

# Electrophysiological recordings of cardiomyocytes isolated from engineered human cardiac tissues derived from pluripotent stem cells

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## Method Article

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# Abstract

Electrophysiological characterization is crucial for validating the function of human cardiomyocytes derived from pluripotent stem cells. To examine the maturity of human cardiomyocytes generated, whole-cell patch clamp recordings for action potentials and IK1 current are required. Here we report a summary of electrophysiological recording procedures using human mature cardiomyocytes isolated from engineered cardiac tissues derived from pluripotent stem cells. As observed in rodent primary myocytes, human mature engineered myocytes could be more difficult for whole-cell patch clamp recordings compared to conventional cardiomyocytes spontaneously contracting, which are differentiated from human pluripotent stem cells. Especially, we found that time window to record the electrical activities in such mature myocytes was narrower than ones in human conventional cardiomyocytes spontaneously beating. The whole workflow could be useful to facilitate electrophysiological characterization of human engineered cardiomyocytes in order to unveil the pathophysiological mechanisms underlying cardiac disease and test drug candidates.

## Reagents

1. Cardiac tissue dissociation solution 20 unit/ml papain \(\Sigma\text{-Aldrich, \#76220, from Caripa papaya}) 1.1mM EDTA \(\text{Thermo Scientific, \#15575038, 0.5 mM solution stock, pH8.0}) 67\mu\text{M 2-Mercaptoethanol \(\Sigma\text{-Aldrich, \#M3148}) 5.5mM L-Cystein HCl \(\Sigma\text{-Aldrich, \#C7880}) 1\times\text{ EBSS \(\text{Thermo Scientific, \#24010043}) 2. Glass-bottom dish coating solution Geltrex \(\text{100x stock, Thermo Scientific, \#A1413302}) in DMEM/F12-GlutaMax I \(\text{Thermo Scientific, \#10565042}) 3. Cardiomyocyte culture media DMEM/F12-GlutaMax I 500ml Fetal bovine serum \(\text{Hyclone, \#SH30070.03, final 5\%}) 27ml Penicillin / streptomycin \(\text{Thermo Fisher, \# 15140122}) 5.3ml MEM Non-essential amino acid \(\text{Thermo Fisher, \# 11140050}) 5.3ml 2-Mercaptoethanol 3.5\mu\text{l 4. External action potential recording solution 140mM NaCl \(\Sigma\text{-Aldrich, \#S7653}) 5.4mM KCl \(\Sigma\text{-Aldrich, \#P9333}) 1mM MgCl}\_2\text{ \(\Sigma\text{-Aldrich, \#M2670}) 10mM D-glucose \(\Sigma\text{-Aldrich, \#G7021}) 1.8mM CaCl}\_2\text{ \(\Sigma\text{-Aldrich, \#C7902}) 10mM HEPES \(\Sigma\text{-Aldrich, \#H3375}) pH7.4 with NaOH at 25 oC 5. Internal/pipette action potential recording solution 120mM K D-gluconate \(\Sigma\text{-Aldrich, \#G4500}) 25mM KCl 4mM MgATP \(\Sigma\text{-Aldrich, \#A9187}) 2mM NaGTP \(\Sigma\text{-Aldrich, \#G8877}) 4mM Na}\_2\text{-phospho-creatin \(\Sigma\text{-Aldrich, \#P7936}) 10mM EGTA \(\Sigma\text{-Aldrich, \#E3889}) 1mM CaCl}\_2\text{ 10mM HEPES pH 7.4 with KOH at 25 oC 6. External IK1 recording solution 160mM NMDG \(\text{(N-Methyl-D-glucamine, \Sigma\text{-Aldrich, \#66930}) 5.4mM KCl 2mM MgCl}\_2\text{ 10mM D-glucose 10}\mu\text{M nisoldipine \(\Sigma\text{-Aldrich, \#N0165}) 1}\mu\text{M E-4031 \(\text{(TOCRIS, \#1808}) 10mM HEPES pH7.2 with HCl at 25 oC 7. BaCl}\_2\text{ \(\text{0.5mM, \Sigma\text{-Aldrich, \#342920}) in the above external solution \(\text{(reagent 6}) 8. Internal IK1 recording solution 150mM K-gluconate 5mM EGTA 1mM MgATP 10mM HEPES pH 7.2 with KOH at 25 oC}

## Equipment

Patch-clamp amplifier \(\text{Molecular Devices, MultiClamp 700B and Digidata 1440}) Inverted microscope equipped with differential interface optics \(\text{Nikon, Ti-U}) on a standard vibration table and faraday cage

Patch clamp and imaging software \ (Molecular Devices, pClamp10 and MetaMorph imaging software MM-40000) EMCCD camera \ (Photometrics Evolve Intelligent, 512X512 EMCCD Digital Monochrome, #EVO-512-M-FW-16-AC) Micropipette puller \ (Sutter Instrument, Model P-97) Borosilicate glass \ (Sutter Instrument, #BF150-110-10) Pipette manipulator \ (Sutter instrument, #MPC-325-2) Heating system \ (Warner instrument, #TC324C) Quick exchange platform for 35-40mm dish \ (Warner instrument, #QE-1) Standard Water bath or heat block \ (for 37 °C tube incubation for tissue dissociation)

## Procedure

1. Prepare the papain-based dissociation solution \ (reagent 1, 10 ml) freshly. Incubate the dissociation solution in 37 °C water bath for 30 min to activate papain. Add DNase solution \ (Worthington, #LS002145, 5 µl/mL) into the papain solution before use.
2. Incubate 35 mm glass-bottom dishes \ (MatTek, P35-1.5-7-c) with Geltrex coating solution \ (reagent 2) for one hour at 37 °C and one hour at room temperature. Aspirate the solution right before plating cardiomyocytes
3. Wash engineered cardiac tissues with 1x PBS \ (phosphate buffered saline, Thermo Fisher, #70011044, no calcium/magnesium addition) twice. Next, incubate the tissues in the papain-based dissociation solution containing DNase for 15 min at 37 °C \ (one cardiac tissue in 10 ml of the papain solution in 15 ml tube). Gently shake the tube every 2 minutes. After 15-min incubation, gently pipette the tissues with 10ml serological plastic pipette 10 times.
4. Terminate the papain activity using the serum-containing culture media \ (reagent 3).
5. Centrifuge the tube, aspirate all of the solution, wash with the myocyte culture media \ (reagent 3) using another centrifugation \ (200xg, 5 min) and add fresh culture media into the tube
6. \ (Critical step) Replate the dissociated single cardiomyocyte \ (≈ 100 µL) on only glass part of the Geltrex-coated dishes \ (7mm diameter). Low cell confluency \ (2-5%) is required to utilize dissociate myocyte efficiently using multiple electrophysiological recording conditions. A dish containing sterile water without cover can be placed next to two myocyte dishes in a 100mm dish in order to keep humidity and prevent the medium from being dried. Twenty four hours after myocyte plating, add 2ml cardiomyocyte culture media \ (reagent 3) into the dishes.
7. Two or three days after myocyte plating, wash the dishes using patch clamp external recording solution \ (reagent 4, at 37 °C in case of action potential recording) once, and add the recording solution \ (~2ml/dish at 37 °C). \ (Critical timing) at the following day, the dissociated myocytes are not fully recovered. More than four days after myocyte dissociation, the cells \ (without any spontaneous contraction) are no longer healthy for electrophysiological recordings. Therefore, the whole-cell patch clamp recordings should be completed in 2-3 days after the myocyte dissociation and plating. Particularly, this optimized protocol enables obtaining healthy patchable single cardiomyocytes without spontaneous beating from early/intensity cardiac tissues \ (see “associated publication”).
8. Find single cardiomyocytes \ (no spontaneous beating) on the dish using 20x objective lens \ (Nikon, #MRD30205, CFI Plan Apochromat Lambda DM 20x Objective Lens NA 0.75 WD 1.00MM).
9. Fill the internal solution \ (reagent 5 in case of action potential recording) into a glass pipette, and install the pipette into the electrode connected with the manipulator holder. Move the pipette in the external solution using the manipulator controller \ (speed 0 or 1).
10. Examine pipette resistance \ (smaller, ~8 MΩ is better, i.e. using a larger pipette with 2-6 MΩ resistance often induces failure of sealing and breaking through and/or

results in leak trouble in cardiomyocytes). (Critical step) Move the pipette tip on the top of the target cell slowly (speed 4) using the 20x objective lens. Also, confirm that there is no bubble inside the glass pipette. If any bubbles or debris are observed, apply pressure using a 10ml syringe to get rid of bubbles and debris after keeping the pipette far from the target cell. 11. Move the pipette down slowly (speed 6, down to ~30µm above the cell). Switch the objective lens to 40x one (Nikon, #MRD00405, CFI Plan Apochromat Lambda 40x Dry Objective Lens NA 0.95 WD 0.21MM). Again confirm that pipette resistance and pressure are stable and there are no bubble and debris inside the pipette. Switch to intermediate magnification changer 1.5X (Nikon). Use PC monitor to see the target myocyte with MetaMorph software and ECDD camera instead of 10x eyepiece of the microscope. 12. Seal the cell using a glass pipette using mouth (gentle pressure). (Critical step) Seal thicker and elastic region of the target cell (but not close to the nucleus). Hold potential at -60 mV after sealing is successful (>3 GΩ). Next, apply a small amount of pressure multiple times using mouth suction (i.e. sharp pulses) to the electrode/pipette in order to break through the plasma membrane of the sealed cardiomyocyte. Immediately after breaking through, the cell capacitance should be measured using the whole cell clamp mode in pClamp 10 software. And then switch the mode to current-clamp to make sure that there is no spontaneous action potentials in the patched cardiomyocytes. Start action potential recordings. 13. In case of IK1 current recording in human pluripotent stem cell-derived cardiomyocyte<sup>1-2</sup>, reagent 6 and 8 are used as the external and internal solutions, respectively. Reagent 7 is used for measuring Ba<sup>2+</sup>-sensitive current to dissect IK1 current in the cardiomyocytes. Use 2-sec long voltage-clamp applied from -130 to +10 mV (holding at -40 mV, 0.1 Hz 2-sec voltage pulse). The IK1 reversal potential (Ba<sup>2+</sup>-sensitive current) had a negative slope conductance consistent with inward rectification, as previously described. The current-voltage plot can be analyzed before and after the addition of 0.5 mM BaCl<sub>2</sub> for 2min.

## References

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## Figures

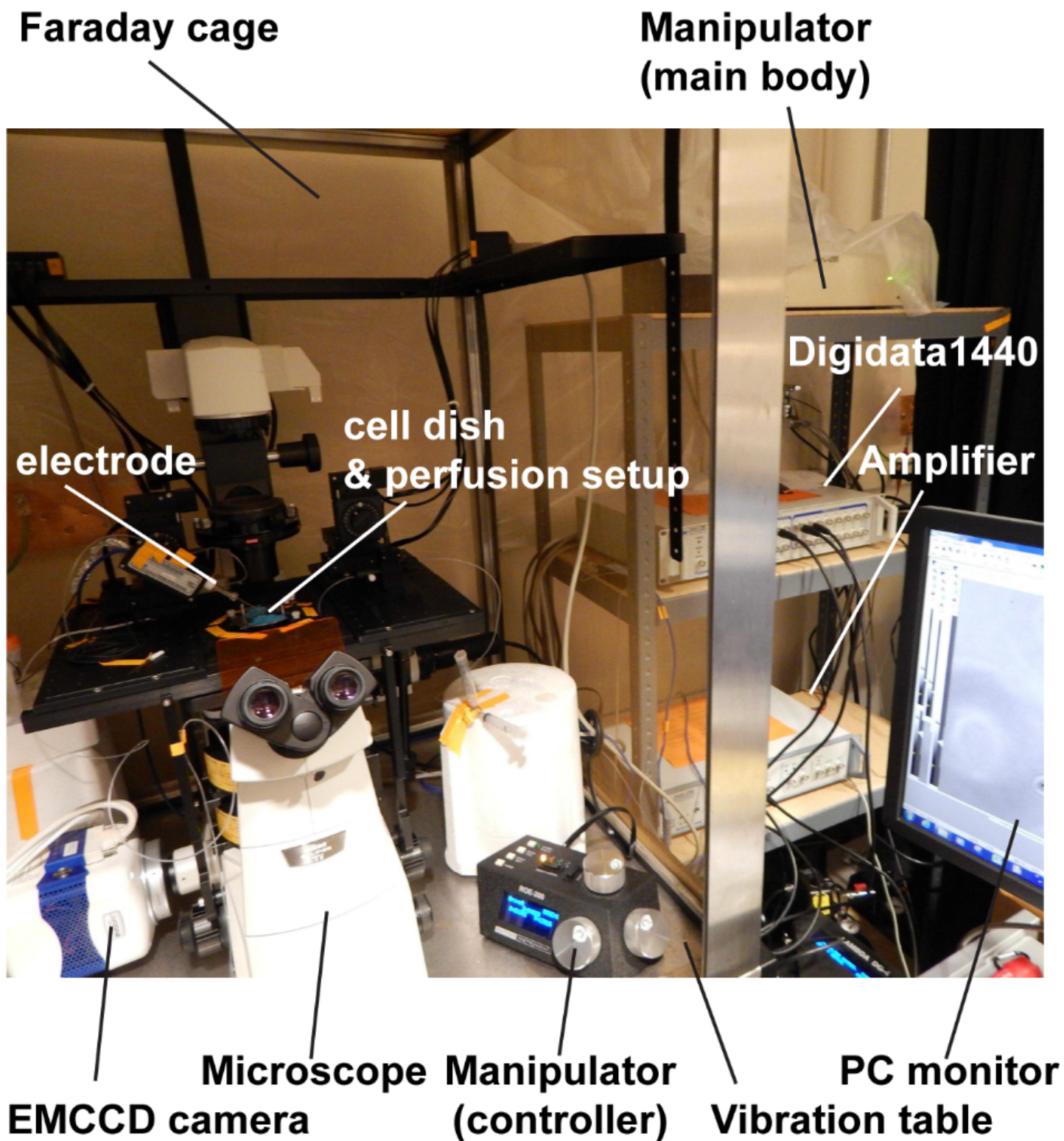


Figure 1

Electrophysiological recording setup

## Supplementary Files

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