



A Hybrid Model for Safety Pharmacology on an Automated Patch Clamp Platform: Using Dynamic Clamp to Join iPSC-Derived Cardiomyocytes and Simulations of I_{K1} Ion Channels in Real-Time

OPEN ACCESS

Edited by:

Blanca Rodriguez,
University of Oxford, United Kingdom

Reviewed by:

David Christini,
Weill Cornell Medical College, Cornell
University, United States
Antonio Zaza,
Università degli studi di Milano
Bicocca, Italy
Patrick M. McDonough,
Vala Sciences, United States

*Correspondence:

Teun P. de Boer
t.p.deboer@umcutrecht.nl

[†]These authors have contributed
equally to this work.

Specialty section:

This article was submitted to
Cardiac Electrophysiology,
a section of the journal
Frontiers in Physiology

Received: 29 June 2017

Accepted: 12 December 2017

Published: 19 January 2018

Citation:

Goversen B, Becker N,
Stoelzle-Feix S, Obergrussberger A,
Vos MA, van Veen TAB, Fertig N and
de Boer TP (2018) A Hybrid Model for
Safety Pharmacology on an
Automated Patch Clamp Platform:
Using Dynamic Clamp to Join
iPSC-Derived Cardiomyocytes and
Simulations of I_{K1} Ion Channels in
Real-Time. *Front. Physiol.* 8:1094.
doi: 10.3389/fphys.2017.01094

Birgit Goversen^{1†}, Nadine Becker^{2†}, Sonja Stoelzle-Feix², Alison Obergrussberger²,
Marc A. Vos¹, Toon A. B. van Veen¹, Niels Fertig² and Teun P. de Boer^{1*}

¹ Division of Heart & Lungs, Department of Medical Physiology, University Medical Center Utrecht, Utrecht, Netherlands,

² Nanion Technologies, Munich, Germany

An important aspect of the Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) proposal is the use of human stem cell-derived cardiomyocytes and the confirmation of their predictive power in drug safety assays. The benefits of this cell source are clear; drugs can be tested *in vitro* on human cardiomyocytes, with patient-specific genotypes if needed, and differentiation efficiencies are generally excellent, resulting in a virtually limitless supply of cardiomyocytes. There are, however, several challenges that will have to be surmounted before successful establishment of hSC-CMs as an all-round predictive model for drug safety assays. An important factor is the relative electrophysiological immaturity of hSC-CMs, which limits arrhythmic responses to unsafe drugs that are pro-arrhythmic in humans. Potentially, immaturity may be improved functionally by creation of hybrid models, in which the dynamic clamp technique joins simulations of lacking cardiac ion channels (e.g., I_{K1}) with hSC-CMs in real-time during patch clamp experiments. This approach has been used successfully in manual patch clamp experiments, but throughput is low. In this study, we combined dynamic clamp with automated patch clamp of iPSC-CMs in current clamp mode, and demonstrate that I_{K1} conductance can be added to iPSC-CMs on an automated patch clamp platform, resulting in an improved electrophysiological maturity.

Keywords: automated patch clamp electrophysiology, cardiomyocyte, stem cell, dynamic clamp, inward rectifying potassium ion channels, safety pharmacology

INTRODUCTION

The Comprehensive *In Vitro* Proarrhythmia Assay (CiPA) initiative aims to find new means of predicting the proarrhythmic risk of newly developed drugs (Gintant et al., 2016), which do not rely exclusively on hERG block, and not on QT prolongation at all. Key aspects are to include results of computer simulations of drug effects on heart rhythm and *in vitro* assays using human stem cell-derived cardiomyocytes (hSC-CMs).

Characterization of the hSC-CM electrophysiological phenotype has so far shown that these CM express most, but not all cardiac ion channels, which has implications for their use in safety pharmacological assays (Jonsson et al., 2012; van den Heuvel et al., 2014). Importantly, the inward rectifying potassium current, I_{K1} (de Boer et al., 2010), that is highly expressed in adult CMs is not, or hardly, expressed by hSC-CMs (Doss et al., 2012; Goversen et al., 2017), while the pacemaker current I_f is expressed consistently. As a result, hSC-CMs display a pacemaker-like phenotype, with a depolarized and unstable resting membrane potential, resulting in spontaneous triggering of action potentials. This is an important issue, as the action potential waveform affects the activity and availability of many cardiac ion channels, as these are voltage-sensitive and rely on a negative resting membrane potential between beats to recover from inactivation after an action potential. For example, in hSC-CMs, sodium channel availability during the action potential is minimal, even though the channels are expressed in sufficient levels. As a consequence, we found in a previous study that, except for drugs blocking the hERG channel, none of the tested drugs that are known to be proarrhythmic in adult cardiomyocytes triggered arrhythmias (early-after-depolarizations) in hSC-CMs (Jonsson et al., 2012). More recent studies have found that iPSC-CMs are not sufficiently mature to detect risks associated with inhibition of the late sodium current (Blinova et al., 2017), or peak sodium current (Ando et al., 2017). Interestingly, adenovirus mediated overexpression of I_{K1} channels in iPSC-CM was demonstrated to improve drug responses (Li et al., 2017).

Several approaches are being adopted to improve the electrophysiological phenotype, for instance overexpression of the I_{K1} channel, which has shown promising results (Vaidyanathan et al., 2016). Another, more controllable approach is to use dynamic clamp to add simulated I_{K1} channels to hSC-CMs (Wilders, 2006; Ortega et al., 2017). The essence of dynamic clamp is that a hybrid model is created by connecting a real cell with a computer simulation of (parts of) a cell. For this to work, one needs a computer simulation that is running in real-time—simultaneously with the experiment on the real cell—so there is an instantaneous interaction between the real cell and the simulation. This works well, and has been described by several groups that applied it in manual patch clamp experiments (Bett et al., 2013; Meijer van Putten et al., 2015). Addition of simulated or overexpressed I_{K1} channels results in a stable, more negative resting membrane potential, increased action potential amplitude, and upstroke velocity, thereby bringing the action potential waveform much closer to that of adult human ventricular cardiomyocytes. The expectation is that this approach will result in a more reliable prediction of drug effects, but this hypothesis has yet to be studied systematically (Goversen et al., 2017).

When considering the use of dynamic clamp in safety pharmacology, the low throughput and complex nature of manual patch clamp in combination with dynamic clamp is problematic. Additionally, as noted in the literature (Meijer van Putten et al., 2015), current implementations require the

simultaneous use of two computers that both require user interaction during the experiment, which is not very practical. In this study, we have developed a remote-controlled dynamic clamp system with the purpose to couple and integrate it with automated patch clamp devices, in order to increase throughput and develop new predictive assays using hSC-CMs that are in line with the aims of the CiPA initiative. In this study, we demonstrate its application by creating hybrid human cardiomyocyte models by the addition of virtual I_{K1} current to single, suspended human iPSC-CMs, and recording action potentials on an automated patch clamp device.

MATERIALS AND METHODS

iPSC-CM Culture and Dissociation

Differentiated iPSC-derived cardiomyocytes (Cor.4U, kindly provided by Axiogenesis AG, Germany and Cellartis Cardiomyocytes, kindly provided by Takara Bio Europe AB, Sweden) were cultured according to the suppliers' instructions. The cells were dissociated by incubating them for 15–30 min in TrypLE (Gibco) until detached from the surface of the culture flask, and then kept at 4°C for 30 min before pipetting them to individualize the cells.

Automated Patch Clamp Electrophysiology

Recordings from single iPSC-CMs were done using a Nanion Patchliner automated patch clamp device at 20°C and standard medium resistance NPC-16 chips. After catching an iPSC-CM, obtaining a gigaseal and breaking into whole cell configuration, several experiments were performed.

From a holding potential of -100 mV, current-voltage recordings were made using voltage steps from -80 to 40 mV for 20 ms increasing in 10 mV steps at 2 s intervals (sodium ion currents), and from -40 to 40 mV for 200 ms increasing in 10 mV steps at 5 s intervals (calcium ion currents), with a 100 ms pre-pulse to -40 mV to inactivate sodium ion currents. I_{K1} current were recorded from a holding potential of -40 mV, with voltage steps from -120 to $+30$ mV for 1,200 ms increasing in 10 mV steps. I_{K1} was blocked by adding $10 \mu\text{M}$ Ba^{2+} , from these recordings we calculated the Ba^{2+} -sensitive steady-state current and report these in I-V diagrams.

Next, the recording mode was switched to current clamp and the effect of adding simulated I_{K1} conductance to the iPSC-CM using dynamic clamp was tested. To this end, a current stimulus was optimized for each cell individually to reliably induce action potentials (APs) at a rate of 0.5 Hz. The stimulus was 1 ms long and ranged from 0.6 to 3 nA. The same stimulus was used to trigger APs while exposing the cells to increasing simulated I_{K1} conductance. If the conductance was set too low, no effect on the AP was detectable, if set too high, no AP could be induced. The simulated I_{K1} conductance range varied considerably between individual cells, 200 to 2,000 pS/pF were used across cells.

All experiments were done using extracellular solution containing (in mmol/L) 140 NaCl, 10 HEPES, 5 Glucose, 4 KCl, 2 CaCl_2 , 1 MgCl_2 , pH 7.4 (NaOH), 298 mOsm, and intracellular solution containing 110 KCl, 10 KCl, 10 NaCl, 10 HEPES,

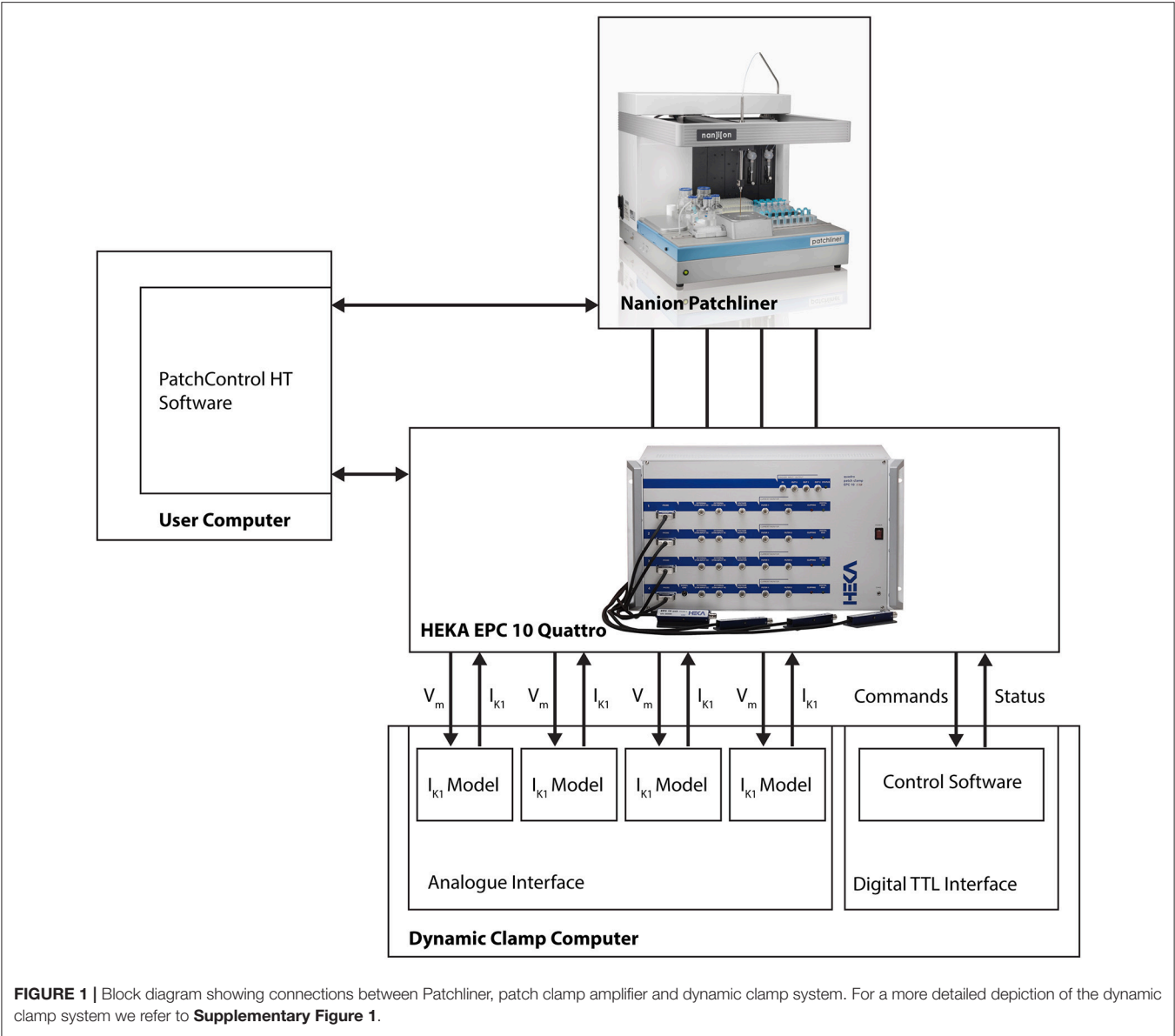


TABLE 1 | iPSC-CMs can be captured efficiently using automated patch clamp and display Na^+ and Ca^{2+} currents.

Capture rate (%)	I_{Na} larger than 50 pA (%)	$I_{\text{Ca,L}}$ larger than 50 pA (%)
58 (28/48)	71 (20/28)	68 (19/28)

Six experiments were performed using a total of 3 chips, therefore 48 potential sites on the chip were available and 28 Cellartis iPSC-CMs were captured (with seal resistance > 150 M Ω) resulting in a success rate of 58% for capture.

10 EGTA, pH7.2 (KOH), 285 mOsm. In some experiments, potassium salts were replaced by cesium salts to expose calcium ion currents otherwise obscured by potassium ion currents. Since potassium currents turned out to be negligible, no difference referring to (real) potassium conductance was observed between those recordings.

TABLE 2 | Average electrophysiological parameters iPSC-CMs.

R_{Seal} (M Ω)	C_M (pF)	R_s (M Ω)	I_{Na} at -30 mV (nA)	$I_{\text{Ca,L}}$ at 10 mV (pA)
976 \pm 144 (28)	37 \pm 6 (28)	6.0 \pm 0.9 (28)	-5.4 \pm 1.5 (7)	-157 \pm 24 (18)

Shown are values for seal resistance (R_{Seal}), cell capacitance (C_M) and series resistance (R_s) for Cellartis Cardiomyocytes captured with seal resistance > 150 M Ω . Na^+ current at -30 mV and Ca^{2+} current at 10 mV is also shown. Number of cells shown in brackets. Note that the average current is taken from the IV curves and not all cells which had a detectable Na^+ current were used for the IV analysis.

Dynamic Clamp System

Experiments were done using a dynamic clamp system developed in house at UMC Utrecht, Utrecht, The Netherlands. The system runs on the Labview RT operating system, and simulates I_{K1} current in response to membrane potential measured

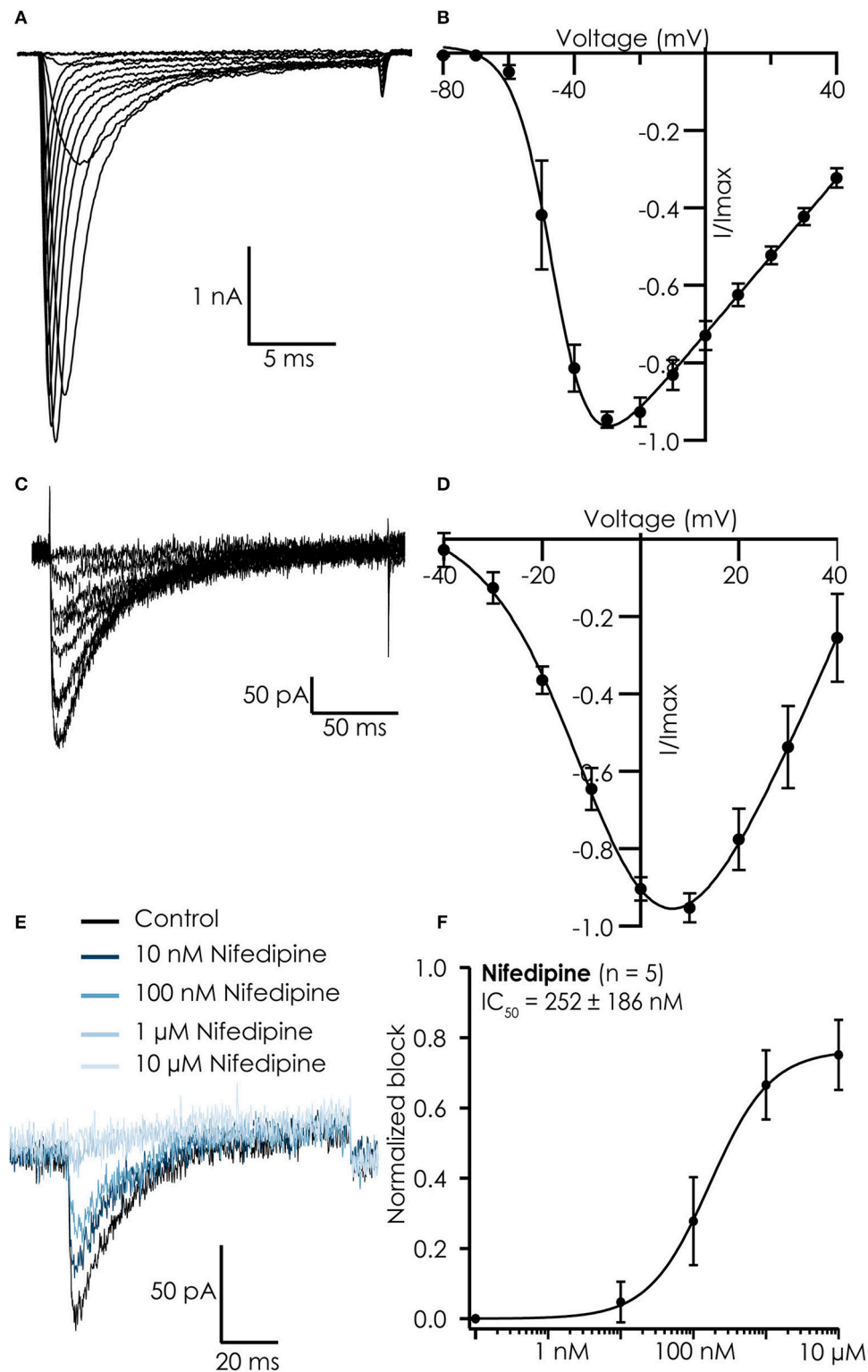


FIGURE 2 | Typical recordings from hiPSC Cellartis Cardiomyocytes recorded on the Patchliner. **(A)** Na^+ currents in response to increasing voltage steps. **(B)** Corresponding current-voltage plot for an average of 7 cells. A Boltzmann fit revealed a V_{half} of activation of -46 mV . **(C)** Ca^{2+} currents in response to increasing voltage steps. **(D)** Corresponding current-voltage plot for an average of 18 cells. A Boltzmann fit revealed a V_{half} of activation of -5.8 mV . **(E)** Raw traces of Ca^{2+} current in control conditions (black) and after inhibition by increasing concentrations of nifedipine (blue). **(F)** The concentration response curve (normalized to maximum block) for nifedipine for an average of 5 cells. The average concentration response curve was fitted with a standard Hill-equation which revealed an $\text{IC}_{50} = 252 \pm 186 \text{ nM}$ ($n = 5$).

from the iPSC-CM. Real-time simulation of the I_{K1} current was done using the model by Ishihara et al. (2009), and simulations were done at 20 kHz, on four independent channels, allowing simultaneous dynamic clamp of four iPSC-CMs. We have included as supplementary information the used I_{K1} model (**Supplementary Data Sheet 1**), a diagram explaining our dynamic clamp implementation (**Supplementary Figure 1**) and a flowchart describing the various steps taken during the dynamic clamp experiments (**Supplementary Figure 2**).

The dynamic clamp system is coupled to the HEKA EPC-10 Quattro amplifier that is part of the Patchliner setup (see **Figure 1**). The connections couple the membrane potential of each iPSC-CM with each I_{K1} simulation channel and return the computed I_{K1} current to the iPSC-CM via the external stimulus input of the EPC-10 amplifier. Remote control is achieved via additional couplings that allow bi-directional communication between the dynamic clamp system and the Patchliner setup. For this we use the standard digital input and output channels of the HEKA EPC-10 Quattro amplifier. These are used to continuously read dynamic clamp system status and set model parameters (e.g., I_{K1} conductance, membrane capacitance or external K^+ ion concentration) when needed. Converting a model parameter to a digital command was done using macros in Patchmaster.

Because of this tight integration with the APC and its software, no direct user interaction with the dynamic clamp setup is necessary. The dynamic clamp system is completely

controlled from within the HEKA PatchMaster or the Patchliner PatchControlHT software used to run experiments, and can be set automatically using the programming features in these software programs (i.e., Protocol Editor in PatchMaster or Tree Editor in PatchControlHT).

Statistics

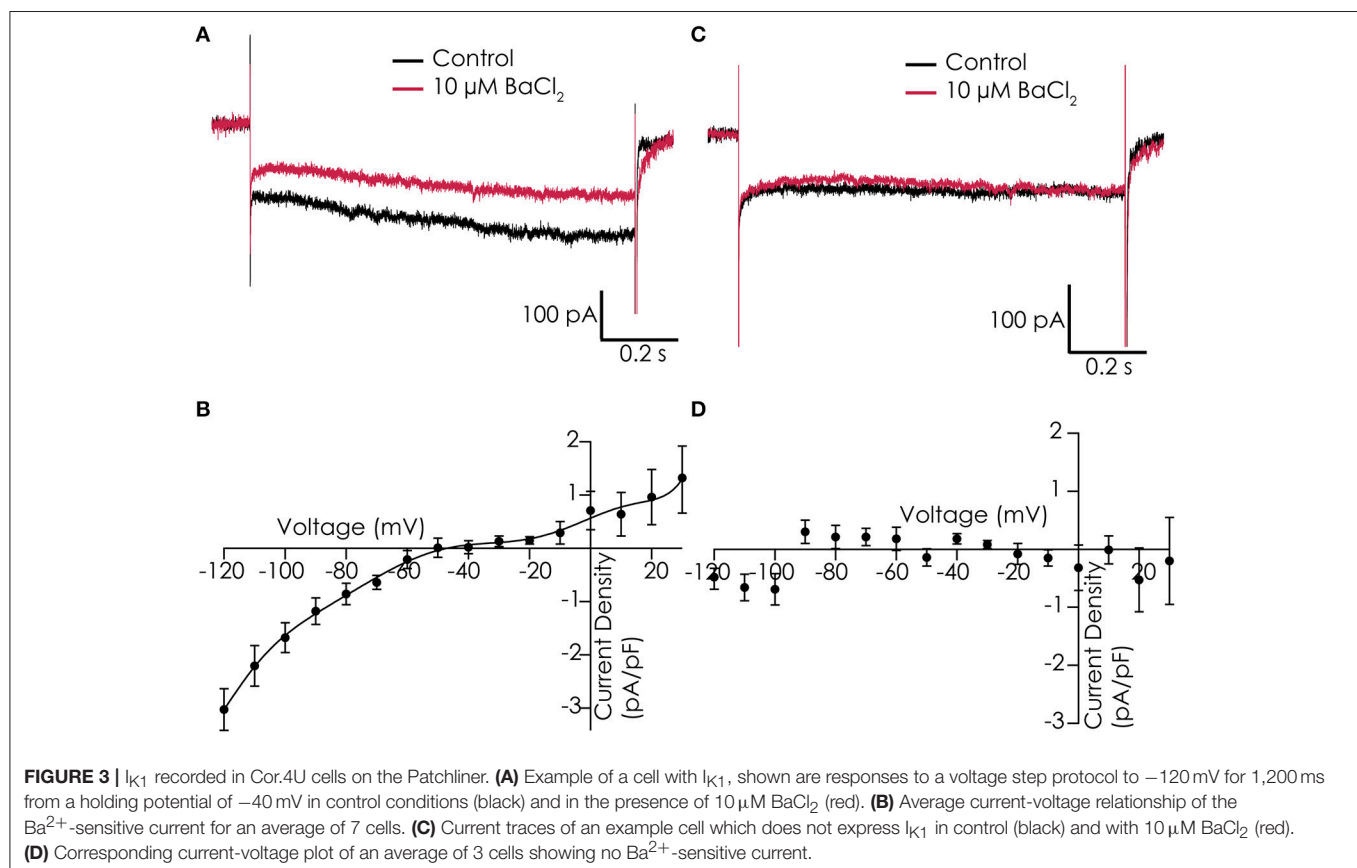
All results are presented as mean \pm standard error of the mean (s.e.m.). Differences in mean outcomes were tested using a One-way ANOVA followed by Tukey's multiple comparisons test, p -values smaller than 0.05 were considered significant.

RESULTS

Na^+ and Ca^{2+} Currents in iPSC-CMs

Single iPSC-CMs dissociated from iPSC-CM cultures were loaded in the automated patch clamp device and studied in voltage clamp mode to record Na^+ and Ca^{2+} currents. Capture of the iPSC-CM in the patch clamp chip was efficient, with appropriate seals in 58% of captured cells (see **Table 1**). After obtaining whole cell configuration, Na^+ and Ca^{2+} currents could be recorded in $\sim 70\%$ of iPSC-CMs (see **Table 2**).

Current-voltage relations of both currents were similar to those reported for iPSC-CMs in manual patch clamp experiments (Ma et al., 2011), showing maximal peak current amplitudes at -30 mV for Na^+ currents (**Figures 2A,B**) and 10 mV for Ca^{2+}



currents (**Figures 2C,D**). The Ca^{2+} currents could be blocked by nifedipine with an IC_{50} of 252 ± 186 nM, confirming we recorded current conducted by L-type Ca^{2+} channels (**Figures 2E,F**).

I_{K1} Currents in iPSC-CMs

Functionality of I_{K1} currents was studied in single dissociated iPSC-CMs that were loaded in the automated patch clamp device. Voltage clamp studies in control solution, and in presence of $10 \mu\text{M}$ Ba^{2+} to block I_{K1} currents, showed that we could record Ba^{2+} -sensitive currents with some resemblance of I_{K1} in 7 out of 12 cells. Examples of cells showing Ba^{2+} -sensitive and insensitive currents can be found in **Figures 3A,C**, respectively. However, current densities were low, and both the reversal potential (which was less negative than expected) and the rectification of the currents were not as observed in adult cardiomyocytes (**Figures 3B,D**).

Single Suspended iPSC-CMs Have Depolarized Membrane Potentials

While the voltage clamp experiments demonstrated that the cardiac Na^{+} and Ca^{2+} channels remain functional after dissociation, recording action potentials from iPSC-CMs in suspension was challenging. After switching to current clamp mode, the resting membrane potential of iPSC-CMs was typically between 0 and -15 mV. A negative resting membrane potential close to the reversal potential of K^{+} could be achieved by injecting a small constant, negative holding current. After that, action potentials could be triggered with a brief depolarizing stimulus. However, the action potentials were very short and

had a monotonic repolarization without a plateau phase, which does not match well with adult human cardiac action potentials (see **Figure 4**, black trace labeled “ -180 pA”). The I_{K1} channels expressed in fully differentiated human cardiomyocytes hyperpolarize the resting membrane potential of these cells, bringing it close to E_K . An important difference with a constant hyperpolarizing current is that I_{K1} channels close upon strong depolarization, allowing development of the plateau phase of the cardiac action potential. In order to improve our methods, and obtain better action potential recordings from suspended single iPSC-CMs, we implemented the dynamic clamp technique on our automated patch clamp device in order to inject simulated I_{K1} .

Hybrid Models of Suspended iPSC-CMs and Simulated I_{K1} Channels Produce Longer Action Potentials with a Plateau Phase

Our dynamic clamp implementation is in many ways similar to other published implementations (Bett et al., 2013; Ortega et al., 2014; Meijer van Putten et al., 2015), but differs in two aspects: the functional integration with existing patch clamp control software,

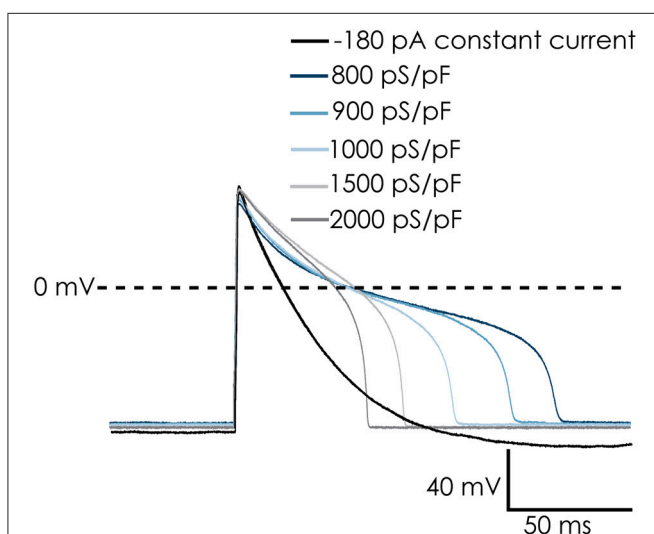


FIGURE 4 | Dynamic clamp used to simulate I_{K1} conductance on action potentials (APs) of Cellartis Cardiomyocytes. The simulated I_{K1} conductance could replace injected current to achieve a native resting membrane potential (RMP) of approximately -94 mV. The cells repolarized faster and the AP duration decreased with increasing I_{K1} conductance. Note: in order to keep a RMP of -94 mV in the absence of simulated I_{K1} conductance, -180 pA of holding current was injected. This was removed upon addition of I_{K1} and RMP remains at -94 mV.

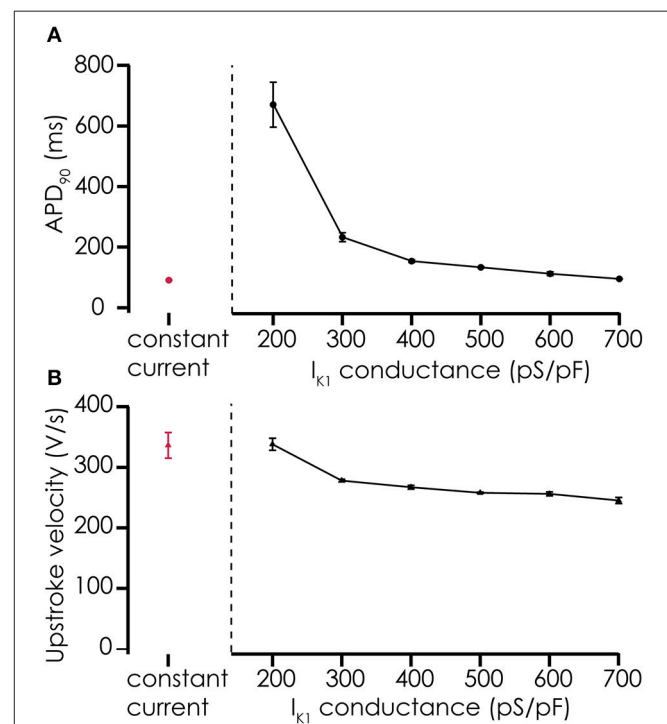


FIGURE 5 | Effects of adding simulated I_{K1} on AP parameters. **(A)** Adding I_{K1} prolongs the APD_{90} of action potentials recorded from Cellartis Cardiomyocytes ($n = 4$) compared to constant current injection. With increasing I_{K1} conductance, the prolongation of the action potential becomes smaller, consistent with the role of I_{K1} in the final repolarization of the cardiac action potential. **(B)** Upstroke velocity of the action potentials after addition of simulated I_{K1} is high, as with constant current injections, and decreases slightly with increasing I_{K1} conductance.

and the model used to compute I_{K1} . Earlier work has used I_{K1} formulations that model the current as a steady-state current, i.e., without time-dependence. The model by Ishihara et al. used in this study is a more detailed, time-dependent model that includes the rectifying effects of Mg^{2+} and polyamines, as well as two modes of channel closure by spermine (Ishihara et al., 2009). Including this more detailed model is relevant, as time and voltage dependent Mg^{2+} block or unblock can significantly affect action potential duration (Ishihara et al., 2002).

In current clamp experiments with suspended iPSC-CMs, we injected virtual I_{K1} current with varying conductance densities (G_{K1}), depending on the specific cell. At depolarized potentials

(> -40 mV), the Ishihara model does not generate much outward current, therefore increasing G_{K1} did not immediately induce hyperpolarization. This could be solved by briefly injecting a hyperpolarizing current, bringing the membrane potential to values inducing a sufficiently large outward I_{K1} current. As a result, the membrane potential was maintained at or close to E_K (which was -95 mV for the simulated I_{K1} channels) due to the injected virtual I_{K1} current. After this, the constant hyperpolarizing current was switched off, as it was only needed to start the experiment.

Action potentials recorded from iPSC-CMs with addition of virtual I_{K1} differed from the earlier recorded brief and

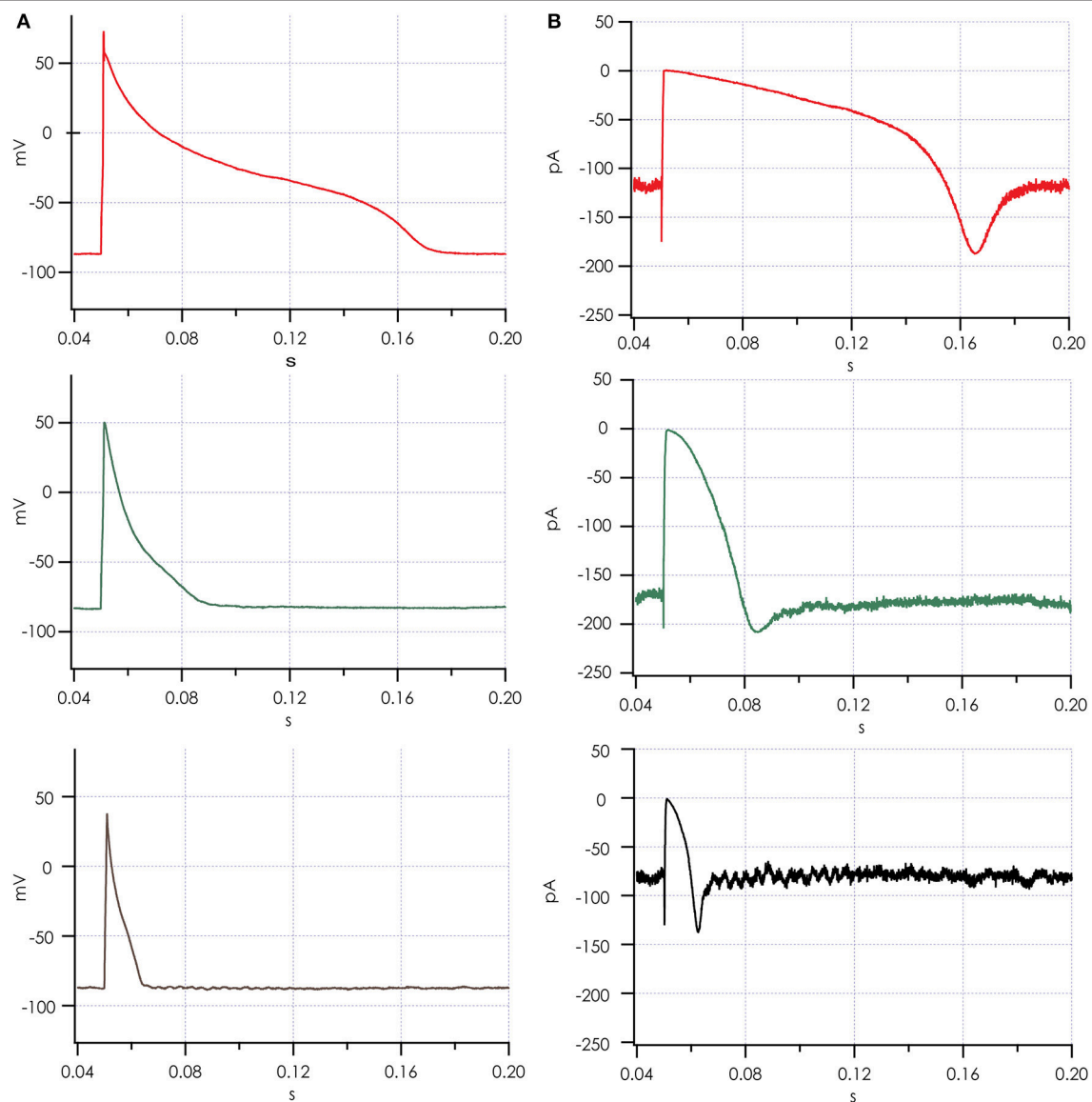


FIGURE 6 | Action potentials recorded simultaneously after adding simulated I_{K1} . **(A)** Action potentials from 3 cells recorded in parallel on a Patchliner Quattro. **(B)** Simulated I_{K1} was recorded for each of the 3 cells and is shown. Note the brief increase in I_{K1} during the upstroke of the action potential, where I_{K1} channels respond with increased current to the depolarization induced by the pacing stimulus, which is in line with the slight decrease observed in upstroke velocity with increasing I_{K1} conductance.

monotonically repolarizing action potentials. Injecting I_{K1} resulted in a stable resting membrane potential, fast upstroke velocities, and prolonged action potential duration with a clear plateau phase (Figure 4). Further increasing G_{K1} , resulting in injection of more I_{K1} , caused a small additional hyperpolarization, but more significantly, also shortened action potential duration (Figure 5A) and slightly decreased upstroke velocity (Figure 5B). This is consistent with the role of I_{K1} in cardiomyocyte electrophysiology, as it contributes to resting membrane potential stability and the final repolarization phase of the action potential (de Boer et al., 2010). A benefit of our newly developed approach is that experimental throughput can be moderately increased as we can record from, and inject I_{K1} into, 4 cells in parallel. In Figure 6 we show an example of an experiment in which we were able to record APs from 3 iPSC-CM in parallel (Figure 6A) and have also plotted the injected I_{K1} (Figure 6B). The fourth channel was available but for this channel no cell was captured successfully. Additional experiments, in which we studied the effect of G_{K1} on upstroke velocity, showed that we could perform a dynamic clamp experiment in 20 out of 28 wells in 7 experimental runs that used 4 wells per run (71% success rate), see Supplementary Figure 3.

Addition of Virtual I_{K1} Channels Restores Sensitivity of the Plateau Phase to Pharmacological Modulation

In earlier experiments, it proved difficult to observe effects of Ca^{2+} channel antagonists or agonists on the action potential shape of suspended iPSC-CMs when injecting a constant hyperpolarizing current (data not shown), most likely due to suppression of the plateau phase. After observing the restoration of the plateau phase when injecting virtual I_{K1} current, we tested if the action potential became again sensitive to manipulation of the L-type Ca^{2+} current. After injecting I_{K1} (on average G_{K1} was 267 ± 42 pS/pF) we observed an APD_{90} of 118 ± 21 ms ($n = 6$), which significantly prolonged after enhancing the L-type Ca^{2+} channel with $1 \mu\text{M}$ BayK-8644 to 155 ± 31 ms ($n = 6$, see Figures 7A,B). In contrast, blocking the L-type Ca^{2+} channel with $30 \mu\text{M}$ nifedipine caused a shortening to 81 ± 14 ms ($n = 6$). These findings demonstrate that enabling the plateau phase of the action potential of suspended iPSC-CMs by addition of virtual I_{K1} channels restores action potential sensitivity to L-type Ca^{2+} channel modulation.

DISCUSSION

In this study, as a proof of principle, we have demonstrated that the dynamic clamp technique can be used in combination with automated patch clamp devices, thereby creating a higher throughput alternative to manual patch clamp. Using dynamic clamp to add virtual I_{K1} channels to suspended iPSC-CMs allowed us to record action potentials with waveforms that are more representative of the human fully differentiated ventricular cardiomyocyte. This is especially relevant to the CiPA initiative, which aims to use hSC-CMs in drug safety testing.

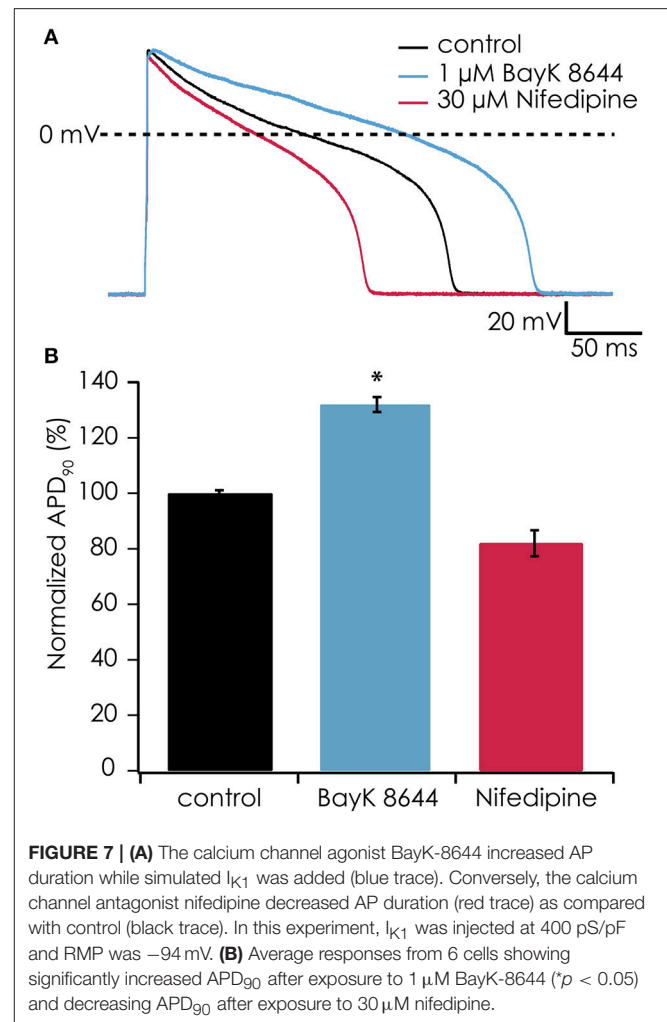


FIGURE 7 | (A) The calcium channel agonist BayK-8644 increased AP duration while simulated I_{K1} was added (blue trace). Conversely, the calcium channel antagonist nifedipine decreased AP duration (red trace) as compared with control (black trace). In this experiment, I_{K1} was injected at 400 pS/pF and RMP was -94 mV. **(B)** Average responses from 6 cells showing significantly increased APD_{90} after exposure to $1 \mu\text{M}$ BayK-8644 (* $p < 0.05$) and decreasing APD_{90} after exposure to $30 \mu\text{M}$ nifedipine.

Achieving higher throughput evaluation of drug effects on action potentials generated by iPSC-CMs will most likely require the use of isolated, suspended cells, as these can be used with automated patch clamp devices. The depolarized resting membrane potential of freshly isolated iPSC-CMs is a challenge that can be overcome, at least to some extent, by using the dynamic clamp technique (this study), but will also require improvement in the dissociation methods used. Dissociation of cardiomyocytes with enzymes disrupting the extracellular matrix, whether native or created in culture, is known to affect the function of I_{Kr} , I_{Ks} , and I_{K1} channels (Yue et al., 1996; Hoshino et al., 2012). Hoshino et al. demonstrated that the approach used to isolate cardiomyocytes from neonatal mouse hearts has a significant impact on I_{K1} channel function and resting membrane potential. Using enzymatic perfusion of the hearts preserved I_{K1} channels, while the chunk digestion method resulted in four to five times smaller I_{K1} currents and a depolarization of ~ 20 mV. The approach used in this and other studies to obtain single, suspended iPSC-CMs is very similar to the chunk digestion method, and we have indeed observed only small Ba^{2+} -sensitive currents, which may be smaller than those observed in studies

using adherent iPSC-CM (Ma et al., 2011; Doss et al., 2012; Nunes et al., 2013). Further improvement of cell dissociation protocols may improve results with iPSC-CMs on automated patch clamp devices. This is supported by recent work by Rajamohan et al. who have used a two-step dissociation protocol and recorded APs from the dissociated iPSC-CMs using both manual and automated patch clamping approaches (same device as used in this study). From the data in this study it appears that the two-step protocol yields cells with a more hyperpolarized membrane potential (Rajamohan et al., 2016).

In the present study, we provide proof-of-principle that automated patch clamp devices and dynamic clamp can be combined successfully. The resulting action potential durations and waveforms are very comparable to those obtained in manual patch clamp experiments in which I_{K1} was added to iPSC-CMs using dynamic clamp (Bett et al., 2013; Meijer van Putten et al., 2015), including the action potential prolongation in response to Bay-K8644. However, more research will be needed to establish the method, and to define its limits and benefits. A better insight into the effects of the specific I_{K1} model that is applied is needed, as well as a well-defined algorithm that allows us to determine which amount of added I_{K1} results in the most predictive results and therefore the best safety pharmacology assay. This should subsequently be demonstrated using the set of drugs defined by CiPA. If these goals can be reached, performing predictive patch clamp experiments with iPSC-CMs with an increased throughput becomes feasible.

AUTHOR CONTRIBUTIONS

BG: data acquisition, data analysis, data interpretation, revising; NB: data acquisition, data analysis, data interpretation, study

REFERENCES

- Ando, H., Yoshinaga, T., Yamamoto, W., Asakura, K., Uda, T., Taniguchi, T., et al. (2017). A new paradigm for drug-induced torsadogenic risk assessment using human iPS cell-derived cardiomyocytes. *J. Pharmacol. Toxicol. Methods* 84, 111–127. doi: 10.1016/j.vascn.2016.12.003
- Bett, G. C. L., Kaplan, A. D., Lis, A., Cimato, T. R., Tzanakakis, E. S., Zhou, Q., et al. (2013). Electronic “expression” of the inward rectifier in cardiocytes derived from human induced pluripotent stem cells. *Heart Rhythm* 10, 1903–1910. doi: 10.1016/j.hrthm.2013.09.061
- Blinova, K., Stohlman, J., Vicente, J., Chan, D., Johannesen, L., Hortigon-Vinagre, M. P., et al. (2017). Comprehensive translational assessment of human-induced pluripotent stem cell derived cardiomyocytes for evaluating drug-induced arrhythmias. *Toxicol. Sci.* 155, 234–247. doi: 10.1093/toxsci/kfw200
- de Boer, T. P., Houtman, M. J. C., Compier, M., and van der Heyden, M. A. G. (2010). The mammalian $K(IR)2.x$ inward rectifier ion channel family: expression pattern and pathophysiology. *Acta Physiol.* 199, 243–256. doi: 10.1111/j.1748-1716.2010.02108.x
- Doss, M. X., Di Diego, J. M., Goodrow, R. J., Wu, Y., Cordeiro, J. M., Nesterenko, V. V., et al. (2012). Maximum diastolic potential of human induced pluripotent stem cell-derived cardiomyocytes depends critically on $I(Kr)$. *PLoS ONE* 7:e40288. doi: 10.1371/journal.pone.0040288
- Gintant, G., Sager, P. T., and Stockbridge, N. (2016). Evolution of strategies to improve preclinical cardiac safety testing. *Nat. Rev. Drug Discov.* 15, 457–471. doi: 10.1038/nrd.2015.34
- Goversen, B., Van der Heyden, M. A. G., van Veen, T. A. B., and de Boer, T. P. (2017). The immature electrophysiological phenotype of iPSC-CMs still hampers *in vitro* drug screening: Special focus on I_{K1} . *Pharmacol. Ther.* doi: 10.1016/j.pharmthera.2017.10.001. [Epub ahead of print].
- Hoshino, S., Omatsu-Kanbe, M., Nakagawa, M., and Matsuura, H. (2012). Postnatal developmental decline in I_{K1} in mouse ventricular myocytes isolated by the Langendorff perfusion method: comparison with the chunk method. *Pflügers Arch. Eur. J. Physiol.* 463, 649–668. doi: 10.1007/s00424-012-1084-0
- Ishihara, K., Sarai, N., Asakura, K., Noma, A., and Matsuoka, S. (2009). Role of Mg^{2+} block of the inward rectifier K^{+} current in cardiac repolarization reserve: A quantitative simulation. *J. Mol. Cell. Cardiol.* 47, 76–84. doi: 10.1016/j.yjmcc.2009.03.008
- Ishihara, K., Yan, D.-H., Yamamoto, S., and Ehara, T. (2002). Inward rectifier K^{2+} current under physiological cytoplasmic conditions in guinea-pig cardiac ventricular cells. *J. Physiol.* 540, 831–841. doi: 10.1113/jphysiol.2001.013470
- Jonsson, M. K. B., Vos, M. A., Mirams, G. R., Duker, G., Sartipy, P., de Boer, T. P., et al. (2012). Application of human stem cell-derived cardiomyocytes in safety pharmacology requires caution beyond hERG. *J. Mol. Cell. Cardiol.* 52, 998–1008. doi: 10.1016/j.yjmcc.2012.02.002
- Li, M., Kanda, Y., Ashihara, T., Sasano, T., Nakai, Y., Kodama, M., et al. (2017). Overexpression of $KCNJ2$ in induced pluripotent stem cell-derived cardiomyocytes for the assessment of QT-prolonging drugs. *J. Pharmacol. Sci.* 134, 75–85. doi: 10.1016/j.jphs.2017.05.004
- Ma, J., Guo, L., Fiene, S. J., Anson, B. D., Thomson, J. A., Kamp, T. J., et al. (2011). High purity human-induced pluripotent stem cell-derived

design, writing; SS-F, AO: data analysis, data interpretation, revising; TvV, MV, NF: data interpretation, revising; TdB: data acquisition, data analysis, data interpretation, study design, writing, revising. All authors meet the following criteria: (i) Substantial contributions to the conception or design of the work; or the acquisition, analysis, or interpretation of data for the work, (2) Drafting the work or revising it critically for important intellectual content, (3) Final approval of the version to be published, (4) Agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

FUNDING

This study is supported by MKMD grants 114021501 and 114022502 from ZonMW (TvV and TdB), and a grant from Utrecht Holdings (TdB).

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: <https://www.frontiersin.org/articles/10.3389/fphys.2017.01094/full#supplementary-material>

Supplementary Figure 1 | Diagram describing implementation of dynamic clamping.

Supplementary Figure 2 | Flowchart describing steps in dynamic clamp experiment.

Supplementary Figure 3 | Influence of I_{K1} conductance on upstroke velocity.

Supplementary Data Sheet 1 | CellML implementation of I_{K1} model used in this study.

- cardiomyocytes: electrophysiological properties of action potentials and ionic currents. *Am. J. Physiol. Heart Circ. Physiol.* 301, H2006–H2017. doi: 10.1152/ajpheart.00694.2011
- Meijer van Putten, R. M. E., Mengarelli, I., Guan, K., Zegers, J. G., van Ginneken, A. C. G., Verkerk, A. O., et al. (2015). Ion channelopathies in human induced pluripotent stem cell derived cardiomyocytes: a dynamic clamp study with virtual I_{K1} . *Front Physiol* 6:7. doi: 10.3389/fphys.2015.00007
- Nunes, S. S., Miklas, J. W., Liu, J., Aschar-Sobbi, R., Xiao, Y., Zhang, B., et al. (2013). Biowire: a platform for maturation of human pluripotent stem cell-derived cardiomyocytes. *Nat. Methods* 10, 781–787. doi: 10.1038/nmeth.2524
- Ortega, F. A., Butera, R. J., Christini, D. J., White, J. A., and Dorval, A. D. (2014). Dynamic clamp in cardiac and neuronal systems using RTXI. *Methods Mol. Biol.* 1183, 327–354. doi: 10.1007/978-1-4939-1096-0_21
- Ortega, F. A., Grandi, E., Krogh-Madsen, T., and Christini, D. J. (2017). Applications of dynamic clamp to cardiac arrhythmia research: Role in drug target discovery and safety pharmacology testing. *Front Physiol.* 8:1094. doi: 10.3389/fphys.2017.01094
- Rajamohan, D., Kalra, S., Duc Hoang, M., George, V., Staniforth, A., Russell, H., et al. (2016). Automated electrophysiological and pharmacological evaluation of human pluripotent stem cell-derived cardiomyocytes. *Stem Cells Dev.* 25, 439–452. doi: 10.1089/scd.2015.0253
- Vaidyanathan, R., Markandeya, Y. S., Kamp, T. J., Makielski, J. C., January, C. T., and Eckhardt, L. L. (2016). I_{K1} -enhanced human-induced pluripotent stem cell-derived cardiomyocytes: an improved cardiomyocyte model to investigate inherited arrhythmia syndromes. *Am. J. Physiol. Heart Circ. Physiol.* 310, H1611–H1621. doi: 10.1152/ajpheart.00481.2015
- van den Heuvel, N. H. L., van Veen, T. A. B., Lim, B., and Jonsson, M. K. B. (2014). Lessons from the heart: Mirroring electrophysiological characteristics during cardiac development to *in vitro* differentiation of stem cell derived cardiomyocytes. *J. Mol. Cell. Cardiol.* 67, 12–25. doi: 10.1016/j.yjmcc.2013.12.011
- Wilders, R. (2006). Dynamic clamp: a powerful tool in cardiac electrophysiology. *J. Physiol.* 576, 349–359. doi: 10.1113/jphysiol.2006.115840
- Yue, L., Feng, J., Li, G. R., and Nattel, S. (1996). Transient outward and delayed rectifier currents in canine atrium: properties and role of isolation methods. *Am. J. Physiol.* 270, H2157–H2168.

Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Copyright © 2018 Goversen, Becker, Stoelele-Feix, Obergrussberger, Vos, van Veen, Fertig and de Boer. This is an open-access article distributed under the terms of the Creative Commons Attribution License (CC BY). The use, distribution or reproduction in other forums is permitted, provided the original author(s) or licensor are credited and that the original publication in this journal is cited, in accordance with accepted academic practice. No use, distribution or reproduction is permitted which does not comply with these terms.