A Novel Model of the Action Potential of Ventricular-like Human Induced Pluripotent Stem Cell-derived Cardiomyocytes

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Abstract

Human induced pluripotent stem cells (hiPSCs) represent nowadays a valuable in-vitro model to study the mechanisms underlying pathologies and focusing on drug treatment in a patient-specific manner. In-silico models can integrate the experimental practice being used as simulation platforms, providing new hints and helping defining new experiments and hypothesis.

We developed a new model of ventricular-like hiPSC-derived cardiomyocyte (hiPSC-CM) based on recently published data and aiming to provide a detailed description of the hiPSC-CM electrophysiology.

Our model reproduced: (i) spontaneous action potentials (APs); (ii) AP features typical of the ventricular-like phenotype such as maximum diastolic potential, AP duration and amplitude; (iii) effects of prototypical current blockers.

In conclusion our new hiPSC-CM model represents a validated description of the electrophysiology of ventricular-like hiPSC-CM and it has potential application in further studies on patient- and disease-specific ion channels mutations in hiPS-CMs.

1. Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are nowadays considered a more and more valuable tool from developmental biology to drug discovery. Although hiPSC-CMs are still not used in clinical applications such as cell therapies, in basic research they are regarded as a valuable in-vitro model. Diverse recent researches are focusing on healthy and mutated hiPSC-CMs for exhaustive electrophysiological characterizations [1–4]: as an example, producing lineages from healthy subjects or from mutation symptomatic/asymptomatic carriers [2] could be used to study the mechanisms underlying pathologies such as LQT syndromes and focusing on drug treatment in a patient-specific manner.

Supporting the in-vitro practice with in-silico models can represent a strategic choice that has potential to reduce the number of costly and time consuming in-vitro experiments. In-silico models could help to verify hypotheses, to formulate new ones as well as to plan new experiments.

In this work we present our novel model describing the action potential (AP) of ventricular-like hiPSC-CMs based on recently published measurements [1] of currents and APs of these cells.

2. Methods

Experimental data applied for model development is based on hiPSC-CMs cultured for 30 - 32 days and then cryopreserved; patch clamp recordings of APs and currents were performed on hiPSC-CMs 4 - 21 days post-thaw [1].

Our model follows the canonical Hodgkin & Huxley formulation:

$$\frac{dV}{dt} = -\frac{I_{ion} - I_{stim}}{C_m}$$

where V and C_m are respectively the membrane potential and capacitance, I_{ion} is the whole membrane current and I_{stim} the external stimulus. The model development process is summarized in the following paragraphs.

2.1. Fitting the experimental current data

We formulated our model by fitting the data of the experimental Na^+ current (I_{Na}), the L-type Ca^{++} current (I_{CaL}), the hyperpolarization-activated cyclic nucleotide-gated current (I_f) and K^+ currents such as the transient outward (I_{to}), the inward rectifying (I_{K1}), and the delayed rectifying rapid (I_{Kr}) and slow (I_{Ks}) currents, recorded on hiPSC-CMs developing towards the ventricular-, atrial- and nodal-like phenotypes [1]. I/V curves were available for each current (step and tail currents for I_{Kr}), activation/inactivation curves for I_{Na}, I_{CaL} and I_f. Figure 1 shows the quality of the fitting indicating the calculated

and measured currents obtained using the same voltage clamp protocols as in [1]

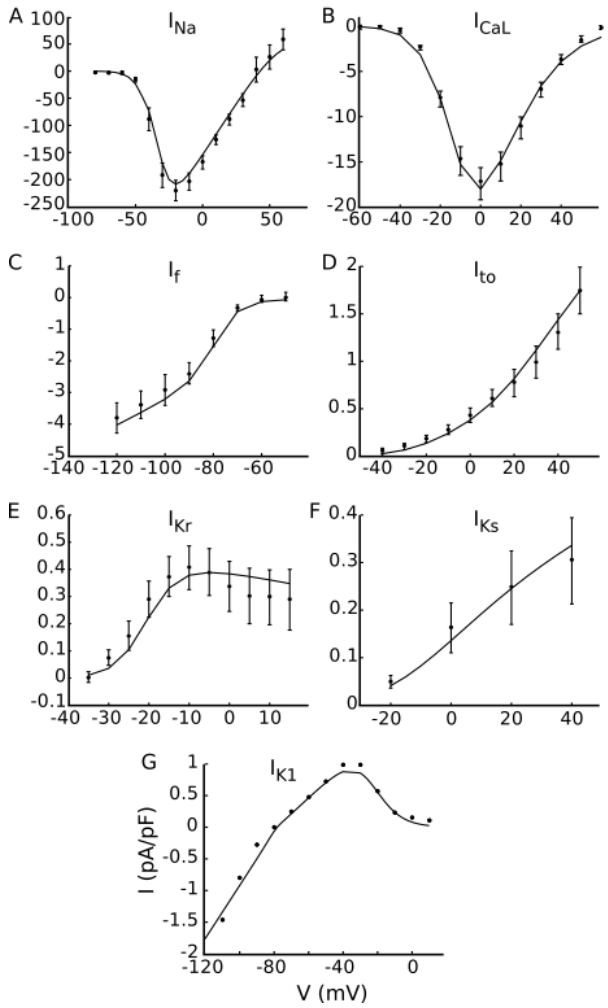

Figure 1. Fitting of the experimental data from [1] used in the hiPSC-CM model. Measures were performed on ensembles of ventricular-, atrial- and nodal-like cells.

2.2. Moving towards the ventricular-like phenotype

Due to the phenotypical heterogeneity affecting the voltage clamp data, equations of I_{Na}, I_{K1} and I_{to}, i.e. currents mostly contributing to the differences in AP shape at the adult stage between ventricular and atrial cells, were slightly tuned according to literature data [5–7]. I_f was scaled as well to take into account the presence of nodal-like cells, characterized by a stronger I_f expression than the ventricular- and atrial-like cells. The ventricular-like cell capacitance C_m was calculated scaling the mean capacitance value of 88.7 pF reported in [1], due to a greater capacitance in ventricular than in atrial cells, as reported by [8]. This aimed to fit the morphological features computed on the experimental ventricular-like spontaneous APs (I_{stim} = 0) [1]. Scaling coefficients are reported in Table 1. Comparison between experimental and simulated APs was performed in terms of the AP morphological features (AP features): rate of spontaneous beating (F), maximum diastolic potential (MDP), peak voltage (Peak), AP amplitude (APA), maximum upstroke velocity (Vmax), AP duration at different percentages of repolarization (APD10, APD30, APD90). As an additional AP feature, the ratio rappAPD computed as

$$rappAPD = \frac{APD_{30} - APD_{40}}{APD_{70} - APD_{80}}$$

was included in Table 2: rappAPD was used as a threshold in discriminating among ventricular-, atrial- and nodal-like phenotypes.

2.3. Current blocker simulations

In [1] effects of prototypical current blockers, namely Tetrodotoxin (TTX), E4031, Nifedipine (Nifed) and 3R4S-Chromanol 293B (Chr), on the shape of APs were assessed during measurements on stimulated ventricular-like hiPSC-CMs.: (i) TTX blocks I_{Na} and shifts the upstroke, (ii) E4031 blocks I_{Kr} and lengthens APD, (iii) Nifed blocks I_{CaL} and shortens APD and (iv) Chr blocks I_{Ks} having only minor effects on the AP shape.

To facilitate the simulations of the effects of TTX, E4031, Nifed and Chr, the maximum conductance of the current targeted by the specific blocker was reduced by 50%, 70% and 90% simulating the diverse blockade levels.

In order to reproduce the experimental protocol [1], our hiPSC-CM was stimulated ($I_{stim} \neq 0$) at a constant pacing rate 1 Hz with depolarizing pulses of 5 ms duration and 550 pA amplitude.

Table 1. Scaling coefficients for the maximum conductances of the currents reported in Figure 1 and the membrane capacity C_m. Coefficients were chosen according to literature data from comparison between atrial (ATR) and ventricular (VEN) cardiomyocytes. Also the nodal (NOD) phenotype was considered while scaling I_f.

Parameter	Phenotype-related differences	Scaling coefficient
G_{Na}	VEN<ATR [5]	0.65
G_{K1}	VEN>ATR [6]	1.1
G_{to}	VEN<ATR [6]	0.5
G_f	VEN,ATR<NOD [7]	0.7
C_m	VEN>ATR [8]	1.113

3. Results

3.1. Spontaneous action potentials

Simulated spontaneous APs are reported in Figure 2, while Figure 3 and 4 show the intracellular Na^+ and Ca^{2+} concentrations and the shape of the main membrane currents. Table 2 reports a comparison between experimental and simulated ventricular-like APs in terms of AP features.

3.2. Current blocker simulations

As a proof of concept, we challenged our model in reproducing the AP shape changes reported in Section 2.3, thus getting potential traces showing good agreement with the experimental data (Figure 5).

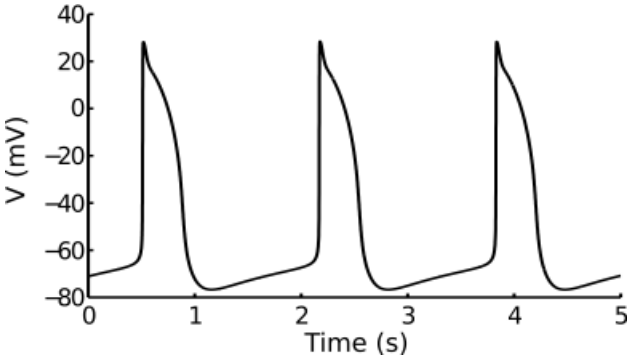

Figure 2. Simulated steady-state ventricular-like hiPSC-CM spontaneous AP (I_{stim} = 0).

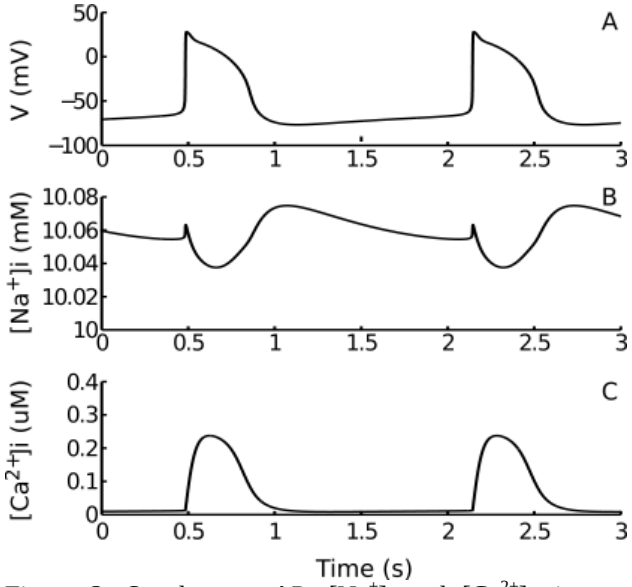

Figure 3. Steady-state AP, $[Na^+]_i$ and $[Ca^{2+}]_i$. in no-stimulus condition (I_{stim} = 0).

Table 2. Morphological features of experimental (EXP) [1] and simulated (SIM) APs. A ventricular-like AP shows rappAPD>1.5.

AP feature		EXP (mean±SEM)	SIM
F	(bpm)	35.3±2.2	36.2
MDP	(mV)	-75.6±1.2	76.7
Peak	(mV)	28.3±1.0	28.0
APA	(mV)	104.0±1.1	104.7
Vmax	(V/s)	27.8±4.8	27.8
APD10	(ms)	74.1±4.8	53.3
APD30	(ms)	180.0±10.7	235.8
APD90	(ms)	414.7±21.8	404.7
rappAPD		2.5±0.2 (>1.5)	3.4

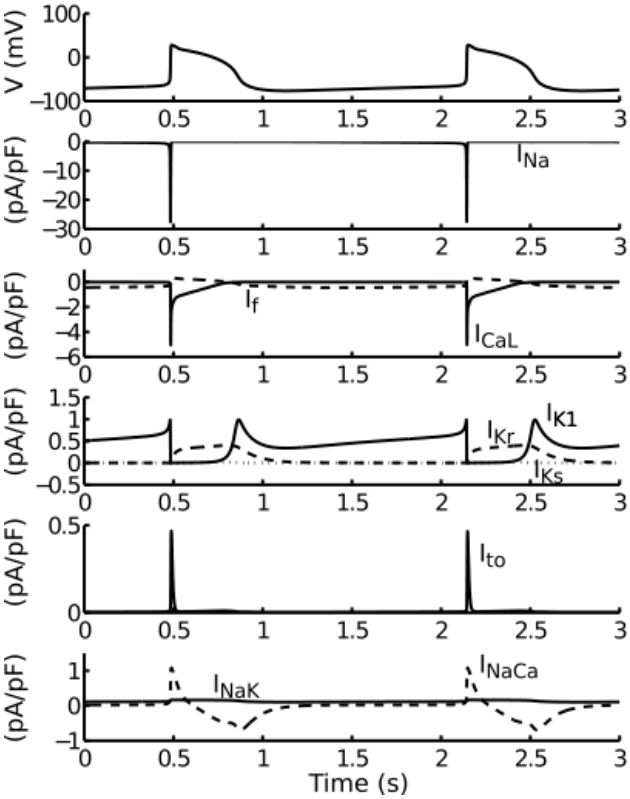

Figure 4. Steady-state AP and major ionic currents in no stimulus condition (I_{stim} = 0).

4. Discussion and conclusions

The aim of this work was developing a model of the hiPSC-CM AP, reproducing the basic electrophysiological properties of these cells. In particular we chose the ventricular-like phenotype, since it was reported to be the most common: in [1] more than 50% of the characterized APs and in [2] 29 out of the 40 tested cells showed to be ventricular-like.

Due to the phenotypical heterogeneity of the cells used for voltage-clamp recordings, we slightly modified the

equations we used to interpolate the experimental data.

In particular we focused on the current expression differences between the ventricular and the atrial phenotype. Moreover the nodal phenotype was taken into account for the I_f edits, since nodal cells are the most counting for the I_f expression.

The simulated spontaneous APs fits the experimentally recorded shape, except for APD10 and APD30. This affects also the simulated rappAPD: our value is greater than the experimental one, but it is fully compliant with the condition for a ventricular-like classification (rappAPD>1.5).

Our simulation about current blockers are in agreement with the experiments. I_{Na} (Figure 5A) acts prevalently during the upstroke and a strong blockade induced by TTX produces an important shift in time of the AP peak, making the I_{CaL} contribution to the upstroke critical. Moreover, I_{CaL} contributes also in sustaining the action potential (Figure 5B), thus an I_{CaL} blockade induced by Nifed causes the reduction of all the APDs and triangulates the AP profile. E4031 is a highly selective I_{Kr} blocker, thus it affects prevalently the APD, especially

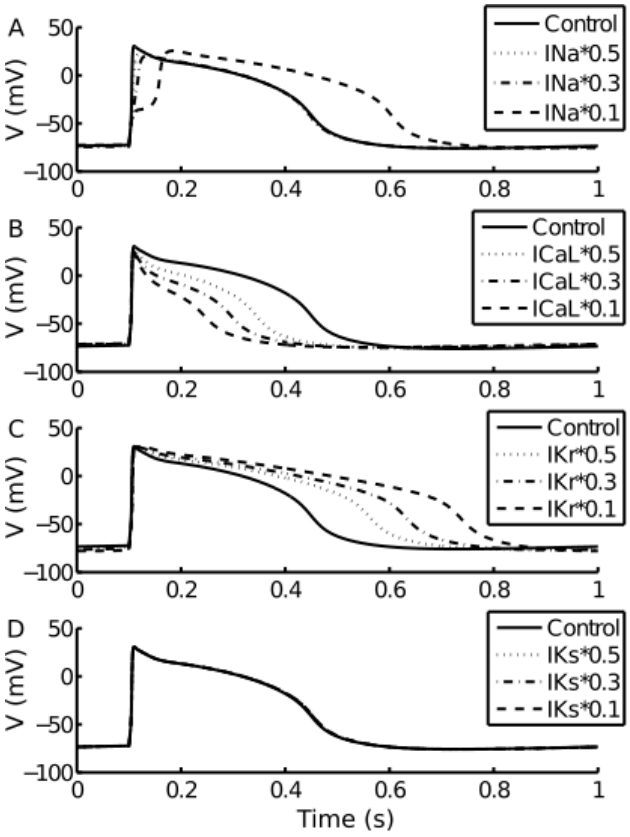

Figure 5. Simulations of different blockade levels of (A) I_{Na} by TTX, (B) I_{CaL} by Nifed, (C) I_{Kr} by E4031and (D) I_{Ks} by Chr. The hiPSC-CM was stimulated ($I_{stim} \neq 0$) at a constant pacing rate 1 Hz with depolarizing pulses of 5 ms duration and 550 pA amplitude.

APD70 and APD90 (Figure 5C). I_{Ks} blockade did not have any significant effect on the AP shape (Figure 5D).

To conclude, our new in-silico hiPS-CM model can reproduce major electrophysiological characteristics including drug effects of the actual cells tested in-vitro.

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