An *in silico* Study of Cardiac hiPSC Electronic Maturation by Dynamic Clamp

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Abstract. Regenerative cardiology recently advanced in patient–specific medicine by employing somatic cells to derive pluripotent stem cells and differentiate them into cardiomyocytes. Resulting populations present an immature phenotype; the Dynamic Clamp technique is a popular experimental manipulation to induce electronic maturation towards an adult phenotype.

In this work, we present a fully virtual framework to study this Dynamic Clamp technique, based on the injection of the inward-rectifier potassium current into the myocyte, taking into account six different current formulations. We investigate the effects of the current injection on the action potential morphology and on three specific biomarkers for different current percentages, and we compare resulting morphologies with the standard transmembrane potential profile of a human adult cardiomyocyte. The results of this quantitative analysis suggest that atrial–like potassium current formulations allow the cell to reach action potential features comparable with the ones of mature cells, preventing the cell to show a non physiological morphology.

Keywords: hiPSC–CMs · Virtual dynamic clamp · Cardiac action potential morphology

1 Introduction

Human–induced pluripotent stem cell–derived cardiomyocytes (hiPSC–CMs) provide a powerful tool to develop reliable human-based *in vitro* models for disease modeling and drug toxicity screening. These cells arise from differentiation protocols, that result in heterogeneous populations of immature CMs consisting predominantly of ventricular–like (VL) cells with a small percentage of atrial–like (AL) cells and nodal–like cells.

Two manipulations are widely used to push hiPSC–CMs toward more adult cardiac phenotypes. First, Retinoic Acid treatment allows to over-express atrial

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markers [4]. Then, through the Dynamic Clamp (DC) technique, the membrane diastolic potential (MDP) hyperpolarizes to values suitable for generating a mature action potential (AP) waveform, allowing the discrimination between atrial and ventricular AP phenotype. Thus, the chamber–specific AP phenotype is more pronounced and this facilitates the separation of AL and VL CMs, as described in [1,15].

Another important difference between cultured hiPSC-CMs and adult myocytes is the low, or even absence of, inward-rectifier potassium current (I_{K1}). To overcome this immature characteristic, we consider a DC technique based on the injection of a virtual I_{K1} current. This electronic maturation improves AP measurements in hiPSC–CMs and makes hiPSC–CMs a more reliable model for investigating cardiac arrhythmias (see [9]).

This study is devoted to investigating the DC technique in a fully computational setting. We carry out an *in silico* study based on Virtual Dynamic Clamp in order to analyze six different I_{K1} current formulations, considering qualitative effects on the cell and evaluating quantitatively AP features with respect to adult CMs in a perspective of cell maturation.

2 Methods

In this section, we first describe the experimental DC setup and its *in silico* rendering (virtual DC), as described in [2]. Furthermore, we will present the innovative adopted hiPSC–CMs ionic model, and the I_{K1} scaled formulations tested in the present work.

2.1 From Experimental to Virtual DC

In cultures of matured hiPSC–CMs, the I_{K1} current can be too low or even lacking, leading to unstable depolarized (MDP), if compared to the mature CMs. These immature electrophysiological conditions correspond to a spontaneous firing activity or a depolarized resting ($\simeq -20$ mV), respectively. For sake of brevity, we will take into account the worst case of lacking native I_{K1}.

DC is a valid and effective approach to overcoming immature characteristics of hiPSC–CM through the injection of a virtual I_{K1} current. In a closed–loop paradigm the transmembrane potential (V) is acquired through traditional patch clamp and used to compute the voltage-dependent I_{K1}, finally injected into the cell with the additional stimulus current, see e.g. [1,9,15]. DC allows the hyperpolarization of MDP to values suitable to generate a mature AP waveform.

The whole interface protocol and the current injection can be performed *in silico* in a fully computational setting, coupling the I_{K1} mathematical equation used in the real–time simulator with the set of ordinary differential equations (ODEs) describing the dynamics of the ionic currents and the resulting hiPSC–CM electrical activity. Then, different I_{K1} formulations can be tested, comparing the physiological responses and giving a mathematical definition of the waveforms' differences.

The physiological I_{K1} lack in experimental conditions is reached in the *in-silico* framework through the native I_{K1} current suppression. Once the depolarized membrane potential reaches the steady state, a novel I_{K1} current can be added to the total ionic current, taken from different formulations existing in the literature.

2.2 Paci 2020 Ionic Model and I_{K1} Tested Currents

hiPSC–CMs show some relevant differences concerning adult myocytes, requiring a new mathematical approach. The first ionic model based on hiPSC–CMs data was created by M. Paci, who developed a primal model in 2013 [11], creating a new line improved in 2018 [12] and 2020 [13].

Paci generation and every other single–cell model existing in literature focused on the ventricular-like phenotype, the predominant phenotype emerging during the differentiation process. Among them, it is possible to deduce a qualitative primitive atrial–specific model from the Paci2013, even if no atrial–specific current is taken into account. Since the DC technique is generally applied to unknown phenotype cells, we will base this analysis on the most recent ventricular-like model, Paci2020 [13], equipped with an improved calcium dynamic formulation with respect to previous models.

The original Paci2020 model, constrained by experimental data, simulated traces of spontaneous electrical activity. The first test we performed was about the suppression of the native I_{K1}. The lack of the potassium current leads V to a quiescent depolarized resting potential, higher than -20 mV. In DC experiments the injection of the additional current is joined with the injection of the applied current I_{app}, thus we considered an external stimulus pacing the model 1 Hz. According to Fabbri [5], the cell could be elicited because a required amount of I_{K1} could bring V to a stable and hyperpolarized MDP ($\simeq -78$ mV).

Simulations were performed in MATLAB using the ODE function ODE15S. We consider the system to be at steady state after 800 s.

Six different I_{K1} formulations available in the literature were taken into account to carry out the *in silico* DC. Four ventricular–specific formulations were tested, from Ten Tusscher (TT) [14], Fink [6], Grandi [7], O'Hara–Rudy (ORd) [10] human ventricular models. Because of the *a priori* unknown cell phenotype, also two atrial–specific formulations have been analyzed, from Koivumäki (K) [8] and Courtemanche (CRN) [3] human atrial models.

All ionic models considered are available in the CellML repository (link).

According to Fabbri et al. [5], each current formulation was scaled as in Fig. 1. The most recent model, Koivumäki, was considered as the target and every other formulation was normalized in order to obtain the same outward peak current density (0.63 pA/pF).

3 Results and Discussion

As described in [2] and [1], I_{K1} injection considering low densities gave rise to an irregular plateau. In this section, we define a novel mathematical criterion to

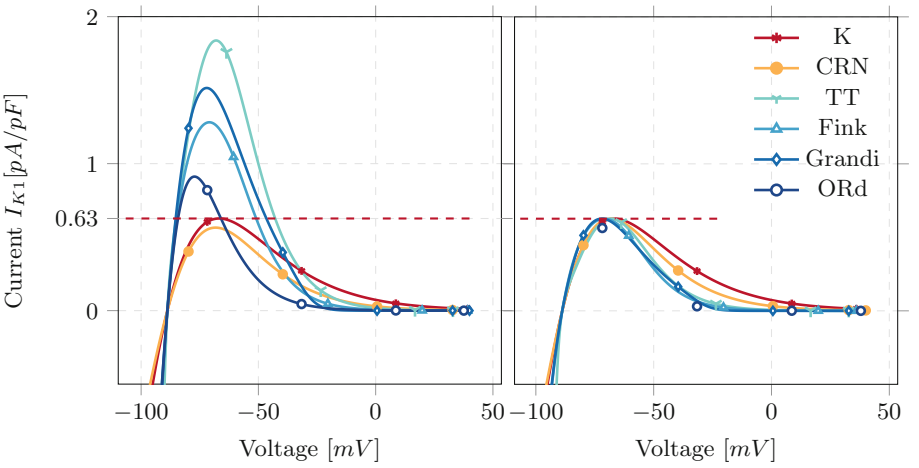

Fig. 1. I_{K1} tested formulations, normalized with respect to Koivumäki outward peak current density (dashed line). Original (left) and scaled (right) currents are shown at steady state, when considering voltages between −120 and 40 mV.

classify the AP morphology and we present resulting curves of some specific AP features (biomarkers) with respect to the injected current density. The comparison with experimental data suggests the choice of AL formulations to improve cell maturation.

3.1 A Novel AP Morphology Classifier

According to *in vitro* AP recordings, see [1], the injection of VL I_{K1} formulations with low densities highlighted a longer AP plateau. From a mathematical point of view, the abnormality corresponds to an extra inflection during phase 3 (repolarization), as depicted in Fig. 2. Thus, considering a subset of the phase 3, we derived the following definition.

Definition 1. *The AP morphology of a hiPSC–CM is physiological if*

$$\frac{d^2V(t)}{dt^2} \leq 0 \qquad \forall t \in [\mathrm{APD}_{40}, \mathrm{APD}_{70}]\,, \tag{1}$$

where APD_X *is the AP duration at* $X\%$ *of repolarization, and the amplitude reference is the difference between the maximum value of* V *and the* MDP.

As described in Fig. 3, every I_{K1} formulation allows the cell to reach a physiological AP morphology, but the required amount of the current is different. Comparing the different results, we observe that atrial I_{K1} formulations, K and CRN, require a lower amount of the injected current to gain a physiological morphology, exactly equal to the normalized current (i.e. 100%). On the other hand, ventricular formulations need a much higher current density to prevent

an abnormal plateau, up to 200% for TT, 300% for Fink, and 600% for Grandi and ORd. Since DC injects an external current, it is reasonable to add as little current as possible, suggesting the use of atrial formulations in the experimental real–time closed–loop.

3.2 Biomarkers Analysis

Since experimental DC is an electronic maturation method, we are interested in evaluating the rate of approximation to an adult human CM when considering the injection of different I_{K1} formulations. To this end, we take into account three different biomarkers: the APD_{30}, the $APDAPD_{90}$, and the membrane diastolic potential (MDP).

In Fig. 4 we present the dependence of these biomarkers on the injected I_{K1} percentage, discriminating between ratios inducing an abnormal AP and ratios supporting a morphological AP shape. The hiPSC–CMs' maturation is then analyzed by comparing these curves with experimental data provided by ORd, [10], referring to a human adult ventricular CM and provided as Mean ± Standard Deviation (St. Dev.).

Every presented biomarker highlights a clear partition between the behaviour of AL formulations (K and CRN) and VL ones.

First of all, MDP portrait (Fig. 4b) suggests that only AL I_{K1} formulations allow the cell to reach almost every experimental value, while the injection of VL currents leads the cell to hyperpolarized values.

Similar observations could concern the APD_{90}, in Fig. 4c: different VL formulations could not reach the experimental bound, except TT. Anyway, this model presents a gap when approaching the experimental bound, and it could perform experimental values only by injecting a huge amount of external I_{K1}.

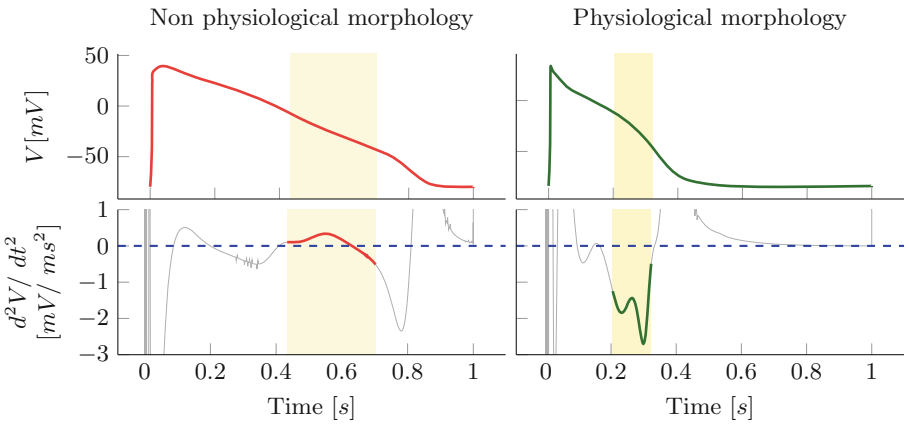

Fig. 2. AP morphology classification: the non physiological morphology (left) presents an extra inflection in the repolarization phase, the physiological morphology (right) is always convex in phase 3.

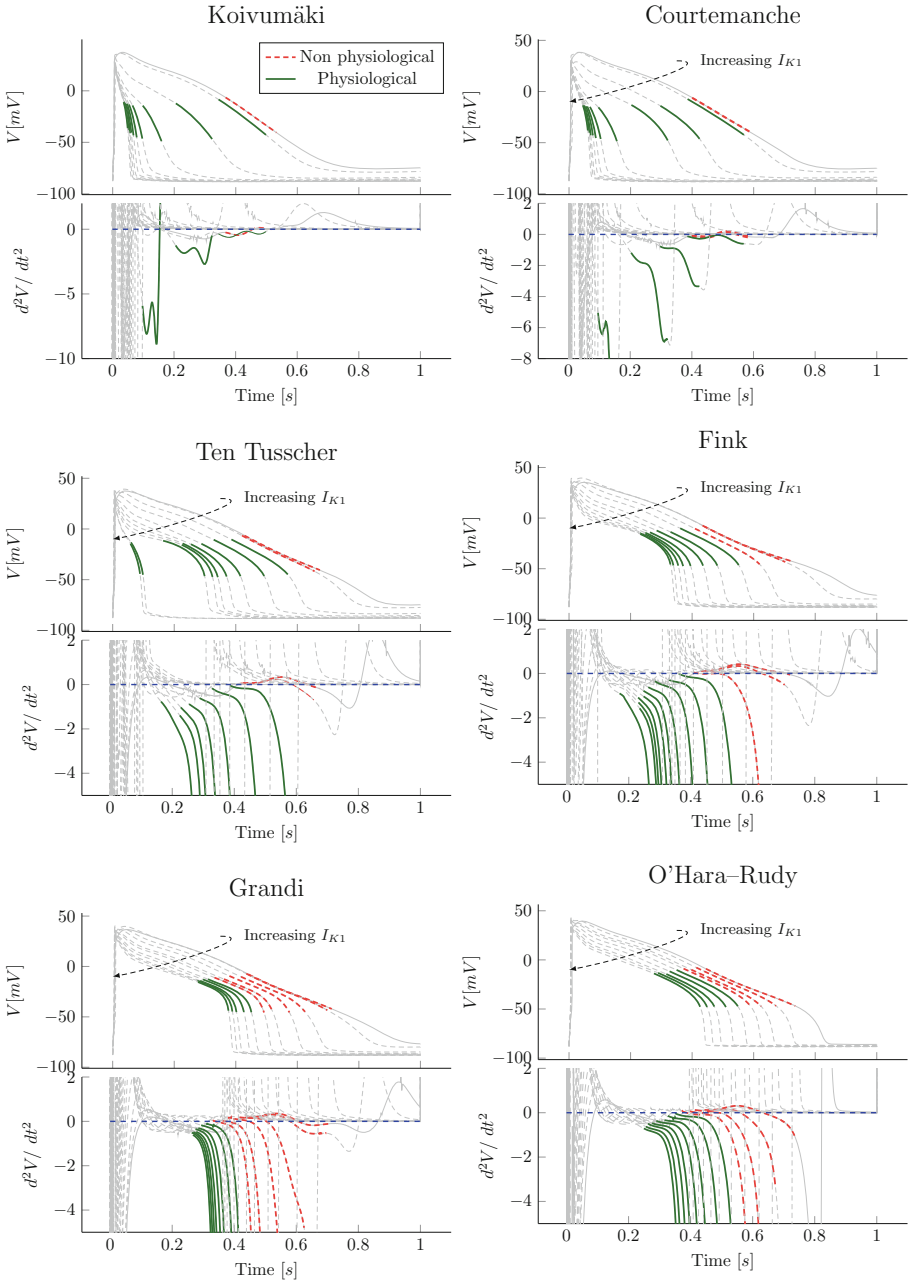

Fig. 3. AP morphologies and relative second derivatives after DC, with the six tested formulations. We considered percentages of the normalized injected current in the set $\{80, 100 : \text{step } 100 : 1300\}$, except for ORd formulation, where the set is $\{200 : \text{step } 100 : 1300\}$, since lower percentages do not trigger an action potential wave.

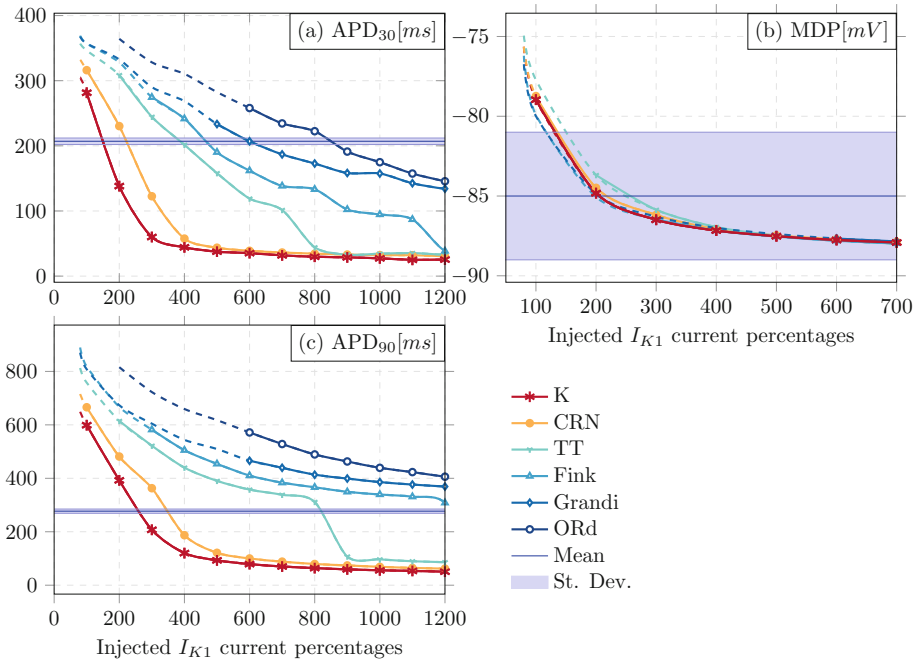

Fig. 4. Biomarkers dependence on the injected current density and comparison with experimental data. The horizontal continuous line and relative bounds stand for the mean and the interval [Mean ± St.Dev.] of ORd experimental data, respectively. Dashed lines refer to current percentages that give rise to non physiological APs.

Finally, VL formulations present the following APD_{30} dynamic (Fig. 4a): they reach the experimental bound, but I_{K1} injected current percentage is too high. Otherwise, when considering both APD_{30} and APD_{90}, K and CRN models allow to reach the experimental bound for lower values of injected current. Among them, K is much better than CRN when considering previous arguments.

4 Conclusion

Briefly, starting from the experimental DC, used for the electronic maturation of stem cells, we implemented an *in silico* tool to perform the current injection as an additional current in the Paci2020 ionic model for VL hiPSC–CMs.

Our numerical simulation shows that the AP morphology changes with respect to the injected current density and the I_{K1} formulation. Thus, we tested six different current models and we defined a mathematical classifier to discriminate a physiological and a non physiological AP repolarization phase.

In conclusion, a virtual analysis of the biomarkers suggests that K and CRN I_{K1} formulations allow the cell to reach AP features comparable with adult and mature values with a minimal amount of additional external current. These

formulations also prevent the cell to show a non physiological morphology for almost any percentage of injected current.

In this work, we considered VL hiPSC–CMs, the main phenotype resulting in cultures. In a future perspective, it could be useful to perform a similar virtual analysis on an AL hiPSC–CMs, whose ionic model, provided by atrial–specific currents, is still missing in the literature.

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