture on each copolymer scaffold, we examined the effects of the copolymer composition on iPSC-CM phenotype by evaluating beating behavior, mitochondrial function and gene expression profiles. Our results indicate that certain combinatorial polymer scaffolds, especially a 4%PEG-96%PCL copolymer, promote the acquisition of several phenotypic features of mature ventricular myocytes including organized sarcomeres, abundant mitochondria, increased contractility and higher expression of cardiac myosin light chain-2v, cardiac troponin I and integrin alpha-7, each of which have been associated with cardiac/ventricular maturation [16–18]. Moreover, 4%PEG-96%PCL was associated with enhanced expression of intermediate filament-associated proteins involved in transducing integrin-mediated mechanical signals to the myofilaments. These results suggest the synthetic biomaterial promoted cardiac maturation by mimicking some features of basement membrane-integrin/sarcolemma interactions seen in normal development. In summary, our study suggests that specific chemical compositions of synthetic extracellular substrates can exert profound influence on *in vitro* maturation of iPSC-CMs.

2. Materials and methods

2.1. Reprogramming of human dermal fibroblasts and maintenance of human iPSCs

A human iPSC line (CC2) was generated from a healthy control subject using an episomal approach and validated, as we have previously described, following the work of Dr. Shinya Yamanaka [19–21]. Maintenance and culture of human iPSCs followed our established methods [19–22]. Pluripotency was validated by PluriTest, a bioinformatics assay [23], using a teratoma-validated line as a positive control, and normal chromosomal karyotype was confirmed (Genetic Associates, Nashville TN) as previously described (Fig. S1a and S1b) [19,23]. The absence of episomal vector genomic integration was confirmed by PCR (data not shown). Immunostaining for pluripotency markers used the following antibodies; OCT4 (mouse monoclonal, Millipore), NANOG (affinity purified anti-goat IgG), and SSEA4 (rabbit monoclonal, Millipore) (Fig. S2a).

2.2. Differentiation of human iPSCs to cardiomyocytes, and isolation of rabbit ventricular myocytes

iPSCs were washed with DMEM/F12 (1:1, Invitrogen) and PBS, followed by incubation with 1 mL/well Versene (Invitrogen) for 10 min at 37 °C. iPSCs were seeded on growth factor reduced Matrigel (BD Biosciences) coated plates at a density of 1 million cells per well in mTeSR1 medium supplemented with 10 μM ROCK inhibitor (Y-27632, CalBiochem). iPSCs were overlaid with mTeSR1 supplemented Matrigel (70–150 μg). After 24 h, medium was changed to RPMI 1640 medium plus B-27 and Activin A (R&D Systems) without insulin supplement (Invitrogen). Fresh Matrigel (83 μg Matrigel/well) mixed with RPMI 1640 basal medium was then overlaid for 30 min on the cells at 24 h post-Activin A treatment at day 0 of cardiac induction. After 20 min of Matrigel gelation, RPMI 1640 basal medium with B-27, BMP4 (5 ng/mL, R&D Systems) and bFGF (5 ng/mL, Invitrogen) without insulin supplement was replaced on the cells (day 1), and then incubated for 4 days without changing medium. At day 5, the medium was changed with RPMI 1640 with B27 complete supplement (Invitrogen) (Fig. S2b) and replaced every 2–3 days. After 15 days of cardiac differentiation, the medium was changed to DMEM/F12 including 2% fetal bovine serum (FBS), 2 mM L-glutamine, 0.1 mM nonessential amino acids, 0.1 mM β-mercaptoethanol, and 50u/mL penicillin/streptomycin for maintenance of iPSC-CMs.

Ventricular myocytes from adult rabbits were isolated by a modified collagenase/protease method, as previously described [24].

2.3. Traction force microscopy

Traction force maps of spontaneously beating iPSC-CMs were generated at room temperature using the traction force microscopy (N = 15 cells from four experimental replicates). Briefly, iPSC-CMs, grown on various culture matrices, were dissociated and replated on Matrigel containing 0.75 μm fluorescence beads (Polysciences) diluted at 1:50. Contraction motion of individual iPSC-CMs was video-captured by phase-contrast and fluorescent imaging, and the particle image velocimetry (PIV) was applied to assess displacement deformation of Matrigel substrate. Fourier transform traction cytometry (FTTC) was used to quantify contractile force using an ImageJ plugin [25,26].

Supplementary video related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2015.07.004.

2.4. Flow cytometry

iPSC-CMs maintained on each matrix for 30 days were detached with Triplexpress (Invitrogen). Cells were lightly vortexed to break up large cell aggregates and quenched with RMPI1640 with complete B27 supplement. Cells were fixed in 1% paraformaldehyde at

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