Numerical Simulations Indicate I_{K1} Dynamic Clamp Can Unveil the Phenotype of Cardiomyocytes Derived from Induced Pluripotent Stem Cells

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Abstract

Cardiomyocytes derived from human induced pluripotent stem cells (hiPSC-CMs) are a virtually endless source of human cardiomyocytes, considerably used in vitro models to test drug toxicity. These cells express the major cardiac markers and ion channels, but they also result in a mix of incompletely mature cardiac cells that can be classified as atrial-like and ventricular-like cardiomyocytes. One of the most popular manipulations used to push towards more adult cardiac phenotypes is the dynamic clamp technique, based on virtual inward - rectifier potassium current (I_{K1}) injection.

In this exploratory in silico study, six different I_{K1} expressions have been virtually analyzed to classify hiPSC-CM phenotypes. Starting from the resulting action potential morphologies, we defined a mathematical criterion to estimate the efficacy of the injected I_{K1} current in terms of the threshold percentage of the current density required to obtain an hiPSC-CM physiological response. It was found that atrial I_{K1} formulations are more reliable than ventricular ones, with the Koivumäki I_{K1} formulation being the most appropriate since it requires the minimal current density to be injected.

1. Introduction

Human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) offer a human-based system for disease modeling and drug discovery. The phenotyping of the generated atrial and ventricular hiPSC-CMs is crucial for a better understanding of subtype-related diseases and chamber-specific drugs effects. Current protocols for efficiently differentiating hiPSC into defined CMs give as a result heterogeneous populations of CMs consisting predominantly of ventricular-like cells with a small percentage of atrial-like cells. In order to differentiate hiPSC-CMs between an atrial phenotype, Retinoic Acid (RA) is widely used in cardiovascular development and *in vitro* experiments show higher percentages of atrial-like CMs by an overexpression of atrial markers, and downregulation of ventricular ones [1].

Another useful tool for improving action potential measurements in hiPSC-CMs is the Dynamic Clamp technique, based on inward - rectifier potassium current (I_{K1}) injection. In a general perspective, this tool makes hiPSC-CMs a reliable model for investigating cardiac arrhythmias (see [2]). Different studies, such as [3, 4], also state that, by employing the dynamic clamp technique, the atrial-like action potential phenotype is more pronounced and this facilitates the separation of atrial-like and ventricular-like action potential phenotypes.

In this work, we carry out an *in silico* study based on virtual dynamic clamp in order to analyze six different I_{K1} current expressions to separate hiPSC-CM phenotypes, showing the importance of selecting an appropriate synthetic formulation. Even though the qualitative effects on the cell are almost the same, the quantitative ranges of injected current, required in order to obtain an hiPSC-CM physiological response, are quite different. Keeping in mind that we are considering non-native currents, we claim that the most appropriate I_{K1} formulation is the one that minimizes the amount of required current.

2. Materials and Methods

We describe first the dynamic clamp experimental setup, then its *in silico* version (virtual dynamic clamp), the hiPSC-CMs ionic model adopted, and the inward-rectifier I_{K1} currents tested in our work.

2.1. Experimental dynamic clamp

hiPSC-CMs are characterized by an immature electrical phenotype. The inward-rectifier potassium current (I_{K1}) in these cells can be too low or even lacking, leading to an unstable and depolarized membrane diastolic potential (MDP). I_{K1} can be overexpressed through injection of a virtual I_{K1} current, a technique known as dynamic clamp (DC). Using a traditional patch clamp, transmembrane potential (V_m) recordings are sampled and fed to a computer running a real time simulation of the V_m-dependent current I_{K1}, which is then added to a stimulus current and sent to a patch clamp amplifier to be injected into the cell, see e.g. [2–4].

2.2. Virtual dynamic clamp

Since in DC technique the injected current can be fully described by mathematical equations, it is useful to perform *in silico* the whole interface protocol and the current injection. In a fully computational set, it is possible to simulate the electrical activity of an hiPSC-CM using existing mathematical models, made up by a set of ordinary differential equations, describing the ionic currents dynamics. Then, it is possible to test different I_{K1} models, compare the physiological responses and choose the best formulation.

In the chosen mathematical membrane model, we first suppressed the native I_{K1} current, in order to reach the immature physiological condition of the cell membrane. We then added to the total ionic current different I_{K1} formulations taken from existing ionic current models, as in the experimental DC current injection procedure.

2.3. Paci2013 model for hiPSC-CMs

Dynamic clamp technique can be applied to both atrial-like (AL) or ventricular-like (VL) hiPSC-CMs. Virtual DC simulations were performed using the computational ionic model developed by Paci et al. [5], which models both AL and VL hiPSC-CMs. The parameter settings of the model allow for the quantitative investigation of the two AL and VL configurations, even if no atrial specific current is present in the model. The original Paci2013 model, constrained by experimental data, simulated traces of spontaneous electrical activity. According to Fabbri [6], the required amount of replaced I_{K1} could bring the cell to a stable and hyperpolarized MDP ($\simeq -78\ mV$).

In this framework, we assessed the resulting AP morphology while pacing the models at 1 Hz.

2.4. Tested inward-rectifier I_{K1} currents

We carried out the virtual dynamic clamp by considering different I_{K1} models available in the literature. In a general perspective, we took into account four ventricular specific I_{K1} formulations, coming from different human ventricular action potential models, Ten Tusscher (TT) [7], Grandi [8], Fink [9], O'Hara-Rudy (ORd) [10] and 2 atrial specific I_{K1} formulations, Koivumäki (K) [11] and Courtemanche (CRN) [12]. As suggested by Fabbri et al. [6], we conduced an *a priori* comparison between the six I_{K1} formulations. First of all, we scaled all of them in order to obtain the same outward peak current density ($0.63\ pA/pF$), corresponding to the peak of the Koivumäki model, this being one of the most recent atrial models. Considering voltages between -120 and $10\ mV$, each normalized models generated the steady state normalized I_{K1} currents shown in Figure 1.

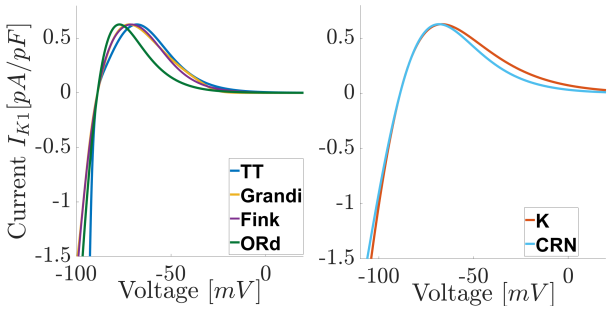

Figure 1: Normalized I_{K1} currents from ventricular (left) and atrial (right) ionic models.

3. Results

We compared the six I_{K1} formulations focusing on the expression of different morphological features with respect to the injected current density.

3.1. Action potential morphology

The action potential (AP) morphology for different atrial and ventricular I_{K1} formulations changed with respect to the density of the injected current. The AP morphology was absolutely non physiological for low percentages of the injected current, while it became physiological for higher densities. This morphology change is shown in Figure 2 for atrial-like cells and in Figure 3 for ventricular-like cells, shown for simplicity the I_{K1} Koivumäki formulation (atrial) and Ten Tusscher formulation (ventricular). Analogous results hold for the other I_{K1} formulations considered.

In order to estimate the minimal amount of injected I_{K1} required to obtain a physiological AP, we defined

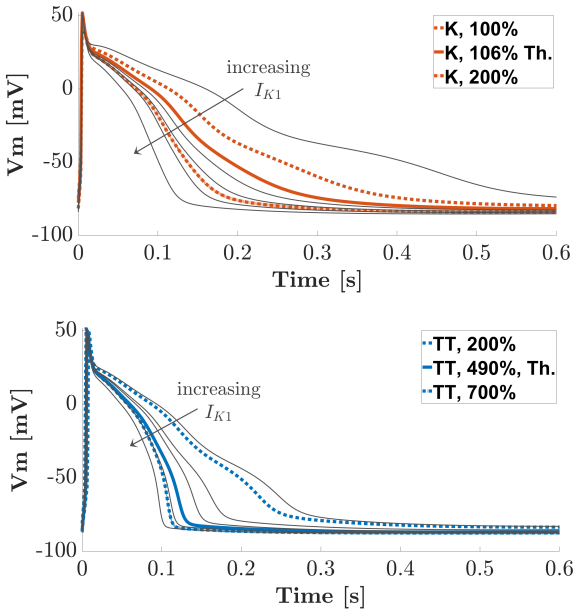

Figure 2: Virtual DC performed on Paci2013 for AL hiPSC-CMs, considering Koivumäki (upper plot) TenTusccher I_{K1} (lower plot) formulation. Three different current densities were expressed, labeled as percentages of the normalized current.

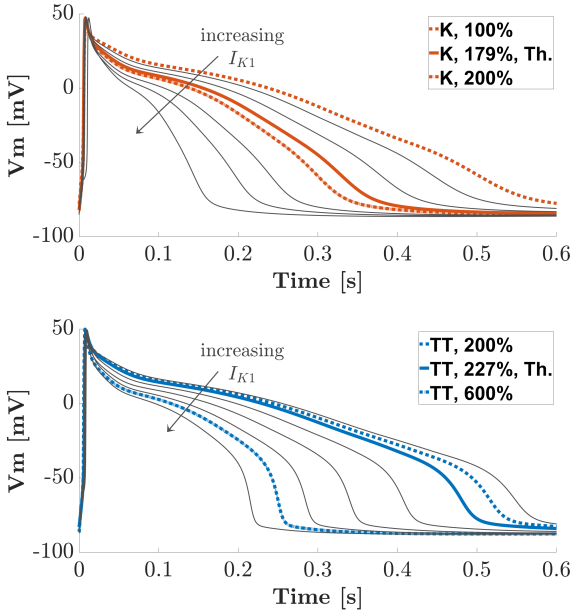

Figure 3: Virtual DC performed on Paci2013 for VL hiPSC-CMs, considering Koivumäki (upper plot) TenTusccher I_{K1} (lower plot) formulation. Three different current densities were expressed, labeled as percentages of the normalized current.

a mathematical criterion to classify the different AP morphologies based on the number n_I of AP inflections (the time where $\frac{\partial^2 V_m}{\partial t^2}(t) = 0$) between the AP peak and the resting MDP. More precisely, we classified the AP morphology as non physiological if $n_I > 6$, while the morphology is physiological if $n_I \leq 6$.

3.2. Conductance thresholds analysis

Using the previous AP morphology classification, we identified for every I_{K1} formulation the threshold conductance G^{th}_{K1} as the minimal conductance required for obtaining a physiological AP (i.e. to suppress the non physiological AP morphology), see Table 1.

Comparing the different results between atrial specific I_{K1} formulations (Koivumäki, CRN) and ventricular ones in Table 1, we see that for both AL and VL hiPSC-CMs, the Koivumäki and Courtemanche threshold conductances are much lower than the thresholds of the ventricular formulations.

In order to gain a better understanding, we also computed the threshold percentages of the normalized I_{K1} current in each original model and we report these values in Figure 4. Once more, ventricular formulations need higher percentages of injected current than atrial formulations in order to prevent the cell from showing a non physiological morphology. A common feature among the different I_{K1} formulations is the increased current density needed by AL cells with respect to VL hiPSC-CMs. Again, the discrepancy decreases when considering the Koivumäki or Courtemanche I_{K1} formulations.

Table 1: Conductance G^{th}_{K1} threshold nominal values, measured in $[nS/pF]$, required to obtain a physiological AP for each model, for both AL (second column) and VL (third column) hiPSC-CMs.

I_{K1} formulation	G^{th}_{K1} AL hiPSC-CMs	G^{th}_{K1} VL hiPSC-CMs
Ten Tusscher	9.0336	4.1855
Fink	2.3693	0.9410
Grandi	2.2761	0.7659
O'Hara-Rudy	1.8066	0.7754
Koivumäki	0.0742	0.1253
Courtemanche	0.3297	0.1738

4. Discussion and conclusions

In silico simulation of the dynamic clamp for AL and VL hiPSC-CMs revealed to be a powerful tool for investigating the *a priori* cell response to different I_{K1} injections.

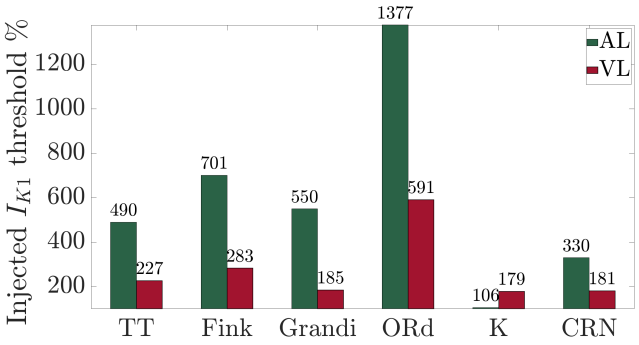

Figure 4: Threshold percentages of the normalized I_{K1} current in each original model, for both AL or VL hiPSC-CMs.

In this exploratory *in silico* study, we virtually performed the dynamic clamp and considered six different I_{K1} formulations. Since DC injects an external current, it is reasonable to add as small a current as possible. Under this assumption, rightly injected threshold current could be seen as an estimation of the efficacy of the I_{K1} current formulation inside the DC technique.

When considering VL hiPSC-CMs, almost every I_{K1} formulation needs a current density lower than 3 times the normalized current density in the literature, i.e. the I_{K1} threshold percentage is lower than 300%. On the other hand, for every tested formulation, the AL simulation shows a physiological morphology when the injected current density reaches higher percentages, and almost every I_{K1} formulation needs a current density higher than 3 times the normalized literature value, i.e. the threshold percentage is higher than 300%.

Keeping in mind that the DC technique is used as a tool to improve action potential measurements in atrial-like hiPSC-CMs, we focused on the effects of I_{K1} injection on AL hiPSC-CMs action potential morphology. Previous results and considerations led us to the statement that atrial current formulations, i.e. Koivumäki and Courtemanche, are preferable to the ventricular ones. In agreement with this, Figure 4 shows that VL hiPSC-CMs reveal the ventricular-like phenotype for low densities independent of the I_{K1} formulation. On the other hand, the atrial-like phenotype easily revealed using only atrial I_{K1} formulations, by using 106% (Koivumäki) and 300% (Courtemanche) of the original normalized literature values, respectively.

In conclusion, in order to characterize atrial specific hiPSC-CMs using the minimal injection of I_{K1}, virtual dynamic clamp suggests Koivumäki I_{K1} as the more reliable I_{K1} formulation, with the minimal current density equal to 106% for atrial-like cells.

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