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Production of Single Contracting Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes: Creating the Matrigel Mattress

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Abstract

This unit describes the published Matrigel Mattress method. Briefly, we describe the application of the mattress, human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) Matrigel Mattress re-plating, and hiPSC-CM mattress maintenance. Adherence to this protocol will yield individual robust shortening hiPSC-CMs, which can be used for downstream applications.

Keywords

Matrigel; hiPSC-CMs; myocytes; cardiac; stem cells

Assessing function in hiSPC-CMs

Cardiovascular disease is the leading cause of mortality worldwide (Anonymous, 2016). In the United States, heart failure is projected to increase by 46% from 2012–2030 thus affecting over 8 million Americans adults (Heidenreich et al., 2013). Ergo, there is an urgent need for appropriate model systems to investigate disease pathophysiology and cardiovascular biology. Advances in hiPSC biology have led to reliable and efficient methods of differentiating hiPSCs into hiPSC-CMs for research and disease modeling (Burridge et al., 2014; Hinson et al., 2015; Mummery et al., 2012). Initially, the majority of physiological readouts using hiPSC-CMs have focused on electrophysiology and calcium handling measurements. Functional assessment of single cell contractility (cell shortening) has been limited due to the lack of cell shortening due to the aberrant spherical morphology of hiPSC-CMs cultured on standard substrates. To circumvent this issue, biophysical

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approaches such as atomic force microscopy, traction force microscopy, and micropost deflection have been employed to assess the contractile forces of single cell hiPSC-CMs (Ahola et al., 2014; Liu et al., 2012; Rodriguez et al., 2014). Although quantitative, these methods are technically challenging and require specialized lab equipment, which limits their adoptability.

Alternatively, tunable synthetic polymers coupled to printed biological substrates have been used to quantify hiPSC-CM single cell contractility (Ribeiro et al., 2015). Our method, the Matrigel Mattress, takes advantage of Matrigel's natural and environmental stiffness (450 Pa)(Li et al., 1994; Soofi et al., 2009) which alleviates the need for microcontacted prints on biomaterials. In this unit, we describe the simple 1 hour protocol for generating the Matrigel Mattress, which generates individual, shortening, hiSPC-CMs within 3–5 days post-mattress plating (Feaster et al., 2015).

Generating Matrigel Mattresses (~1 hour)

The protocol outlines the hiPSC-CM dissociation, Matrigel Mattress application, and hiPSC-CMs re-plating steps. Successful completion of the protocol will yield functional cardiomyocytes that will be ready for downstream applications in 3–5 days post-plating.

Note: The protocol detailed below must be performed using proper aseptic technique in a class II, type A2 biological safety cabinet.

Note: This protocol describes the dissociation from cultured differentiated hiSPC-CMs at day 30 of cardiac induction.

Note: Frozen hiPSC-CMs should be thawed and cultured for 3–5 days before dissociation.

Note: Check status of cultured hiPSC-CMs before dissociation by assessing viability and contractility.

Note: hiPSC-CM purity is important, as non-CMs will also adhere to the Matrigel mattress. We recommend metabolic selection via glucose deprivation at day 10 of induction for two days to remove contaminating cell types as in Feaster et. al.(Feaster et al., 2015) (Media composition, RPMI 1640 (Life Technologies, 11879–020) supplemented with 2% B27-minus insulin (Life Technologies, A1895601).

Note: The protocol described outlines the removal and re-plating of hiSPC-CMs from a 35 mm cell culture dish containing approximately 2×10^6 hiPSC-CMs.

Note: The protocol describes the generation of 3 Matrigel Mattress dishes (Delta TPG DISH)

Materials

HiPSC-CMs in culture

HiPSC-CM media (See Recipe)

Matrigel Growth Factor Reduced Basement Membrane Matrix (Corning, Product ##356230)

TrypLE Express (Life Technologies, 12604013)

D-PBS without CaCl₂ or MgCl₂ (Life Technologies, 14190144)

Falcon™ 15mL Conical Centrifuge Tubes (Fisher Scientific)

Small Cell Scraper (Corning, #3010)

2µl Gilson pipet with tips

Timer

Light microscope

Incubator

Delta TPG DISH (Fisher Scientific, Delta TPG DISH (50/Pack) 0.17mm black, 12-071-34) for experiments (typically 3 dishes per group)

Reagents and Solutions

HiPSC-CM media: RPMI 1640 with glucose (Invitrogen, cat# 11875); 2% B27 with insulin (Invitrogen, cat#17504-044); 1 % Pen-Strep (Invitrogen, cat#17504-044).

HiPSC-CM dissociation Timing: 30 minutes

Note: Thaw an aliquot of pure undiluted Matrigel on ice before beginning

- 1 Wash hiPSC-CMs by adding 2 mL D-PBS without to the 35 mm dish.
- 2 Aspirate solution.
- 3 Add 1 mL TrypLE Express (room temperature) to the 35 mm dish and incubate at 37°C for 15 minutes (Perform step 4 during incubation).
- 4 Add 10 mL of hiPSC-CMs culture media to a 15 mL conical centrifuge tube.
- 5 After incubation, gently remove TrypLE Express from the dish and transfer it to the 15 mL conical centrifuge tube.
- 6 Add 1 mL of hiPSC-CM media to the culture dish.
- 7 Gently pipette hiPSC-CM media and eject it onto the cell culture dish surface to dislodge remaining adherent cells.
- 8 Repeat 3 times.
- 9 Use a cell scraper to dislodge any attached hiPSC-CMs.
- 10 Transfer entire contents of the dish to the 15 mL conical centrifuge tube.
- 11 Add 3 mL of hiPSC-CM media the 15 mL conical centrifuge tube to bring the total volume to 15 mL.
- 12 Place the 15 mL conical tube into the centrifuge and spin for 5 minutes at 200g.

- 13 Decant the conical tube. Be careful not to disrupt the cell pellet.
- 14 Re-suspend the pellet using 3 mL of cardiomyocyte media.
- 15 Bring to a final volume of 5 mL
- 16 Count the cardiomyocytes using a hemacytometer.
- 17 Adjust to 2×10^5 cardiomyocytes per 1 mL of cardiomyocyte media.
- 18 Incubate cardiomyocytes at room temp during Matrigel preparation.

Matrigel Matrigel Preparation and hiSPC-CM re-plating: Timing: 30 minutes

Note: It may be helpful to practice ahead of time. Also to prepare a smaller number of Matrigel Matrigel to start (~4) until proper technique is established.

- 19 Mix the Matrigel by carefully flicking the microcentrifuge tube.
- 20 Place the Matrigel tube on ice.
- 21 Start a timer with application of the first Matrigel
- 22 Chill the pipet tip by pipetting 1 μ L of chilled Matrigel up and down 2–3 times
- 23 Apply 1 μ L of undiluted Matrigel over the length of 20 mm line. (Fig 1)
 - a. Be sure to pipette a 45-degree angle.
 - b. Apply 4, 1 μ L lines in parallel per dish. Each line (1 μ L) represents one Matrigel.

Note: Aim to spend 2 minutes preparing 12 Matrigel Matrigel.

Note: To help with orientation during experimentation, plate all Matrigel lines in parallel.

Note: Matrigel Matrigel shorter than 10 mm are too thick resulting in an unstable Matrigel

Note: Work quickly to avoid Matrigel solidifying in the pipette tip. If Matrigel solidifies in the pipette tip, discard the tip, re-chill a new pipette tip, and continue.

- 24 Incubate the Matrigel Matrigel at room temperature for 8–10 minutes.

Note: An 8 minute incubation allows for enough time for the Matrigel to solidify. Incubation longer than 10 minutes results in a stiff Matrigel which impedes cell shortening. Matrigel Matrigel incubated under 8 minutes are unstable.

- 25 Add ~40,000 cardiomyocytes in 200 μ L hiSPC-CM media directly to each dish.

Note: Addition of cardiomyocytes and media halts Matrigel polymerization.

- 26 Incubate at room temperature for 5 minutes. (Do not disturb the dish as the cells are adhering to the matrigel)
- 27 Using a light microscope, visually inspect the dish to ensure the cells are not too dense. i.e. hiSPC-CMs do not touch. If the hiSPC-CMs are touching, make new Matrigel Matrigel and repeat plating at a lower density.

- 28 Gently pipette 1 mL of hiPSC-CM media onto each dish.
- 29 Incubate at 37°C for 3–5 days until cell shortening begins

Note: Visually inspect for morphology changes. Cell rounding should begin immediately. By 12 hours cardiomyocytes should exhibit distinct morphologic changes and robust cell shortening at 24 hours. (Fig 2)

- 30 Change the hiPSC-CM media

Commentary

Background Information

Matrigel is a solubilized basement membrane matrix extracted from the Engelbreth-Holm-Swarm mouse sarcoma (Kleinman et al., 1982). This tumor is rich in extracellular proteins such as procollagen IV, laminin and heparin sulfate proteoglycans (Kleinman et al., 1982). This tumor is also rich in growth factors such as transforming growth factor β (TGF- β), epidermal growth factor (EGF), insulin-like growth factor 1, bovine fibroblast growth factor (bFGF), and platelet-derived growth factor (PDGF) (Vukicevic et al., 1992). Although, we don't know the exact mechanism that causes the change in hiPSC-CM morphology and function, but we postulate a combination of both growth factors and membrane hydrogel stiffness, that allows for the robust hiPSC-CM shortening. Future research should identify which combination of factors and substrates mimic the Matrigel Mattress in order to establish a chemical and synthetic hydrogel to promote functional maturation.

Critical Parameters

It is critical to identify hiPSC-CM health, as poorly differentiated cells may not adopt the Matrigel Mattress phenotype. Be sure to evaluate the hiPSC-CMs for rhythmic beating, purity and viability.

Troubleshooting

Refer to Table 1 for common Matrigel Mattress problems and solutions.

Anticipated Results

This protocol yields individually contracting cardiomyocytes within 3–5 days on the Matrigel Mattress.

Time Considerations

HiPSC-CM dissociation: Timing: 30 minutes

Matrigel Mattress Preparation and hiSPC-CM re-plating: Timing: 30 minutes

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Significance Statement

The Matrigel Mattress is an innovative platform that promotes single cell shortening in hiPSC-CMs.

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Figure 1. Sample Delta TPG dish with 4 Matrigel Mattresses drawn. Mattresses are indicated by red arrows.

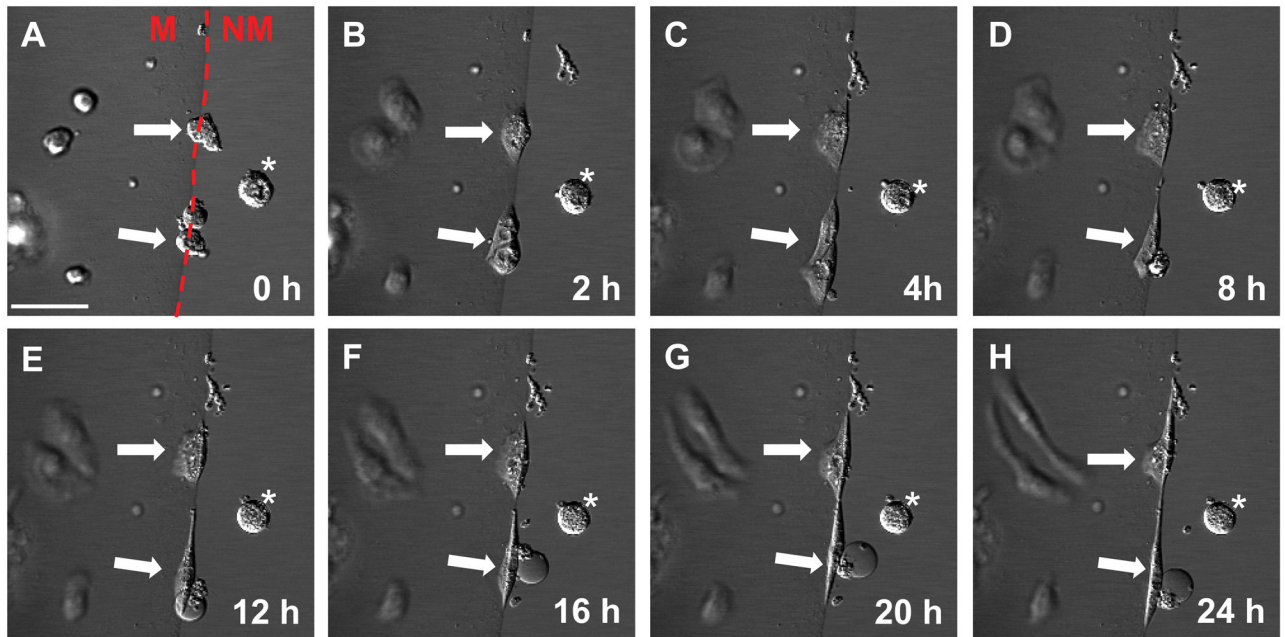


Figure 2. Time-lapse imaging of hiPSC-CMs on the Matrigel Mattress. Mattress hiPSC-CMs adopt elongated morphology ~12 hours post-plating. Arrows indicate hiPSC-CMs on the Mattress. Asterisk indicate cell not on the mattress. The red dashed line separates the Matrigel mattress from non-mattress area. M indicates mattress, NM indicates non-mattress, scale bar is 50 μm .

Table 1

List of potential Matrigel Mattress problems and solutions.

Problem	Possible Reason	Solution
Matrigel solidifies in pipette tip	Matrigel has reached room temperature	Work quickly
Matrigel in oblique line	Pipetting too quickly	Slow down
Poor quality mattresses	Preparing too many mattress simultaneously	Prepare less until proper technique is established
Matrigel implodes	Short incubation	Incubate mattresses for 8–10 minutes prior to seeding hiPSC-CMs
hiPSC-CMs do not contract on mattress	Mattresses are too thick or thin	Apply Matrigel at a thickness of 0.4 mm
Cells dissociate from mattress	Perfusion or aspiration too strong during daily media changes	Decrease aspiration strength
Matrigel ruptures or dislodges from dish	Media forcefully applied to dish	Gently add media to the corner of the dish; not directly on the mattress