

## **ELECTRONIC EXPRESSION OF THE INWARD RECTIFIER IN CARDIOCYTES DERIVED FROM HUMAN INDUCED PLURIPOTENT STEM CELLS**

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### **CROSS REFERENCE TO RELATED APPLICATIONS**

**[0001]** This application claims priority to U.S. Provisional application no.  
5 61/824,507, filed on May 17, 2013, the disclosure of which is incorporated herein by  
reference.

### **STATEMENT REGARDING FEDERAL FUNDING**

**[0002]** This invention was made with government support under grant numbers  
HL093631 and HL062465 from the National Institutes of Health. The government has certain  
10 rights in the invention.

### **BACKGROUND OF THE INVENTION**

**[0003]** The term “Induced pluripotent stem cells,” or iPSCs, refers to a type of  
pluripotent stem cell artificially prepared from a non-pluripotent cell. Cardiac myocytes  
derived from iPSCs are a useful experimental system which has great potential. They offer an  
15 innovative human preparation for cardiac repair, drug safety design and testing, clinical  
diagnosis, and research. Cardiac myocytes derived from iPSCs offer the opportunity to work  
on cells which recapitulate the activity of healthy human cardiac myocytes, which are  
otherwise rarely available for comprehensive experimental investigation. Human iPSC-  
derived cardiac myocytes offer the ability to develop predictive tools for cardiac function.

20 **[0004]** Despite the possibilities of cardiac myocytes derived from iPSCs, problems  
with this approach have been noted, leading to serious concerns about their use in studying  
arrhythmogenic mechanisms and drug safety screening. Action potentials (APs) from human  
cardiac myocytes derived from iPSCs are often referred to as an “immature phenotype.” The  
lack of the expected classic spike and dome type of AP morphology has led to serious  
25 concerns over the ability of human cardiac myocytes derived from iPSCs to be used to study  
the genetic basis of Brugada syndrome and other J-wave or early repolarization related  
arrhythmias. In addition to an apparently less complicated repolarization profile, iPSC  
cardiac myocytes also show spontaneous contractile activity. This contractile activity is  
accompanied by corresponding diastolic depolarization resulting in spontaneous APs. Thus,  
30 while iPSCs can be induced to develop into cardiac myocyte-like cells, they are not able to  
display electrophysiological properties that would allow use of these cells in evaluation of  
cardiac cell function or use for screening of drugs affecting cardiac cells.

## SUMMARY OF THE INVENTION

[0005] In the present disclosure, we evaluate the role of reduced inward rectifying current ( $I_{K1}$ ) in human iPSC-derived myocytes by electronically adding an  $I_{K1}$  component to these cardiac myocytes under current clamp. This approach indicates the strong influence of  $I_{K1}$  and the degree to which its expression produces major physiological differences in repolarization and potentially arrhythmogenic behavior. We demonstrate that artificial replacement of  $I_{K1}$  produces human iPSC-derived cardiac myocytes with APs which much more closely resemble the behavior of APs from freshly isolated human cardiac myocytes.

[0006] In one aspect, this disclosure provides a system to improve action potential morphology in iPSC-derived cardiac myocytes. Improved AP morphology may include, for example, physiological resting membrane potentials, and spike and dome action potentials. The system comprises a patch clamp having an electrode for measuring a membrane voltage of the myocyte and a generating circuit in electrical communication with the electrode. The generating circuit is configured to calculate a value of an inward rectifying current based on the membrane voltage and apply a synthetic inward rectifying current to the myocyte based on the calculated value.

[0007] This method has many applications, and will enable comprehensive analysis of this electrophysiological system and also allow screening of drugs specific for atrial or for ventricular cell defects. iPSC cardiac myocytes of the present disclosure can be used in the interpretation of channelopathies, drug screening, and the assessment of potential arrhythmogenesis. By eliminating many of the non-physiological consequences of spontaneous activity and unstable behavior, this tool will be useful for studying the dynamics of repolarization. It has applications for research studies looking at genetic regulation of repolarization in disease states, as well as serving as an aid in the interpretation of data from drug studies and the proarrhythmic potential of safety screens. It could also be used to modify treatment regimes based on the data obtained.

[0008] In one aspect, this disclosure provides a method for producing action potentials having improved morphology in iPSC-derived cardiac myocytes. The method comprises the steps of measuring a membrane voltage of a myocyte; calculating a value of an inward rectifying current based on the measured membrane voltage; and applying a synthetic inward rectifying current to the myocyte thereby causing the myocyte to produce action potentials having improved morphology.

[0009] In one aspect, this disclosure provides a method for distinguishing iPSC-derived cardiac myocytes having atrial cell-like electrical features from iPSC-derived cardiac myocytes displaying ventricular cell-like electrical features.

[0010] In one aspect, this disclosure provides a method for screening candidate agents  
5 that can affect the electrical characteristics of atrial cells or ventricular cells. The method comprises identifying whether an iPSC-derived cardiac myocyte is displaying an atrial or a ventricular phenotype and then testing the effect of putative drugs that affect atrial or ventricular function.

[0011] iPSC cardiac myocytes will be able to be used in the interpretation of  
10 channelopathies, drug screening, and the assessment of potential arrhythmogenesis. By eliminating many of the non-physiological consequences of spontaneous activity and unstable behavior, this tool will be useful for studying the dynamics of repolarization. It has applications for research studies looking at genetic regulation of repolarization in disease states, as well as serving as an aid in the interpretation of data from drug studies and the  
15 proarrhythmic potential of safety screens. It could also be used to modify treatment regimes based on the data obtained.

#### BRIEF DESCRIPTION OF THE FIGURES

[0012] **Figure 1: Organization of the electronic  $I_{K1}$  current expression system.**  
 $I_{K1, Synthetic}$  is generated in real time in response to the myocyte membrane potential. For all  
20 cells in this study the potentiometer was set to provide a standard outward current of 150 pA at -75 mV

[0013] **Figure 2: Spontaneous APs from iPSC-derived cardiac myocytes. (A)**  
Typical spontaneous APs, with relatively slow diastolic depolarization typical of pacemaker type cells and a very slow upstroke ( $dV/dt_{max}$ ). **(B)** Example of irregular and DAD-like  
25 behavior. Generally, spontaneous activity during current clamp showed substantial irregularity. This irregularity often had a diastolic interval which was largely flat with noisy behavior and sudden spontaneous increases in potential preceding initiation of the action potential. **(C,D)**: Examples of EAD-like behavior in the same cells. In c, there is a high voltage prolongation of the AP in a single beat around 0 mV. In d, the cell spent a prolonged  
30 time in a low voltage (-20 to -40mV) EAD-like oscillation.

[0014] **Figure 3. Pacing quiescent and spontaneously active cells. (A)** Typical resting potential of a quiescent cell without any stimulation. **(B)** Stimulating (0.5Hz for 4 beats) a quiescent cell (same as in (A)) initiates complex behavior and automaticity. **(C)**

Expanded time scale showing stimulated APs. **(D)** Spontaneous activity, highly variable in frequency, diastolic potential, overshoot and shape. **(E)** Pacing (0.25Hz for 4 beats) a spontaneously active cell (myocyte as in **(D)**), produces a more consistent after-hyperpolarization potential and changes spontaneous activity. **(F)** The after-hyperpolarization can be seen more readily on an expanded time scale.

**[0015] Figure 4: APs before and after synthetic  $I_{K1}$  expression and pacing. (A)**

Spontaneous ventricular-type cells. These cells show a rounded AP, but are difficult to distinguish because the relatively high diastolic potential reduces  $dV/dt_{max}$ . The “shoulder” of the AP is blurred by slow phase IV repolarization. **(B)** Electronic expression of  $I_{K1}$  in **(A)** restores a normal resting potential which removes  $I_{Na}$  inactivation, thereby increasing  $dV/dt_{max}$  and resetting other currents. The resetting of currents such as  $I_{to}$  produces the classical “spike and dome” morphology of ventricular cells. **(C)** Spontaneously beating atrial-like cells, which have a morphology which is difficult to distinguish from ventricular or nodal cells. **(D)** Electronic expression of  $I_{K1}$  reveals the atrial nature of the cells in panel c. As with ventricular cells, the negative resting potential restores a normal  $dV/dt$  and reveals the characteristic spike and low more triangular repolarization phase typical of atrial cells. **(E,F)** Details of repolarization of **(E)** atrial-like and **(F)** ventricular-like APs on expanded time scale. **(G)** Scatterplot of the ratio  $APD_{30}/APD_{90}$  vs.  $APD_{30}$  for cells with electronic expression of  $I_{K1}$ .

**[0016] Figure 5. Effects of calcium channel agonist BayK8644. (A)** APs stimulated

at a cycle length of 4s. Intrinsic automaticity caused some APs to be very shortened, and some stimuli occurred during repolarization from a preceding spontaneous beat. Three traces are overlaid. **(B)** Same cell, with 1  $\mu$ M BayK8644. BayK8644 caused calcium loading which terminated spontaneous activity. Stimulated APs showed abnormal behavior with severely shortened repolarization and little evidence of regenerative depolarization. Three traces are overlaid. **(C)** Same cell as **(A,B)** but with electronic expression of  $I_{K1,synthetic}$ . The AP has normal spike and dome morphology, and consistent  $APD_{90}$ . These traces were recorded prior to **B**. **(D)** Same cell as in **(A,B,C)**, with  $I_{K1,synthetic}$  and 1  $\mu$ M of BayK8644. APD is prolonged, and the arrhythmogenic phenomenon of alternans is observed. Some APs were interrupted by the subsequent stimulus. Despite the time invariant nature of  $I_{K1}$ , electronic expression of  $I_{K1}$  makes the observation of the dynamic phenomenon of alternans readily observable. In addition to QT prolongation, changes in the threshold for alternans are an important cellular index of pro-arrhythmic potential. **(E)**  $APD_{90}$  is little changed by  $I_{K1,synthetic}$  ( $n=7$ ). This may seem paradoxical, but the net effect of the addition of  $I_{K1}$  is prolongation of the dome part of

repolarization and increasing the rate of the final stages (or foot) of repolarization. The result is only a modest change in APD<sub>90</sub>. **(F)** Ratio of APD<sub>90</sub> with/without BayK8644 shows a major increase in APD with I<sub>K1,synthetic</sub> expression and an anomalous decrease in APD<sub>90</sub> without it.

5   **[0017]**       Figure 6 is a flowchart depicting a method according to an embodiment of the present invention. Figure 7 is a flowchart depicting a method according to another embodiment of the present invention. Figure 8 is a flowchart depicting a method according to another embodiment of the present invention.

#### DETAILED DESCRIPTION OF THE INVENTION

10   **[0018]**       The present disclosure provides a method of imparting physiological action potential morphology, relative to native cells, to iPSC-derived cardiac myocytes. The method comprises electronically adding an I<sub>K1</sub> component to cardiac myocytes under current clamp. We demonstrate that artificial replacement of I<sub>K1</sub> produces human iPSC-derived cardiac myocytes with APs which much more closely resemble the behavior of APs from freshly  
15   isolated human cardiac myocytes.

**[0019]**       The iPSCs can be prepared from a non-pluripotent cell, typically an adult somatic cell, or terminally differentiated cell, such as fibroblast, a hematopoietic cell, a myocyte, a neuron, an epidermal cell, or the like, by introducing into the cells or contacting the cells with reprogramming factors. Induced pluripotent stem cells can be differentiated  
20   into cardiac myocytes. For example, adult human fibroblasts can be transfected with transcription factors Oct4, Sox2, c-Myc and/or Klf4 using retroviral transduction, or with Oct4, Sox2, Nanog and Lin28 using lentivirus transduction. For a discussion of iPSCs derived cardiac myocytes, see U.S. Patent No. 8,415,155, the disclosure of which is incorporated herein by reference.

25   **[0020]**       After reprogramming factors are introduced into somatic cells, these cells may be cultured in a medium sufficient to maintain the pluripotency and the undifferentiated state. Culturing methods and media for the maintaining the iPSCs in culture are well-known.

**[0021]**       Cardiomyocyte lineage cells can be obtained from undifferentiated stem cells by culturing or differentiating in a special growth environment that enriches for cells with the  
30   desired phenotype. For example, this can be achieved by outgrowth of the desired cells, or by inhibition or killing of other cell types. Details can be found in U.S. Patent 8,415,155. This provides iPSC-derived cardiac myocytes. Additionally, iPSC-derived cardiac myocytes are also commercially available.

[0022] To record electrical activity from the iPSCs derived cardiac myocytes, whole cell voltage or current clamp techniques can be used. Generally, these techniques involve formation of a gigaseal using conventional methods.

[0023] The iPSC-derived cardiac myocytes typically generate action potentials having a “rounded” morphology with a sluggish upstroke. The high MDP reduces the  $dV/dt_{max}$  and it is difficult to distinguish ventricular from atrial cells based on the AP morphology.

[0024] With reference to Fig. 1, the present disclosure may be embodied as a system **10** for improving action potential morphology in an iPSC-derived cardiac myocyte **90**. Improved action potential morphology includes, for example, a stable physiological resting membrane potential and/or physiological action potential morphology. The system **10** comprises a patch clamp **12** as is known in the art. The patch clamp **12** is a single-electrode apparatus configured for whole-cell measurement. As such, the patch clamp **12** comprises an electrode **14**. The electrode **14** may be, for example, a glass micropipette filled with a conductive solution. Such an exemplary electrode may be configured to form a gigaohm seal with the cell to be measured. The patch clamp **12** is configured to measure a membrane voltage ( $V_m$ ) of the myocyte **90**.

[0025] The patch clamp **12** is configured to measure the action potential of the myocyte **90**. In some embodiments, the patch clamp **12** is configured as a current clamp and provides a stimulation current ( $I_{STIM}$ ) to the cell **90** by way of the electrode **14**. As such the patch clamp **12** may be configured to record the action potential of the cell **90** over time by sampling (measuring) the membrane voltage at a sampling frequency.

[0026] The system **10** comprises a generating circuit **20** in electrical communication with the electrode **14** of the patch clamp **12**. The generating circuit **20** is configured to receive an electrical signal from the electrode **14**, where the electrical signal indicates a measured membrane voltage. In this way, the generating circuit **20** receives a measured membrane voltage from the electrode **14**. The generating circuit **20** is configured to calculate a value of an inward rectifying current ( $I_{K1}$ ) based on a membrane voltage from the electrode **14**. For example, the inward rectifying current may be calculated from the membrane voltage according to the equation:

$$I_{K1} = 0.5 \left( \frac{V_m + 85}{1 + e^{0.0896(V_m + 85)}} \right) + 0.01(V_m + 85). \quad (1)$$

Other relationships between membrane voltage and inward rectifying current will be apparent in light of the present disclosure and should be included in the scope of some embodiments.

[0027] The generating circuit **20** is further configured to provide a synthetic inward rectifying current ( $I_{SYNTHETIC}$ ) to the electrode **14**. The synthetic inward rectifying current provided by the generating circuit **20** corresponds to the value of  $I_{K1}$  calculated by the generating circuit **20**. In some embodiments the synthetic inward rectifying current is equal to the calculated value of the inward rectifying current ( $I_{SYNTHETIC} = I_{K1}$ ). In some  
 5 embodiments  $I_{SYNTHETIC}$  may be different from  $I_{K1}$ . For example, the generating circuit **20** may further comprise a potentiometer **22**, and the provided current ( $I_{SYNTHETIC}$ ) may be attenuated such that  $I_{SYNTHETIC} < I_{K1}$ .

[0028] In some embodiments, the generating circuit **20** comprises a processor, and the  
 10 processor is programmed to calculate the value of the inward rectifying current. It will be apparent in light of the present disclosure that the generating circuit **20** may be implemented using a processor (e.g., with software), discrete electronic components, application-specific integrated circuits, field-programmable gate arrays, or combinations of these or other configurations.

[0029] The system **10** may comprise a summing circuit **30** configured to add the  
 15 synthetic inward rectifying current ( $I_{SYNTHETIC}$ ) to the stimulation current ( $I_{STIM}$ ) of the patch clamp **12**, producing an input current ( $I_{INPUT}$ ). In this way, the synthetic current is provided to the electrode **14** as a component of the input current.

[0030] The system **10** may be used continually such that, in an embodiment having a  
 20 digital implementation, the membrane voltage is repeatedly measured over time at a sampling frequency, and corresponding synthetic inward rectifying currents are provided to the cell **90** by way of the electrode **14**. It should be noted that a plurality of currents may be implemented as a continuous electrical current that varies over time according to the synthetic current for each time period.

[0031] The generating circuit **20** may further comprise a switch **24** for selectively  
 25 disconnecting the generating circuit **20** such that no synthetic inward rectifying current is provided to the electrode **14**.

[0032] The present disclosure may be embodied as a method **100** for producing  
 regular action potentials from an iPSC-derived myocyte (*see, e.g.*, Fig. 6). The method **100**  
 30 comprises the step of measuring **103** a membrane voltage ( $V_m$ ) of the myocyte. For example, a patch clamp may be used in a whole-cell configuration to measure **103** the membrane voltage. A value of an inward rectifying current ( $I_{K1}$ ) is calculated **106** based on the measured membrane voltage. In some embodiments, the value is calculated **106** according to equation

(1), above. In some embodiments, the value is calculated **106** using a processor. For example, a computer may be configured to receive the measured **103** membrane voltage and programmed to calculate **106** an inward rectifying current based on the received membrane voltage. Other techniques for calculating **106** the inward rectifying current will be apparent in light of the present disclosure.

[0033] The method **100** further comprises the step of applying **109** a synthetic inward rectifying current ( $I_{SYNTHETIC}$ ) to the myocyte thereby causing the myocyte to produce regular action potentials. The current may be applied **109** by way of an electrode of a patch clamp. The synthetic inward rectifying current corresponds to the calculated **106** value. In some embodiments, the applied **109** synthetic current is equal to the calculated **106** inward rectifying current. In some embodiments, the applied **109** synthetic current is less than the calculated **106** value, for example, where a potentiometer is used to attenuate the current.

[0034] In some embodiments, a patch clamp is provided **150** and configured to measure **103** a membrane voltage of the myocyte using an electrode. The patch clamp can be configured as a current clamp, and a stimulation current ( $I_{STIM}$ ) is applied to the myocyte. In such an embodiment, the synthetic inward rectifying current may be added **153** to the stimulation current to produce an input current  $I_{INPUT}$  which is applied to the myocyte by way of the electrode. As such, the synthetic current is applied **109** to the myocyte as a component of the input current.

[0035] The method may further be used to determine whether an iPSC-derived myocyte exhibits ventricular features or atrial features. For example, a method **200** may include the step of sampling **203** a membrane voltage of an iPSC-derived myocyte over a period of time and at a sampling frequency (*see, e.g.,* Fig. 7). A plurality of values of synthetic inward rectifying currents is calculated **206**, each value corresponding to a sampled **203** membrane voltage. The values may be calculated **206** according to equation (1). The method **200** comprises the step of applying **209** a synthetic inward rectifying current to the myocyte according to the calculated **206** values of inward rectifying current. The myocyte may be determined **212** to exhibit ventricular features or atrial features based on the action potentials based on the morphology of the resulting action potentials.

[0036] If the myocyte is determined **212** to exhibit ventricular features, the method **200** may include the step of modulating **215** the applied **209** synthetic inward rectifying current such that the action potential of the myocyte more closely matches the action potential of a ventricular myocyte. If the myocyte is determined **212** to exhibit

ventricular features, the method **200** may include the step of modulating **218** the applied **209** synthetic inward rectifying current such that the action potential of the myocyte more closely matches the action potential of a ventricular myocyte.

**[0037]** The present disclosure may be embodied as a method **300** for identifying test  
5 agents for drug development (*see, e.g.*, Fig. 8). The method **300** comprises the steps of providing **303** iPSC-derived cardiac myocytes and providing **306** a system for applying a synthetic inward rectifying current, such as the system **10** described above. A pattern of action potentials having improved morphology is generated **309** using the system. The myocyte may exhibit action potentials characteristic of a ventricular myocyte or an atrial  
10 myocyte. The myocyte is contacted **312** with a test agent. The test agent is identified **315** as a candidate for drug development based on characteristics of the action potentials of the myocyte. For example, the test agent may be identified **315** as a candidate if the characteristics of individual action potentials is changed such that action potentials become more or less triangular in shape, become prolonged or shortened, the regularity of the action  
15 potentials is changed, there are changes in the refractory period, or there are changes in the restitution behavior. In other examples, without limitation, the action potential is changed in its duration, degree of triangulation, refractory period, restitution steepness, threshold for development of alternans, or threshold for unstable or chaotic behavior. The method **300** may further comprise the step of contacting **318** the iPSC-derived myocyte to a second test agent  
20 (after contacting **312** the myocyte with the (first) test agent as described above). The second test agent may be selected to restore the changed characteristics of the myocyte to the characteristics prior to contact **312** with the first test agent.

**[0038]** The described methods have many applications. For example, a method will allow comprehensive analysis of human electrophysiological system. iPSC cardiocytes can  
25 be used in the interpretation of channelopathies, drug screening, and the assessment of potential arrhythmogenesis. By eliminating many of the potentially confounding non-physiological consequences of spontaneous activity and unstable behavior of human iPSC-derived cardiac myocytes, this method provides a useful tool for studying the dynamics of repolarization. It has applications for research studies looking at genetic regulation of  
30 repolarization in disease states, as well as serving as an aid in the interpretation of data from drug studies and the proarrhythmic potential of safety screens.

**[0039]** The system and methods described in the present disclosure can be used for iPSCs from humans or from other animals, including, for example, mammals.

**[0040]** The following example is provided to further illustrate the invention.

## EXAMPLE

### Methods

#### [0041] *Cell Preparation*

5 [0042] Commercially-available iPSC-derived cardiac myocytes (iCell Dynamics, WI), were prepared according to manufacturer's instructions. Briefly, iCell cardiac myocytes (cryopreserved single-cell suspensions in 1 ml cryovials) were stored in liquid nitrogen. Vials were thawed in a waterbath (37°C). Cells were washed then rinsed with 1 ml of RT "iCell Cardiomyocyte Plating Medium." An additional 8 ml of plating medium was added and cells  
10 mixed with solution by gentle inversion. Cardiocytes were seeded on 15 mm cover-slips coated with 0.01% (w/v) gelatin solution in 12-well plates. Cardiocytes were seeded (20,000-40,000/dish) in 2ml of RT plating medium, permitting single cell culture, and incubated for 2+ days at 37°C, 7%CO<sub>2</sub>. Non adherent cells were removed by rinsing with "iCell Cardiomyocyte Maintenance Medium." A further 2ml of maintenance medium was added.  
15 Maintenance medium was changed every 2 days, and cells were used within 1 week.

#### [0043] *Electrophysiology*

[0044] iPSC-derived cardiocytes were voltage clamped. Currents were recorded using whole-cell clamp at RT. Cells in the recording chamber (300µl) were continuously perfused  
20 with Tyrode. Pipettes fabricated from borosilicate glass (Flaming/Brown horizontal micropipette puller) and tips heat-polished (Narishige microforge). Pipettes contained (mM): 10 NaCl, 125 KCl, 1 MgSO<sub>4</sub>, 5 EGTA, 5 ATP(Mg salt), 5 Tris-creatinephosphate, 0.3 GTP, 10 HEPES, pH 7.2. Extracellular solution (mM): 150 NaCl, 5.4 KCl, 0.5 Na<sub>2</sub>HPO<sub>4</sub>, 1 MgCl<sub>2</sub>, 2 CaCl<sub>2</sub>, 10 glucose, 10 HEPES, pH7.4. After establishing a seal (1-10Gohm), suction and  
25 voltage pulses were used to rupture the patch. Currents were recorded (Axopatch-1D, Molecular Dynamics, CA) and interfaced to a Pentium 4 computer via a Digidata 1322A (Molecular Dynamics, CA). PClamp 9.0 (Molecular Dynamics, CA) was used to control protocols and data acquisition.

#### [0045] *In Silico Interface*

30 [0046] We used an Analog and Digital I/O board PCIe-DAS1602/16 (Measurement Computing Corporation) installed on a on a Dell Precision T7500 workstation with 2 Intel Xeon CPU E5520 to transfer the V<sub>m</sub> signal to the signal of I<sub>K1</sub> current. Source code is in the supplement.

[0047] *Data Analysis.*

[0048] Analysis was performed using pClamp 9 (Molecular Dynamics). Action potential duration (APD) was computed for 50% (APD<sub>50</sub>) and 90% (APD<sub>90</sub>) of repolarization in ms. Maximum rate of rise of the AP upstroke was determined ( $dV_m/dt_{max}$ ) in mV/ms. Resting membrane potential was recorded prior to each AP.

For spontaneous APs, the diastolic characteristics were reported for the period of time between complete AP repolarization and the beginning of depolarization in the subsequent AP. Duration, and minimum/mean potentials were computed for the diastolic period.

10 [0049] *Statistical Analysis*

[0050] Results are reported as mean $\pm$ s.e.m. Differences were analyzed by t-test or ANOVA as appropriate and  $p < 0.05$  considered statistically significant.

## Results

[0051] In order to overcome the lack of  $I_{K1}$  in h-iPSC-derived cardiac myocytes, we developed a variation of the dynamic clamp approach to inject a synthetic version of  $I_{K1}$  (Figure 1). The center of the system is the isolated h-iPSC-derived cardiac myocyte under whole cell voltage or current clamp. Whole cell clamp is accomplished with a gigaseal under conventional current clamp mode. The membrane voltage of the cardiac myocyte is output from the amplifier and fed into a computer via a digitizer which calculates the inward rectifier current for the membrane potential, according to the equation shown in Figure 1. The calculated value of  $I_{K1, Synthetic}$  is passed through a potentiometer (used to adjust amplitude without sacrificing digital resolution) to scale  $I_{K1}$  and then summed with the standard stimulation input from pClamp. A switch allows rapid changes between conventional current clamp and summed input. When the summed input is switched on, the myocyte receives the sum of two inputs: the standard AP stimulus current plus  $I_{K1, Synthetic}$  from the real-time current simulator. The standard voltage clamp electronics can be driven via the summing circuit without modification of conventional voltage clamp and data recording with only minor software selection changes of input/output channels. The result is a cell that behaves as though it were expressing a native  $I_{K1}$  that had instantaneously been “transfected” into the cell.

[0052] *Properties and Variability of iPSC-derived cardiac myocyte APs.*

[0053] The majority of reports from iPSC-derived cardiocytes indicate they are generally spontaneously active, with a few quiescent cells, which is consistent with our findings. Consequently, most AP studies to date on iPSC-derived cardiocytes have reported

parameters based on analysis of spontaneous AP behavior. Given the high degree of cell to cell variability, large numbers of cells have been used to produce statistically significant results in changes in AP parameters. High throughput screening methods have even been employed to examine cellular properties of commercially-available iPSC-derived cardiac myocytes. This reflects at least in part, variability between cell types, i.e., ventricular, atrial, and nodal cells. However, some of this variability also results from the consequences of recording from spontaneously active cells. The average maximum diastolic potential for spontaneously active cells was  $-59 \pm 2 \text{ mV}$  ( $n=33$ ). Spontaneous activity was usually irregular and occurred over a broad range of diastolic intervals from a few hundred ms to 8 s (average:  $2068 \pm 415 \text{ ms}$ ,  $n=25$ ) and included a high beat to beat variability for any given single cell (see **Fig 2**). Not surprisingly, variability in heart rate was accompanied by high variability in AP duration and shape. As shown in **Table 1**, the spontaneous APs tended to have a relatively short amplitude and a very slow upstroke (**Fig 2A,B**). This irregular behavior was often accompanied by spontaneous events resembling EADs and low and high threshold DADs (**Fig 2C,D**).

**[0054]** One strategy to reduce variability in electrical activity is through paced external stimulation. This can regularize slow spontaneous activity and potentially induce regenerative electrical behavior in quiescent cells. Not all iPSC-derived cardiac myocytes were spontaneously active (out of 33 cells tested, 8 were not spontaneously active). However, even cells which are not spontaneously active do not exhibit normal AP characteristics associated with human adult myocardial cells from the working myocardium. The lack of spontaneous contractile function can be due to a stable normal resting potential (around  $-85 \text{ mV}$ ) or may be due to a relatively depolarized potential, which results in an abnormal resting state. The majority of the non-spontaneous cells did not have a hyperpolarized resting membrane potential typical of working myocardium, but were in an abnormally depolarized state. Three out of 8 non-spontaneously active cells had a resting potential above  $-60 \text{ mV}$  and only 1 had a resting membrane potential below  $-70 \text{ mV}$ . APs and contractile activity could be initiated in quiescent cells by pacing with a stimulus current. The elicited APs resembled those from spontaneously active cells. In some quiescent cells, pacing could elicit a following period of spontaneous behavior (**Fig 3A-C**), indicating that delayed rectifier currents produce the diastolic potential in human iPSC-derived cardiac myocytes. Pacing of spontaneously active tissue altered beat to beat variability (**Fig 3 D-F**) and improved some other parameters, particularly  $dV/dt_{\text{max}}$  (**Table 1**).

[0055] Regardless of whether APs arose from spontaneous activity or stimulated pacing, they had prolonged repolarization ( $APD_{90}$  was  $955 \pm 103$  ms ( $n=38$ ) and  $967 \pm 141$  ms ( $n=30$ ) respectively), diminished amplitude and a reduced maximum upstroke ( $dV/dt_{max}$ ) compared with APs from freshly isolated human atrial or ventricular cardiocytes. They also lacked the spike and dome morphology typical of ventricular and atrial myocytes. This was somewhat surprising since the cells uniformly express robust  $I_{to}$  that gives rise to this AP morphology. However, the  $Kv4.2/4.3$  mediated channels that give rise to this current have substantial closed state inactivation and may be largely inactivated by the depolarized state of the spontaneous and stimulated APs. Similarly,  $I_{Na}$  will also be inactivated at these potentials and cannot contribute fully to the normal rapid upstroke or the spike and dome behavior. The lack of these behaviors will lead to non-physiological function and greatly diminish the predictive value of any screens for pro-arrhythmic or anti-arrhythmic behavior of mutant channels or candidate drugs. However, when we injected  $I_{K1, Synthetic}$ , a stable resting membrane potential was established and there was a marked change in AP shape and regularity (**Fig 4**). Spike and dome morphology is clearly seen, and the difference between ventricular-like and atrial-like myocytes is readily apparent, suggesting that this method allows efficient discrimination between cell types. **Fig 4G** shows that cells fall into two distinct populations, based on action potential shape and duration. The variance between APs both from beat to beat and from cell to cell was reduced and the rapid upstroke ( $dV/dt_{max}$ ) of the rising phase of the AP was prominent and consistent (**Table 1**). Clearly, reconstitution of  $I_{K1}$  restores many important properties to the h-iPSC-derived cardiac myocytes.

[0056] *Pharmacological Manipulation of Repolarization*

[0057] Reconstitution of the inward rectifier normalizes many of the properties of h-iPSC-derived cardiocyte APs into a form more clearly resembling working cardiac myocytes.

Furthermore, it relieves many of the uncontrolled properties associated with free-running or stimulated APs. The functional utility of this property can be seen by assessing the calcium channel agonist BayK-8644, which increases the amplitude and disrupts inactivation of the L-type calcium channel, resulting in a profound increase in APD. We examined the effect of 1  $\mu$ M BayK-8644 on paced h-iPSC-derived cardiac myocytes with and without electronic  $I_{K1}$  (**Fig 5**). Application of 1  $\mu$ M BayK-8644 had profound effects in both cases. However, AP prolongation by BayK-8644 was not uniformly observed in paced cells. Rather, a termination of spontaneous full scale AP activity occurred due to membrane depolarization after stimulation of a very foreshortened AP. Increased low level fibrillatory-like electrical oscillations were also observed. In contrast, when  $I_{K1}$  was electronically inserted, cellular

activity was regularized, variability was reduced and the AP was more physiological, and the fibrillatory-like electrical behavior was suppressed. Application of BayK-8644 then resulted in clear AP prolongation. At shorted pacing intervals, some APs were prolonged beyond the pacing rate (**Fig 5D**). When viewed using paired data, this qualitative difference in predicted  
5 arrhythmogenic potential is clearly evident (**Fig 5E**) and emphasizes the ability of electronic  $I_{K1}$  expression to improve the predictive ability of the human iPSC-derived cardiac myocyte system as a tool for drug safety and the study of the molecular basis of arrhythmias.

**[0058]** Although the present invention has been described with respect to one or more particular embodiments, it will be understood that other embodiments of the present  
10 invention may be made without departing from the spirit and scope of the present invention. Hence, the present invention is deemed limited only by the appended claims and the reasonable interpretation thereof.

We claim:

1. A system for producing improved action potential morphology from an iPSC-derived cardiac myocyte, the system comprising:

a patch clamp comprising an electrode configured to measure a membrane voltage ( $V_m$ ) of the myocyte;

a generating circuit in electronic communication with the electrode, the generating circuit configured to:

calculate a value of an inward rectifying current ( $I_{K1}$ ) based on the membrane voltage;

provide to the electrode, a synthetic inward rectifying current according to the

determined inward rectifying current, to produce action potentials having improved morphology from the myocyte.

2. The system of claim 1, wherein the value of the synthetic inward rectifying current is determined according to the equation:

$$I_{K1} = 0.5 \left( \frac{V_m + 85}{1 + e^{0.0896(V_m + 85)}} \right) + 0.01(V_m + 85).$$

3. The system of claim 1, wherein the generating circuit is configured to repeatedly calculate a value and provide a current at a sampling frequency.

4. The system of claim 1, wherein the generating circuit comprises a processor programmed to:

calculate a value of a synthetic inward rectifying current based on a membrane voltage ( $I_{K1}$ ) based on the membrane voltage; and

provide to the electrode, a synthetic inward rectifying current according to the determined value to produce action potentials having improved morphology from the myocyte.

5. The system of claim 1, wherein the system is configured to measure the action potential of the myocyte.

6. The system of claim 5, wherein the patch clamp is configured as a current clamp.

7. The system of claim 1, further comprising a summing circuit to add the synthetic inward rectifying current to a stimulation current of the patch clamp to produce an input current, and

the synthetic inward rectifying circuit is provided to the electrode as a component of the input current.

8. The system of claim 7, wherein the generating circuit further comprises a potentiometer for attenuating the synthetic inward rectifying current before the summing circuit.

5 9. A method for producing improved action potential morphology from an iPSC-derived cardiac myocyte, the method comprising the steps of:

measuring a membrane voltage ( $V_m$ ) of the myocyte;

calculating a value of an inward rectifying current ( $I_{K1}$ ) based on the membrane voltage of the myocyte; and

10 applying a synthetic inward rectifying current to the myocyte according to the calculated value of the inward rectifying current, to produce action potentials having improved morphology.

10. The method of claim 9, wherein the value of the inward rectifying current is determined according to the equation:

$$I_{K1} = 0.5 \left( \frac{V_m + 85}{1 + e^{0.0896(V_m + 85)}} \right) + 0.01(V_m + 85).$$

15 11. The method of claim 9, further comprising the step of recording the membrane voltage over time as an action potential of the myocyte.

12. A method for determining whether an iPSC-derived cardiac myocyte exhibits ventricular features or atrial features, the method comprising the steps of:

20 sampling a membrane voltage ( $V_m$ ) of the myocyte over a period of time at a sampling frequency;

calculating a plurality of values of synthetic inward rectifying currents ( $I_{K1}$ ), each value based on a membrane voltage sample, the values calculated according to the equation

$$I_{K1} = 0.5 \left( \frac{V_m + 85}{1 + e^{0.0896(V_m + 85)}} \right) + 0.01(V_m + 85);$$

applying synthetic inward rectifying currents to the myocyte according to the calculated values to produce action potentials having improved morphology from the myocyte;

25 and

determining from the produced regular action potentials whether the myocyte is a ventricular or atrial myocyte.

13. The method of claim 12, further comprising the step of modulating the applied synthetic inward rectifying current such that the action potential of the myocyte more closely matches  
5 the action potential of a ventricular myocyte.

14. The method of claim 12, further comprising the step of modulating the applied synthetic inward rectifying current such that the action potential of the myocyte more closely matches the action potential of a atrial myocyte.

15. A method for identifying test agents for drug development comprising:

- 10 a) providing iPSC-derived cardiac myocytes;
- b) providing the system of claim 1;
- c) generating a pattern of action potentials having improved morphology in the iPSC-derived myocyte using the system in b);
- d) contacting the iPSC-derived cardiac myocyte with a test agent; and
- 15 e) identifying the test agent as a candidate for drug development if the characteristics of the individual action potential or the regularity of action potentials are changed.

16. The method of claim 15, wherein the iPSC-derived derived cardiac myocyte exhibits action potentials characteristic of the action potential of an atrial myocyte.

20 17. The method of claim 15, wherein the iPSC-derived derived cardiac myocyte exhibits action potentials characteristic of the action potential of a ventricular myocyte.

18. The method of claim 15, wherein the individual action potential is changed in its duration, degree of triangulation, refractory period, restitution steepness, threshold for development of alternans, or threshold for unstable or chaotic behavior.

25 19. The method of claim 15, wherein the test agent in step d) is a first test agent and further comprising the step of contacting the iPSC-derived myocyte from step e) to a second test

agent that can restore the changed characteristics to the characteristics prior to contact with the first test agent.

20. The method of claim 15, wherein the provided iPSC-derived cardiac myocytes are human.

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