Acute 3D hiPSC-CM Contractility Tool

PROTOCOL:

1. Preparation of plates and media

1.1. In a sterile tissue culture hood, prepare a sterile 6-well plate by transferring 2 mL of 0.1% gelatin (**Table of Materials**) to each well. Place the lid on the 6-well plate and allow the coated plate to incubate at 37 °C for a minimum of 1 h.

1.2. One day before seeding hiPSC-CMs and primary human ventricular cardiac fibroblasts on ECT (Engineered cardiac tissue) molds to form 3D tissues, thaw hydrogel (**Table of Materials**) aliquot in the refrigerator, on ice.

1.3. Prepare cardiomyocyte medium (**Table of Materials**) according to the manufacturer's instructions.

1.4. Prepare cardiac fibroblast medium (**Table of Materials**) according to the manufacturer's instructions.

2. Seeding of cryopreserved hiPSC-CMs

2.1. Two days before seeding hiPSC-CMs on ECT molds, pre-plate hiPSC-CMs on 0.1% gelatin-coated, sterile 6-well plates. Thaw hiPSC-CMs using standard thawing protocol [1,2].

2.2. Then, plate 1,500,000 total hiPSC-CMs (**Table of Materials**) per well according to the manufacturer's instructions [3].

2.3. Culture hiPSC-CMs in standard cardiomyocyte media for 2 days to allow hiPSC-CMs to recover from cryopreservation at 37 °C, 5% CO_2. Refresh the supernatant with 100% cardiomyocyte medium every 48 h.

3. Seeding of cryopreserved cardiac fibroblasts

3.1. Two days before seeding cardiac fibroblasts on ECT molds, pre-plate cardiac fibroblasts on 0.1% gelatin-coated, sterile 6-well plates. Thaw cardiac fibroblasts using standard thawing protocol [1,2].

3.2. Then, plate 250,000 total cardiac fibroblasts (**Table of Materials**) per well according to the manufacturer's instructions [3].

3.3. Culture cardiac fibroblasts in standard cardiac fibroblast medium for 2 days at 37 °C, 5% CO_2. Refresh the supernatant with 100% cardiac fibroblast medium every 48 h.

4. Dissociation and counting pre-plated hiPSC-CMs and cardiac fibroblast.

4.1. Check the status of hiPSC-CMs before dissociation. Evaluate hiPSC-CM health ensuring viability and stable beating.

NOTE: The purity of the hiPSC-CM population is important (e.g., >90 % Cardiac Troponin T) [4]. A cardiomyocyte selection method (e.g., metabolic selection or sorting) is recommended to reduce

disruption by non-cardiomyocyte cells [2,3].

4.2. Wash hiPSC-CMs 2x with 4 mL per well of D-PBS without $CaCl_2$ or $MgCl_2$ (**Table of Materials**). Aspirate D-PBS and add 1 mL of room temperature dissociation reagent to each well then incubate for 15 min at 37 °C[5].
4.3. Add 10 mL of cardiomyocyte medium to a sterile 15 mL conical tube.

4.4. Dissociate hiPSC-CMs from the 6-well plate with a 1,000 µL pipette (**Figure 1B**). Add the cell suspension to the 15 mL conical tube [3].

4.5. Rinse the well with 1 mL of fresh cardiomyocyte medium to collect any residual hiPSC-CMs and add to the 15 mL conical tube. Bring the final volume of the conical tube to 15 mL.

4.6. Centrifuge for 5 min (200 × g). Remove the supernatant up to the 1 mL mark. Resuspend the cells in cardiomyocyte medium to a final volume of 5 mL.

4.6.1. Count hiPSC-CMs with a manual or automated cell counter.

4.7. Incubate the hiPSC-CM suspension at room temperature while the cardiac fibroblast are dissociated (30 min maximum).

4.8. Dissociate and count cardiac fibroblast as above.

5. Generation of Engineered Cardiac Tissues[6]

5.1. Combine hiPSC-CMs and cardiac fibroblasts at a 10:1 ratio (i.e., 100,000 hiPSC-CM:10,000 cardiac fibroblast)[5,6].

5.2. Combine cell mixture and Extracellular Matrix hydrogel components (**Table of Materials**).

5.3. Seed cell-hydrogel suspension into each well of ECT molds.

5.4. Culture tissues for 7 days to enable remodeling and compaction at 37 °C, 5% CO_2 refresh medium every 48 h.

5.5. Expose tissues to 7-week electrical condition stimulation protocol[5,6].

NOTE: Electrical stimulation protocol of weekly 1 Hz increase in frequency is recommended for ventricular functional maturation until positive force-frequency response is observed[5,6].

6. Contraction recording and analysis[7]

6.1. Prepare CCM assay medium, Tyrode's solution containing (in mmol/L): $CaCl_2$ 1.8, NaCl 134, KCl 5.4, $MgCl_2$ 1, glucose 10, and HEPES 10, pH adjusted to 7.4 with NaOH, and equilibrate to 37 °C in a water bath.

6.2. Place tissue in tissue bath (1 cm × 5 cm, 600 µl) recording chamber.

6.3. Perfuse 37 °C CCM assay medium at 600 µL per chamber at a flow rate of 4 ml/min.

6.4. Cut and attached one end of the tissue to a force transducer (**Table of Materials**)[7].

6.5. Immobilize the other end of the tissue using stainless steel wires attached to a micromanipulator[7].

6.6. Connect a silicon-based strain gauge with two piezoresistive elements (**Table of Materials**) to the amplifier to convert resistance change to voltage signals. Convert voltage to force measurements.

NOTE: Prior to experimentation the strain gauge was pre-calibrated with known weights and a conversion factor of 104.4 µN/mV was established[7].

6.7. Use camera (**Table of Materials**) to capture contraction videos and force transducer to measure contractility. Field stimulate the tissue with a commercial pulse generator (**Table of Materials**) to electrically pace the tissue. Pace tissue at 1.5x threshold at 1 Hz with baseline pulse parameters (e.g., monophasic square wave pacing pulses with a 2 ms stimulus pulse duration (~10 V/cm)[7,8].

6.8. Record the baseline, pacing only (i.e., before CCM) contraction for a minimum of 2 min [8,9].

6.9. Then, stimulate the tissue with an experimental electrical signal. To follow this protocol, use the standard CCM stimulation parameters: two symmetrical biphasic pulses of **5.14 ms phase duration** (**20.56 ms total duration**), **~28 V/cm** (**phase amplitude**), **zero interphase interval** and **30 ms delay** (i.e., time from the end of the pacing pulse and the beginning of the CCM pulse) [10,11] and record CCM-induced contraction for a minimum of 2 min[7].

6.10. Turn off the CCM signal and stimulate with baseline pacing pulse and record contraction of the recovery period (i.e., after CCM) for a minimum of 2 min.

6.11. Enable contractile properties to return to baseline then use contractility tool to evaluate various experimental electrical signals.

6.12. Use standard contraction software to analyze contraction videos and conversation factor to quantify contraction force (e.g., **contraction/force amplitude**, **contraction slope**, **relaxation slope**, **time to peak**, **time to baseline 90%**, and **contraction duration 50%**) [4,7,8,12,13].

Table of Materials

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
0.1% Gelatin	STEMCELL Technologies	7903	Pre-plating Culture Substrate
6-well Plate	Thermofisher	140675	hiPSC-CM Culture, Plastic
Calcium Chloride dihydrate ($CaCl_2$)	Fisher Scientific	c70-500	Tyrode's solution
Conical tube 15 mL	Corning	352099	Dissociation
Digital CMOS Camera	Hamamatsu	C11440-42U30	Contraction Video Recording

D-PBS	Life Technologies	14190-144	Cell Wash
Glucose	Sigma-Aldrich	G8270-1kg	Tyrode's solution
Hemocytometer	Fisher Scientific	22-600-107	Cell Counting
HEPES	Sigma-Aldrich	H3375	Tyrode's solution
iCell Cardiomyocytes Plating Medium	Fujifilm Cellular Dynamic, Inc.	M1001	hiPSC-CM Plating Media
iCell Cardiomyocytes^2, 01434	Fujifilm Cellular Dynamic, Inc.	R1017	hiPSC-CMs
Incubator (37 °C, 5% CO2)	Thermofisher	50116047	Maintain cells and tissues
Inverted Microscope	Olympus	IX73	Imaging ECTs
Magnesium Chloride hexahydrate ($MgCl_2$)	Fisher Scientific	m33-500	Tyrode's solution
Matrigel Basement Membrane Matrix	Corning	354230	ECM Component
Microcentrifuge tubes 1.5 ml	Fisher Scientific	05-408-129	Substrate Aliquot
Model 3800 Mulit-channel Power Stimulator	AM-Systems	Model 3800	Pulse Generator
Pen-Strep	Invitrogen	15140-122	Cardiomyocyte Media
Pipette L-20	Rainin	17014392	Pipette
Pipette P1000	Fisher Scientific	F123602G	Pipette
Pipette tips, 1000 ul	Fisher Scientific	02-707-509	Pipette
Pipette tips, 20 ul	Rainin	GPS-L10S	Pipette
Potassium Chloride (KCl)	Fisher Scientific	P330-500	Tyrode's solution
Sodium Chloride (NaCl)	Fisher Scientific	s641-212	Tyrode's solution
Sodium Hydroxide (NaOH)	Sigma-Aldrich	221465	Tyrode's solution
Stimulation Electrodes	--	--	Pacing and CCM Stimulation
Trypan Blue Stain	Life Technologies	T10282	Cell Counting
TrypLE Express	Life Technologies	12605-010	Cell Dissociation
Fibrin	Sigma-Aldrich	9001-31-4	ECM Component
Collagenase type 2	Worthington	4176	ECM Component
Multi-Purpose Sensor Element	Kronex Technologies	AE801	Silicon-based strain gauge/force transducer
NHCF-V – Human Ventricular Cardiac Fibroblasts	Lonza Bioscience	CC-2904	Cardiac fibroblasts
FGM™-3 Cardiac Fibroblast Growth Medium-3 BulletKit	Lonza Bioscience	CC-4526	Cardiac fibroblast medium

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