An *In Silico* hiPSC-derived Cardiomyocyte Model Built with Genetic Algorithm

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Abstract

The formulation of *in silico* biophysical models generally require optimization strategies for reproducing experimentally observed phenomena. In electrophysiological modeling, robust nonlinear regressive methods are often crucial for guaranteeing high fidelity models. Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs), though nascent, have proven useful in cardiac safety pharmacology, regenerative medicine, and in the implementation of patient-specific test benches for investigating inherited cardiac disorders. This study demonstrates the potency of heuristic techniques at formulating biophysical models, with emphasis on a hiPSC-CM model using a novel genetic algorithm (GA) recipe we propose. The proposed GA protocol was used to develop a hiPSC-CM biophysical computer model by fitting mathematical formulations to experimental data for five ionic currents recorded in hiPSC-CMs. The maximum conductances of the remaining ionic channels were scaled based on recommendations from literature to accurately reproduce the experimentally observed hiPSC-CM action potential metrics.Near-optimal parameter fitting was achieved for the GA-fitted ionic currents. The resulting model recapitulated experimental action potential parameters such as action potential durations (APD50, APD75, and APD90), maximum diastolic potential and frequency of automaticity. The outcome of this work has implications for validating the biophysics of hiPSC-CMs in their use as viable substitutes for human cardiomyocytes, particularly in cardiac safety pharmacology and in the study of inherited cardiac disorders. This study presents a novel GA protocol useful at formulating robust numerical biophysical models. The proposed protocol is used to develop an hiPSC-CM model with implications for cardiac safety pharmacology.

# Introduction

High fidelity numerical biophysical models have the potential to provide the missing mechanistic link between the experimental observations and their clinical implications. Formulating such models are primarily optimization problems with the experimental data as targets. Metaheuristic algorithms such as genetic algorithms [1] are well suited for this task due to their inherent stochastic and judicious exploration of the solution space. In electrophysiological studies, single- and multi-cell mathematical models have aided efforts at elucidating various biological processes such as perception, cognition and cardiac function, which is the focus of this study.

Human induced pluripotent stem cells (hiPSCs), since their discovery by Yamanaka et al. [2] in 2007, have been instrumental at the development of ethically sound patient-specific human cell and tissue models. These models have in turn allowed scientists to investigate the underpinnings of congenital and drug-induced disorders. A prominent derivative of this cell type is the human induced pluripotent stem cell-derived cardiomyocyte (hiPSC-CM). hiPSC-CMs have recently proven both useful and promising in cardiac safety pharmacology, where these cells are adopted as test benches for studying drug effects on cardiac function [3]. hiPSC-CMs have also been used to better understand drug-induced and inherited cardiac disorders such as Long QT syndrome [4], [5] and catecholaminergic polymorphic ventricular tachycardia (CPVT) [6], [7]. Prior to the advent of hiPSC-CMs, researchers often relied on animal models to make extrapolations of disease effects in human cells. This route possesses a significant caveat of dissimilar genotypic representation by these models. While freshly excised human cardiac cells are the ideal candidates for inductive human cardiac studies, obtaining these uncommon cells is highly invasive relative to hiPSC-CMs, which can even be derived from superficial somatic cells such as skin cells. Although hiPSC-CM is apparently a convenient choice for extensive long- and short-term cardiac studies, its electrophysiology is not well-established. There are ongoing attempts by scientists to better understand these cells and validate them as viable substitutes for human cardiomyocytes. These attempts are mainly *in vitro* and *in silico*. For instance, the Comprehensive In vitro Proarrhythmia Assay (CIPA) initiative, first presented in 2013, stipulates research directions around the use of hiPSC-CM experimental data and mathematical modeling in proarrhythmia risk assessment [8].

Since the pioneering work by Hodgkin and Huxley [9] in 1952 involving the formulation of a biophysical mathematical model of the squid giant axon, there have been numerous attempts at creating biophysical models for different cell types [10]–[13]. These models have been helpful at elucidating the electrical dynamics of native cardiomyocytes. Recently, there have been few attempts at formulating in silico hiPSC-CM models [14]–[17]. However, the inherent variability in these cells pose challenges to deriving formulations that could reproduce the wide range of behaviors of hiPSC-CMs. One recent approach is to combine experimental data from multiple labs to derive model formulations on averaged data [15]. However, care must be taken while combining data from disparate sources because the inconsistencies in cell origins [18], culturing environments, experimental protocols and conditions as well as cell maturation levels [19] may introduce unwanted deviations in the model. This study joins in the efforts at formulating a robust in silico hiPSC-CM model mostly based on the experimental data from a single lab for maintaining phenotypical consistency. In achieving this, we present a novel customizable genetic algorithm protocol employed for near-optimal fitting of model ionic current formulations to experimental data.

Genetic algorithm is a heuristic optimization method inspired by Darwinian evolution and natural selection [1]. The process, similar to the biological counterpart, involves population (i.e., sets of possible solutions termed chromosomes) initialization; crossover, which involves a combinatorial shuffling of parameters (termed genes) about a specified number of pivot points (termed crossover points) between two parent solutions to generate offspring. Offspring solutions may then undergo mutation, typically done in an additive or multiplicative fashion. A fitness criterion must be defined to facilitate the propagation of near-optimal solutions over the specified number of iterations termed generations. Metaheuristic optimization methods offer resilience against local optima and saddle point convergence relative to gradient-based nonlinear optimization methods, such as the Newton-Raphson and Levenberg-Marquardt methods [20]. Genetic algorithm-based parametrization has been used to fine-tune mathematical models of murine myocytes [21], [22] and canine atrial cells [23] to incorporate cell-specific experimental data.

Our previous work [24] demonstrated the feasibility of using genetic algorithm to fit cardiac cell biophysical model formulations. In this study, we demonstrate how the GA-based ionic current fitting outcomes can be further integrated into the biophysical numerical model development process. An improved GA-based parametrization protocol was developed to build a complete *in silico* biophysical model of an hiPSC-CM.

# Materials and Methods

## Preparations of hiPSC-CMs

Human induced pluripotent stem cells usage was approved by the Institutional Stem Cell Research Oversight (SCRO) committee at Masonic Medical Research Institute (MMRI). hiPSCs were maintained on growth factor-reduced Matrigel coated plates in E8 medium with E8 supplement. Cardiac differentiation was induced by 6 μM CHIR99021, 10 ng/ml Activin A and 5 nM rapamycin in RPMI1640 medium containing B27 (minus insulin) and 50 μg/ml L-ascorbic acid (cell culture tested powder) as a basal medium. After 24 hours, media was changed in the same basal medium. The following day cells were kept in RPMI1640/B27 (-insulin) with the addition of 5 μM XAV939 and 10 μM KY0211 for days 2–6 changing media every other day. Afterwards cells were kept in RPMI1640/B27 (+insulin) with 50 μg/mL L-ascorbic acid for days 8–10 followed by a purification medium, RPMI 1640 without glucose and supplemented with 4 mM sodium L-lactate. Cells were kept in purification medium for days 12–14, then back to basal medium of RPMI/B27(+insulin) supplemented with 20 ng/ml triiodothyronine (T3), and 1 μM dexamethasone until day 30. For single myocytes, the monolayers were then dissociated around day 25 with 0.05% trypsin, 1 mg/ml collagenase II and plated onto Matrigel coated dishes. All voltage clamp recordings were made 5 days after recovery. Experiments were typically performed on hiPSC-CMs at least 20 days post- differentiation.

## Voltage Clamp Recordings

Whole-cell patch clamp recordings were obtained for five ionic currents namely, fast sodium current, INa; transient outward potassium current, Ito; L-type calcium current, ICaL; rapid delayed rectifier potassium, IKr; and hyperpolarization-activated current, If. Figure 1 presents experimental current-voltage (IV) plots for all five currents.

INa was measured as described previously [25]. Briefly, the bath solution consisted of 2mM CaCl2, 10mM glucose, 1mM MgCl2, 105mM N-Methyl D Glucamine (NMDG), 40mM NaCl, and 10mM HEPES free acid. pH adjusted to 7.4 with HCl. Also, a 300μM CdCl2 is added to block the calcium currents which may interfere with INa recording. The pipette solution was composed of 1mM MgCl2, 15mM NaCl, 5mM KCl, 120mM CsF, 10mM HEPES, 10mM EGTA. Before the experiments, 5mM Na2ATP was added and pH adjusted to 7.2 by addition of CsOH. INa was recorded by applying command voltages (50ms-long pulses) in steps of 5mV over the range of -80mV to 35mV from a holding potential of -120mV. All INa measurements (n=8-15) were taken at lower extracellular (bath) sodium concentration of 40mM to ensure adequate voltage control. Temperature and concentration extrapolation facilitated by the Q10 (temperature adjustment factor) correction and Goldman-Katz constant field equation was predicted by Cordeiro et al. [25] to be a factor of 7 and was subsequently adopted in INa curve fitting. More details about the INa experimental protocol can be found in [25]. IKr was recorded as described previously in [26]. In recording the potassium currents, the conventional K+ pipette solution of 90mM K+-aspartate, 45mM KCl, 10mM NaCl, 1mM MgCl2, 10mM HEPES, 5mM EGTA, 5mM MgATP and pH of 7.2 (maintained by added KOH) was used. IKr was recorded by applying 300ms long test pulses between -40mV and +60mV in steps of 20mV from a -80mV holding potential as described in [27]. ICaL was recorded using 300ms long test pulses applied between -40mV to 60mV in steps of 10mV from a holding potential of -40mV. Ito was measured as described in [28]. Briefly, the voltage clamp protocol consisted of a holding potential of -80mV, followed by a brief -50mV potential to ensure all sodium channels were inactivated. This is important as Ito closely follows the depolarization facilitated by sodium influx. Test pulses were then applied in steps of 10mV from -40mV to 50mV for each clamp voltage. The half-inactivation voltage of Ito, and a slope, was used in our model based on previous studies [28]. If was recorded using a holding potential of -40mV, followed by pulses from -110mV to -40 in steps of 10mV.

All voltage-clamp recordings were made using a MultiClamp 700A (Molecular Devices, Sunnyvale, CA). Whole cell patch pipettes were made from glass capillary tubes (1.5mm O.D., Fisher Scientific, Pittsburg, PA) and pulled on a gravity puller (Narishige Corporation, East Meadow, NY). The resistance ranged from 1.0-3.0 MΩ and electronic compensation of series resistance was applied (~60–70%). Capacitance of the hiPSC-CMs was measured by applying −5 mV voltage steps. Signals were acquired at sampling rate of 10–25 kHz, filtered at 4–6 kHz and digitized with a Digidata 1322 converter (Molecular Devices, Sunnyvale, CA). Acquisition and analysis were performed using pClamp9 software. All hiPSC-CM experiments were performed at 36 °C except peak INa which was recorded at room temperature (20 °C).

Pooled data are presented as Mean ± SEM. Statistical analysis was performed using an ANOVA followed by a Student–Newman–Keuls test using SigmaStat software.

## Mathematical Model of hiPSC-CM

Formulations from six existing cardiac cell models were adopted based on being representative of human cardiomyocyte electrophysiology and/or reproducing spontaneous activity. The formulations of five key currents (INa, Ito, ICaL, IKr and If) were optimized by genetic algorithm based on the experimental data acquired in our lab. The rest of the current formulations, namely ultrarapid and slow delayed potassium rectifier currents (IKur and IKs), sodium-calcium exchanger current (INCX), sodium-potassium exchange pump current (INaK), calcium pump current (IpCa), background sodium and calcium currents (IbNa and IbCa), acetylcholine-activated inward-rectifying potassium current (IKAch), and inward rectifier current (IK1) were adjusted through proportional scaling based on the published literature. The INa formulation was adopted from Luo-Rudy (LRd) model [11] which is a widely adopted mammalian ventricular myocyte model formulated based on the Hodgkin-Huxley formalism. The Ito formulation was adopted from the Grandi-Pandit (GP) human atrial cell model built by Grandi et al. [29] by modifying an earlier human ventricular cell model [30] built by the same group. The choice of the GP model for Ito was due to the atrial-like AP seen in whole-cell patch clamping experiments performed by Cordeiro et al. [28]. The If formulation was adopted from human cardiac Purkinje cell model by Stewart et al. [31]. ICaL and IKr were formulated based on mammalian sinoatrial nodal cell model by Kurata et al. [12]. The choice of this model is due to the inherent automaticity exhibited by hiPSC-CMs similar to that of nodal cells. The intracellular calcium dynamics in hiPSC-CMs is similar to the nodal cells due to lack of mature T-tubules structure [32]. Therefore, the intracellular calcium handing (involving the Ca2+ release and uptake fluxes between the sarcoplasmic reticulum (SR) and the cytosol as well as the intra-SR Ca2+ transfer flux occurring between the junctional SR and network SR) were adopted from Kurata model as well. Figure 2 presents a schematic of our hiPSC-CM model depicting the constituent ionic currents and fluxes. Table 1 lists references to adopted ionic current formulations. The transmembrane voltage, *Vm*, is given by the following first order differential equation.

(1)

is the membrane capacitance and is an instance of the 14 ionic current formulations constituting the model in this study.

## Numerical Implementations: Ionic Current Formulations and Whole Cell Integration

In the *in silico* implementation of current formulations, computational equivalents of the experimental voltage clamping protocols for the five ionic currents mentioned earlier were implemented. An initial Forward Euler implementation was done for all five currents, various time steps were tried and where a sufficiently small time step for computational stability meant prolonged runtime, as in the case of the rapid INa and ICaL currents, we deferred to an adaptive solver. The INa model was simulated using the implicit backward difference (BDF) integration [33] by implementing voltage test pulses from -80mV to +35mV in the steps of 5mV with a duration of 25ms (similar to experimental protocol). The peak inward current values were then identified and used to produce IV plots. The BDF employed an adaptive computational time step; where signal gradients were high (as in the case of the rapid depolarization), a suitably small time-step is automatically adopted and vice versa. This allowed for an accelerated computational runtime with minimal approximation error in single run and multi-generational GA fitting processes. The IKr voltage clamp simulations relied on a forward Euler integration with computational time step of 0.2ms. The chosen time values ensured a trade-off between high resolution time discretization and extended time spans. Voltage pulse intensities applied from a -80mV holding potential ranged from -40mV to 60mV in steps of 20mV at a 300ms pulse duration. Peak IKr tail currents were measured at -50mV. Voltage clamping for ICaL was simulated using the implicit, adaptive time step, BDF integration. The characteristic equations were modified based on Ten-Tusscher et al. [34] to allow the dependence on a dynamic Ca2+ reversal potential, rather than the fixed-Ca2+-potential formalism adopted in the original Kurata model [12]. Application of 500ms voltage pulses ranging from -40mV to 60mV were applied from a holding potential of -30mV, similar to the experimental protocol. Ito voltage clamp simulations were performed by the explicit forward Euler integration with a fixed time step of 0.5ms. Voltage pulse intensities applied from a holding potential of -80mV followed a brief -50mV potential (as described in the experimental protocol) ranged from -40mV to 50mV in steps of 10mV. Pulse duration of 500ms was adopted. If implementation was executed by the forward Euler integration with a fixed time step of 10ms. 500ms long voltage pulses were applied from a holding potential of -40mV over -110mV to -40mV range in 10 mV increments.

We performed whole cell simulations using the implicit Radau adaptive integration method provided by the solve\_ivp module in the SciPy python package. Alternatively, we implemented a faster C/C++ version, where a forward Euler integration was employed.

To enable pacing of the model by external stimulus of varying frequencies, the spontaneous firing of APs was disabled by decreasing the maximum conductance of If by 50%. The effects of adrenergic stimulation using isoproterenol were simulated by altering the maximum conductance of five currents as described previously [35]. Briefly, conductance of L-type Ca2+ channel (ICaL), Na+/K+ pump (INaK), NA+/Ca2+ exchanger (INaCa) and SR Ca ATPase (SERCA) were upregulated by 100%, 30%, 30% and 20%, respectively, while IK1 was downregulated by 20%. The effects of vagal stimulation using carbachol (CCh) were simulated by scaling the maximum conductance of IKACh by 200%. The effects of 4-aminopyridine (4-AP) were simulated by varying levels of IKur block.

## Model Parametrization

Characteristic equations for the five currents were parametrized over which the optimization was done. For each ionic current optimization, parametrization was executed by converting scaling coefficients ) and half-activation/ inactivation voltages () and slopes () of the sigmoidal gating equations into free parameters as presented in Equation 2 below.

(2)

Parameter sets for each current were bundled as chromosomes and optimized heuristically by the genetic algorithm (described next). Accordingly, 20, 18, 20, 6, and 7 free parameters were created for equations of INa, Ito, IKr, ICaL and If, respectively. Detailed parametric equations for each current are given in the Online Supplement.

## The Genetic Algorithm Protocol

Myriads of GA protocols can be formulated by manipulating population size, fitness criterion, number of generations, crossover, and mutation schemes. A description of the GA protocol adopted, and its implementation are discussed in this section.

A starting population was initialized by generating individuals composed of genes selected randomly from a uniformly distributed interval. Constraints (interval limits) were chosen such that the existing model parameter values were contained in the range. The rationale behind this was to initialize the search from a physiologically feasible solution space, as there exists the caveat of multiple individual solutions producing the same/ similar model effects. The initial population is, thus, governed mathematically by;

(3)

where is the stochastic-drawn initial population parameter set, is the initial population gene range width determination constant and is the original model parameter. The condition was applied to genes in all chromosomes in the generation. Superscripts in format through this paper indicates the generation number.

Population size of 10 × *Chromosome size* was chosen based on the recommendation by Storn et al. [36]. However, an exception of reduced population size was made in the case of extended computational runtime, which in turn was limited by the number of processors available (28 cores per node for the Ohio Supercomputer (OSC) HPC clusters [37] and 24 cores per node for the Extreme Science and Engineering Discovery Environment (XSEDE) HPC [38]). A detailed algorithmic description of the GA protocol used in fitting the ionic currents is presented in Figure 3.

The SSE loss or RMSE loss for the IV plot points between model output and experimental data was adopted as the objective function to be minimized to ensure a robust curve fitting. For adaptive time-step, ordinary differential equation (ODE) solvers available in the SciPy solve\_ivp python module – implicit backward difference and Radau [39] methods as well as explicit Runge-Kutta methods [40][41] – were used. The ionic current models were implemented as importable modules to allow parallel computation using the python multiprocessing module.

Mathematically, the objective function for individual fitness computation, can be expressed as;

(4)

(5)

where is symbolic of a parameter set, *X* is a set of experimental data points and represent the ionic current modeled as a function of the parameter set and *T* is the number of data points (IV plots).

Single- and multi-point crossover scheme was adopted in fitting the five currents. For each offspring production, crossover points were stochastically drawn from a uniformly distributed interval of positive integers not exceeding the chromosomal length. The convention was to use a larger number of crossover points to compensate for a small population size relative to the number of parameters. Crossover involved the selection of multiple mating pairs from the pool of the best performing individuals. Genes in these mating pairs were then shuffled about the selected crossover-points. The convention chosen here was to generate the same number of offspring as the number of crossover points. This convention, though arbitrary, was sufficient at producing reasonable fitting. It, however, does not indicate the maximum allowable number of unique offspring. The mutation protocol adopted in this work is an additive scheme involving addition of positive or diminutive proportion of the present offspring parameters to the offspring. The value of these proportions, like in the case of the initial population generation, are randomly selected from a uniformly distributed range. This can be expressed in a matrix form as:

(6)

where *M* is the resulting mutant matrix, *O* is the offspring matrix, and is the proportion matrix. The constraints (mutation coefficient range width determination constant) for the elements in the proportion matrix is given mathematically as:

(7)

The proportion-offspring multiplication in Equation 6 is not a traditional matrix multiplication, but rather the Hadamard element-wise multiplication ( and are row and column indices respectively whereas is generation/ iteration number). This way, multi-gene mutations involving all genes per chromosome are executed by random proportions simultaneously. After the two operations – element-wise multiplication and matrix addition, the mutant matrix picks on the offspring size, ; where is the parent population size. The parent-offspring-mutant ratio is therefore 1:: and consequently the population size, . A summary of the various GA protocols (including) adopted in curve fitting for the five experimental-data-complemented ionic currents are presented in Table 2. In all fittings, the GA was run for 100 generations.

To quantitatively ascertain the goodness of fit at the end of the GA, the coefficient of determination, , was used. This statistical measure gives the degree of variability in data accounted for by the fitted model. The mathematical formula for is given in Equation 8. This value mathematically typically ranges between 0 and 1 (it may also be negative in the case of nonlinear regression), with 1 interpreted as an absolute fit accounting for 100% of the variability in data captured by the fitted model,

(8)

Where is experimental (actual) current values, is the model fitted current value, is the mean of the experimental values. SSE and SST are the model residual sum of squared error and total sum of squared errors for the experimental data, respectively.

Once the above five currents were optimized based on GA, the rest of the currents were manually scaled based on published literature to obtain the experimentally recorded AP morphology. The scaling used for the ionic currents and cell related constants are listed in Tables S6 and S7 in the Online Supplement. Code Implementation of the GA parameter optimization and the hiPSC-CM biophysical model are available at <https://github.com/Adakwaboah/hiPSC-CM_Computational_Model>.

# Results

## GA-based Optimizations

Using the protocols described in Methods, parameters of the five ionic current formulations (INa, Ito, ICaL, IKr and If) were optimized to reproduce the experimental data. The GA process for each ionic current being fitted were performed multiple times to ensure consistency and reproducibility of this meta-heuristic. For each of the fittings the initial and final model IV plots (representative) are shown in Figure 4 (left panels). In addition, representative time course plots for the fitted model have been shown to validate the fitting outcomes as physiologically relevant (middle panels). The right panels in Figure 4 show the near-optimal parameter convergence in the form of losses over 100 generations for the various GA trails per current. The apparent non-uniform decreasing trend in loss plots is evident of the stochastic yet perpetual search for the global minimum. Table 3 summarizes the initial and final fitted loss metrics (mean and standard deviations for multiple trials, n=5) for all currents. Improvement in fitting can be seen in the increasing R2 values toward unity. The original and fitted parameter values for all currents along with corresponding detailed formulations are given in the Online Supplement (Tables S1-S5). Figures S1-S5 in the Online Supplement compare the current activation/inactivation kinetics and corresponding time constants in our optimized model with two recent hiPSC-CM numerical models, namely, Paci et al. [14] and Kernik et al. [15]. Computational runtimes for single GA trial over the 100-generation period with parallel fitness computation over 28 processors were 11 min, 13 min, 7 min, 12 min, and 12 min for INa, ICaL, If, Ito, and IKr respectively.

## Simulated Action Potentials

The model was simulated for 10 mins to achieve the steady states for all current, fluxes and ionic concentrations. The steady state (initial conditions) values of the model variables have been reported in Table S8 of the Online Supplement. Figure 5 shows a comparison of spontaneous action potentials (APs) simulated by the hiPSC-CM model as compared to the experimentally recorded ones and corresponding calcium transients. Our model was able to accurately reproduce the experimentally observed AP morphology as well as automaticity of hiPSC-CMs. Time course metrics such as the AP duration at 50%, 75% and 90% repolarization, i.e., APD50, APD75, and APD90, respectively; cycle length (CL), i.e., AP peak-to-peak duration which includes the diastolic resting phase duration; maximum diastolic potential (MDP), beats per minute (BPM) were used to assess the model AP morphology compared to the experimental counterpart. Table 4 presents the comparison of AP metrics produced by the model to those recorded in experiments, whereas Table 5 lists the calcium transient parameters such as rise time, decay time and maximum amplitude. Table Sxx in the Online Supplement compares the AP metrics and calcium transients of our model to those of Paci et al. [14] and Kernik et al. [15]. It should be noted that the experimental AP traces used in our model differ considerably than those used in the other models. Figure 6 shows the spontaneous AP generation and corresponding transient plots of the constituent ionic currents. Figure 7 shows the intracellular ionic concentrations ([Na+]i, [K+]i and [Ca2+]i) as well as Ca2+ concentrations in NSR ([Ca2+]up), JSR ([Ca2+]rel) and subspace ([Ca2+]sub) during spontaneous APs. Figure S6 in the Online Supplement shows various transient ionic currents during multiple spontaneous AP firing. Figure S7 presents a comparison of spontaneous APs vs. stimulus-elicited APs and corresponding calcium transients in our model. The stimulus-elicited APs have steeper Phase 0 depolarization and higher AP magnitude due to higher INa amplitude. The diastolic depolarization seen in spontaneous APs is absent in paced APs due to partial block of If. However, the amplitude of calcium transients is higher in spontaneous APs than the paced APs.

## Sensitivity Analysis

To analyze the sensitivity of the baseline hiPSC-CM model, the maximum conductances of the various ions currents were varied from 0% (complete block) to 200% (2× enhancement) and their effects on the AP parameters such as APD (APD50, APD75, APD90), AP magnitude, MDP, CL and spontaneous firing rate (BPM) were studied. Scaling factors from 0% to 200% of the baseline channel conductance values were used in computing the correlation coefficients between various AP parameters and corresponding ionic current alteration. Figure 8 shows the outcome of the systematic sensitivity analysis of the model. The figure shows a strong positive correlation coefficient between MDP and the ionic currents If and Ito which indicates that an increase in either of these currents causes an increase in the MDP. IKACh on the other hand shows a strong negative correlation with MDP while showing a positive correlation with CL. IKr expectedly shoes a strong negative correlation with APD (APD90, APD75, and APD50). This also has implications on the CL which is revealed by a strong negative correlation. Varying the INa reveals its strong effect on the AP amplitude corroborated by a strong positive correlation coefficient. It however shows an almost neutral correlation with the APD. If shows a strong positive correlation with MDP implying a direct relationship between the two. A similar relationship exists between If and BPM. If also has a strong negative correlation with CL. The IKACh shows a strong negative correlation with MDP and APD while showing a positive correlation with CL.

Figure 9 shows specific cases of ionic current blockades and their effects on the AP morphology (Panels A-F), AP durations (Panels G-I), and CL (Panels J-L). IKr is the principal repolarization current in the rapid repolarization phase in hiPSC-CMs and its blockade prolongs the AP in the Phase 3 as shown in Panel A. With varying extent of IKr block, APD successively increases (Panel H) and cycle length decreases (Panel K). Figure 9B shows the reduction in AP magnitude as a result of INa block. Excessive blockage of INa (beyond 40%) however prevents the initiation of a spontaneous AP. The pacemaker current, If, plays a major role in maintaining spontaneous activity. A slight block in this current reduces the frequency of automaticity, lowers the MDP and the magnitude of AP (Panel E). More severe blocks (beyond 50%) of If inhibits the spontaneous AP generation. Blocking ICaL significantly shortens the AP (Panels F and I) thereby reducing the CL (Panel L). Blocking IKACh prolongs the AP in Phase 2 and 3 as shown in Panels D and G. Subsequently, there is elevation of MDP (not shown) with higher extent of IKACh block. Interestingly, although AP was prolonged, the CL was seen decreasing monotonically with the extent of IKACh block (Panel J). It was observed that in the diastolic phase (Phase 4), a reduced repolarization effect of IKACh favors diastolic depolarization offered by If.

We further investigated the contribution of two atria-specific currents, namely, IKur and IKACh, which have been recorded in atrial-like and nodal-like hiPSC-CMs [42]. Blocking IKur as a result of simulating the effects of 4-aminopyridine (4-AP; 50 µM) reduced phase-1 repolarization resulting in AP prolongation and increased AP magnitude as shown in Fig 10A. The extent of AP prolongation was proportional to the extent of IKur block. The spontaneous firing rate (bpm), however, remained unchanged. The AP prolongation in all phases of repolarization in our model is in agreement with the recent experimental findings [43]. Figure 10B shows the effect of vagal stimulation by carbachol (CCh; 10 µM). The 200% enhancement of IKACh slowed down the spontaneous activity by xx% without any significant effect on the APD.

Figure 11A shows the model behavior in hyperkalemia conditions. Our model showed increased spontaneous firing rate and diastolic depolarization when extracellular K+ was increased from 5.4 mM to 8 mM. This behavior is in agreement with several experimental studies in nodal cells [44]–[46]. Figure 11B shows the effects of completely blocking INaCa. Inhibition of INaCa reduces the spontaneous firing rate and hyperpolarizes the membrane potential in our model. However, it does not abolish the spontaneous activity as reported in Kim et al. [55].

3.4 Delayed Afterdepolarizations (DADs):

The model was challenged with stressors such as isoproterenol stimulation and elevated extracellular calcium levels for occurrence of DADs. The model was burst paced at 5 Hz for 5 seconds to overload the SR with Ca2+ in presence of isoproterenol effects. The model exhibited several spontaneous DAD-triggered APs post burst-pacing as shown in Figure 12A (black arrows). Figure 12B shows the model behavior in hypercalcemia conditions ([Ca2+]o = 6 mM). The model AP exhibited oscillations (black arrow) after 2.5 sec which temporarily suppressed the spontaneous activity. The spontaneous AP resumed after the intracellular Ca2+ levels lowered. During the quiescent period, the NCX works in forward mode to extrude the excessive Ca2+, thus causing an inward current which slowly depolarizes the membrane and eventually restarts the spontaneous firing of APs.

# Discussion

We present a robust approach to fit experimental electrophysiological data to theoretical formulations in order to develop high-fidelity numerical biophysical models. We demonstrate the effectiveness of genetic algorithm-based optimization method to develop in silico model of hiPSC-CMs that accurately reproduces the experimental measurements. The model behavior was extensively validated based on action potential morphology, sensitivity analysis and various ion channel blocking mechanisms.

The experimental electrophysiological data in hiPSC-CMs shows a wide range of variability, presumably due to the different techniques used to direct the hiPSCs to the cardiac lineage [47]. This imposes additional challenges in fitting the experimental data to model equations. We utilized the GA method to attain model optimization which is an evolutionary metaheuristic method inspired by Darwinian evolution and natural selection. Optimization, by this approach, is executed in a stochastic combinatorial manner which makes it less susceptible to getting stuck at local minima, unlike the gradient-based methods, and tend to converge at the global optimized solution [1]. Previous attempts at implementing GA-based fitting of cardiac models mostly focused on simple fitting of maximum channel conductance to recapitulate the desired AP morphology [21]–[23], [48], [49]. We, on the other hand, performed GA optimization at the underlying ionic current level, which is more realistic and robust approach. Our approach ensures that the model ionic formulations adhere correctly to the experimental recordings and is especially more suitable for modeling hiPSC cells which exhibit a wide range of phenomenological variation. Smirnov et al. [48] modified the GA protocol by Bot et al. [22] by adopting vector mutation terms from the Cauchy distribution that promote drastic variance between the mutants and their uncorrelated parent proportions. Tomek et al.[49], on the other hand, used a multiobjective GA approach for parameter fitting. These works, however, do not mention constraining the range of each gene to a defined neighborhood of the respective original model parameters. Since, uniqueness of solution is often not guaranteed in such non-convex optimization problems, physiological relevance must be upheld. We address this by imposing the customizable constraint, λ𝑎, which symmetrically bounds the range from which the initial parameter values (from published physiologically validated models) are drawn. The significance of this constraint, in conjunction with the population size, is the ability to define the initial search resolution. A wider unconstrained parameter range is likely to be initially underrepresented if the population size is not large enough, which in turn may favor speed of convergence (offered by extreme chromosomal variance) over physiological relevance. Another novelty in our GA protocol, to the best of our knowledge, is the correlation between the parent proportion and the number of crossover points. To maintain sufficient diversity, more offspring and mutants are produced as the number of crossover points increase. Furthermore, an increase in the crossover points offer an extensive combinatorial gene shuffling during crossover per generation; we therefore propose a proportionate increase in the population size to accommodate the resulting diverse offspring and mutants.

There have been very few attempts at implementing hiPSC-CM biophysical models due to inconsistent experimental data. Earlier model by Paci et al. [15] was formulated based on limited data at that time. More recent models by Paci et al. [13][50] and Koivumaki et al. [16] incorporated more realistic calcium handling. The study by Kernik et al. [14] adopted experimental data from multiple sources in an attempt to cover the range of variability seen in these cells. However, the unresolved inconsistencies in recording and clamping protocols used by the various sources have the potential to introduce unwarranted deviations in the hiPSC-CM electrophysiological parameter range as well as the generalizability of the baseline model. Our model recapitulates experimentally recorded hiPSC-CM AP morphology with high fidelity. Moreover, it is able to qualitatively reproduce the experimentally reported [51] effects of: i) APD prolongation caused by IKr block, ii) Reduction in AP magnitude and rate of change of upstroke voltage as a result of Na+ channel block, iii) loss of notch-shaped AP (Phase I repolarization) due to Ito blockade, iv) triangulation of AP due to IKr or Ito blockades, v) significant AP shortening due to ICaL block, vi) loss of automaticity as a result of If blockade, and vii) alterations in spontaneous firing rate as a result of INCX block and hyperkalemia conditions. Our model was also able to produce the experimentally observed effects of channel blocks on frequency of spontaneous APs (cycle length) and maximum diastolic potentials in hiPSC cells. In presence of adrenergic stimulation and hypercalcemia, our model was able to generate DAD-induced triggered activity as a result of SR Ca2+ overload.

One of the advantages of our model over the existing ones is the inclusion of atria-specific ionic channels, IKur and IKACh. which have recently been found to be present and functional in hiPSC-CMs [52]. The inclusion of IKACh allows for the investigation of the variability in the spontaneous beating frequency influenced by parasympathetic influences and/ or the presence of acetylcholine which have been found to reduce the heart rate. Our model can qualitatively reproduce the effects of atria-specific drugs such as carbachol and 4-AP. IKACh is actively involved in maintenance of atrial fibrillation, including chronic atrial fibrillation [53]. Recent advances in cell differentiation techniques use IKACh and IKur as markers to identify atrial-like hiPSC-CMs which were preferentially produced by retinoic acid treatment [54]. the atrial-like CMs are being increasingly used for disease modeling and pre-clinical screening of antiarrhythmic drugs [43]. As such, our model is valuable in disease modeling and simulations of atrial phenotypes.

We adopted simpler Hodgkin-Huxley type current formulations to limit the number of optimization parameters and to keep the simulations computationally tractable. It also avoided overfitting of the experimental data. The main aim of this study was to demonstrate the feasibility of GA-based parametrization of model equations which was done by optimizing five ionic current formulations based on experimental data. The remaining components of the model, including the intracellular calcium handling were adopted from nodal cell model which has a very close morphological resemblance with the hiPSC-CMs. Nonetheless the GA-based optimization can be seamlessly extended to the whole cell level as more and more experimental data becomes available. The calcium handling in our model is based on nodal cell formulations and may not represent true calcium transients in hiPSC-CMs. Our model is able to reproduce DADs, but does not produce other arrhythmogenic events such as EADs and alternans. Our model also does not reproduce the hypocalcemia-induced slowing of spontaneous activity as shown by Kim et al. [55]. Notwithstanding these limitations, our model behavior is consistent with the findings of various in vitro drug tests, in which such arrhythmic markers were observed only in a portion of the pluripotent cells used for testing [55].

# Conclusion

hiPSC-CMs have received significant attention lately with applications in regenerative medicine, cardiac safety pharmacology and the implementation of patient specific models for studying drug-induced and inherited cardiac disease. We present a genetic algorithm-based model parametrization methodology to incorporate experimental data into numerical models. The proposed method was utilized to formulate a biophysical computer model of hiPSC-CMs based on experimental data and available literature, as a potential tool for studying and simulating the dynamics of hiPSC-CM electrophysiology. Ionic current formulations of five key currents, namely, fast sodium current (INa), transient outward potassium current (Ito), L-type calcium current (ICaL), rapid delayed rectifier current (IKr) and hyperpolarization-activated current (If) – were optimized by the genetic algorithm protocol to fit to the experimental data. This was then combined with adjusted formulations for nine other currents imported from existing models to faithfully reproduce experimentally obtained hiPSC-CM action potential morphology and spontaneous activity. The model was able to accurately reproduce the experimentally recorded AP characteristics and channel blocking drug effects. The outcome of this work has implications on validating the biophysics of hiPSC-CMs in their use as viable substitutes for human cardiomyocytes. Specifically, in the study of inherited cardiac disorders and in cardiac safety pharmacology, where drug-induced cardiac disorders are investigated.

# Supplementary Materials

Supplementary materials include detailed equations for model ionic currents and final optimized parameter values. Supplementary material also includes maximum conductance values of the unoptimized currents, cell-related constants, and steady state values of the model variables.

# Author Contributions

ADA performed model formulations, genetic algorithm-based parameterization and model simulations. BT performed single cell simulations and sensitivity analysis. PY performed model assessment and data analysis, JAT and JMC performed electrophysiology experiments and experimental data analysis, JMC also provided experimental and clinical guidance wherever needed. MBH reviewed and modified the mathematical formulations. MD conceived the study, assessed the model, guided the students and confirmed the model outcomes.

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# References

[1] A. E. Smith, M. Gulsen, and D. M. Tate, “A genetic algorithm approach to curve fitting,” *Int. J. Prod. Res.*, vol. 33, no. 7, pp. 1911–1923, 1995.

[2] K. Takahashi *et al.*, “Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors,” *Cell*, vol. 131, no. 5, pp. 861–872, Nov. 2007.

[3] G. Gintant *et al.*, “Use of Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes in Preclinical Cancer Drug Cardiotoxicity Testing: A Scientific Statement From the American Heart Association,” *Circ. Res.*, vol. 125, no. 10, pp. e75–e92, 2019.

[4] A. Moretti *et al.*, “Patient-specific induced pluripotent stem-cell models for long-QT syndrome,” *N. Engl. J. Med.*, vol. 363, no. 15, pp. 1397–1409, 2010.

[5] T. Egashira *et al.*, “Disease characterization using LQTS-specific induced pluripotent stem cells,” *Cardiovasc. Res.*, vol. 95, no. 4, pp. 419–429, 2012.

[6] A. Fatima *et al.*, “In vitro modeling of ryanodine receptor 2 dysfunction using human induced pluripotent stem cells,” *Cell. Physiol. Biochem.*, vol. 28, no. 4, pp. 579–592, 2011.

[7] C. B. Jung *et al.*, “Dantrolene rescues arrhythmogenic RYR2 defect in a patient-specific stem cell model of catecholaminergic polymorphic ventricular tachycardia,” *EMBO Mol. Med.*, vol. 4, no. 3, pp. 180–191, 2012.

[8] G. Gintant, B. Fermini, N. Stockbridge, and D. Strauss, “The Evolving Roles of Human iPSC-Derived Cardiomyocytes in Drug Safety and Discovery,” *Cell Stem Cell*, vol. 21, no. 1, pp. 14–17, 2017.

[9] A. L. HODGKIN and A. F. HUXLEY, “A quantitative description of membrane current and its application to conduction and excitation in nerve.,” *J. Physiol.*, vol. 117, no. 4, pp. 500–44, Aug. 1952.

[10] D. Di Francesco and D. Noble, “A model of cardiac electrical activity incorporating ionic pumps and concentration changes,” *Philos. Trans. R. Soc. London. B, Biol. Sci.*, vol. 307, no. 1133, pp. 353–398, 1985.

[11] C. H. Luo and Y. Rudy, “A dynamic model of the cardiac ventricular action potential: I. Simulations of ionic currents and concentration changes,” *Circ. Res.*, vol. 74, no. 6, pp. 1071–1096, 1994.

[12] Y. Kurata, I. Hisatome, S. Imanishi, and T. Shibamoto, “Dynamical description of sinoatrial node pacemaking: Improved mathematical model for primary pacemaker cell,” *Am. J. Physiol. - Hear. Circ. Physiol.*, vol. 283, no. 5 52-5, pp. 2074–2101, 2002.

[13] M. Courtemanche, R. J. Ramirez, and S. Nattel, “Ionic mechanisms underlying human atrial action potential properties: Insights from a mathematical model,” *Am. J. Physiol. - Hear. Circ. Physiol.*, vol. 275, no. 1 44-1, 1998.

[14] M. Paci *et al.*, “Automatic optimization of an in silico model of human iPSC derived cardiomyocytes recapitulating calcium handling abnormalities,” *Front. Physiol.*, vol. 9, no. JUN, Jun. 2018.

[15] D. C. Kernik *et al.*, “A computational model of induced pluripotent stem-cell derived cardiomyocytes incorporating experimental variability from multiple data sources,” *J. Physiol.*, vol. 597, no. 17, pp. 4533–4564, 2019.

[16] M. Paci, J. Hyttinen, K. Aalto-Setälä, and S. Severi, “Computational models of ventricular-and atrial-like human induced pluripotent stem cell derived cardiomyocytes,” *Ann. Biomed. Eng.*, vol. 41, no. 11, pp. 2334–2348, 2013.

[17] J. T. Koivumäki *et al.*, “Structural immaturity of human iPSC-derived cardiomyocytes: In silico investigation of effects on function and disease modeling,” *Front. Physiol.*, vol. 9, no. FEB, Feb. 2018.

[18] H. S. Hwang *et al.*, “Comparable calcium handling of human iPSC-derived cardiomyocytes generated by multiple laboratories,” *J. Mol. Cell. Cardiol.*, vol. 85, pp. 79–88, Aug. 2015.

[19] K. H. Narsinh *et al.*, “Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells,” *J. Clin. Invest.*, vol. 121, no. 3, pp. 1217–1221, Mar. 2011.

[20] R. R. Rhinehart, *Nonlinear regression modeling for engineering applications: Modeling, model validation, and enabling design of experiments*. wiley, 2016.

[21] W. Groenendaal, F. A. Ortega, A. R. Kherlopian, A. C. Zygmunt, T. Krogh-Madsen, and D. J. Christini, “Cell-Specific Cardiac Electrophysiology Models,” *PLoS Comput. Biol.*, vol. 11, no. 4, Apr. 2015.

[22] C. T. Bot, A. R. Kherlopian, F. A. Ortega, D. J. Christini, and T. Krogh-Madsen, “Rapid genetic algorithm optimization of a mouse computational model: Benefits for anthropomorphization of neonatal mouse cardiomyocytes,” *Front. Physiol.*, vol. 3 NOV, 2012.

[23] Z. Syed, E. Vigmond, S. Nattel, and L. J. Leon, “Atrial cell action potential parameter fitting using genetic algorithms,” *Med. Biol. Eng. Comput.*, vol. 43, no. 5, pp. 561–571, Sep. 2005.

[24] A. D. Akwaboah, P. Yamlome, J. A. Treat, J. M. Cordeiro, and M. Deo, “Genetic Algorithm For Fitting Cardiac Cell Biophysical Model Formulations,” in *2020 42nd Annual International Conference of the IEEE Engineering in Medicine & Biology Society (EMBC)*, 2020, pp. 2463–2466.

[25] R. J. Goodrow *et al.*, “Biophysical comparison of sodium currents in native cardiac myocytes and human induced pluripotent stem cell-derived cardiomyocytes,” *J. Pharmacol. Toxicol. Methods*, vol. 90, no. September 2017, pp. 19–30, 2018.

[26] J. A. Treat, R. J. Goodrow, C. T. Bot, R. J. Haedo, and J. M. Cordeiro, “Pharmacological enhancement of repolarization reserve in human induced pluripotent stem cells derived cardiomyocytes,” *Biochem. Pharmacol.*, vol. 169, p. 113608, Nov. 2019.

[27] M. X. Doss *et al.*, “Maximum diastolic potential of human induced pluripotent stem cell-derived cardiomyocytes depends critically on IKr,” *PLoS One*, vol. 7, no. 7, Jul. 2012.

[28] J. M. Cordeiro *et al.*, “Identification and characterization of a transient outward K+ current in human induced pluripotent stem cell-derived cardiomyocytes.,” *J. Mol. Cell. Cardiol.*, vol. 60, pp. 36–46, Jul. 2013.

[29] E. Grandi *et al.*, “Human atrial action potential and Ca 2+ model: Sinus rhythm and chronic atrial fibrillation,” *Circ. Res.*, vol. 109, no. 9, pp. 1055–1066, Oct. 2011.

[30] E. Grandi, F. S. Pasqualini, and D. M. Bers, “A novel computational model of the human ventricular action potential and Ca transient,” *J. Mol. Cell. Cardiol.*, vol. 48, no. 1, pp. 112–121, Jan. 2010.

[31] P. Stewart, O. V Aslanidi, D. Noble, P. J. Noble, M. R. Boyett, and H. Zhang, “Mathematical models of the electrical action potential of Purkinje fibre cells.,” *Philos. Trans. A. Math. Phys. Eng. Sci.*, vol. 367, no. 1896, pp. 2225–55, Jun. 2009.

[32] S. Li, G. Chen, and R. A. Li, “Calcium signalling of human pluripotent stem cell-derived cardiomyocytes,” *Journal of Physiology*, vol. 591, no. 21. John Wiley & Sons, Ltd, pp. 5279–5290, Nov-2013.

[33] A. Iserles, *A First Course in the Numerical Analysis of Differential Equations: Finite difference schemes*. 2008.

[34] K. H. W. J. ten Tusscher, D. Noble, P. J. Noble, and A. V Panfilov, “A model for human ventricular tissue.,” *Am. J. Physiol. Heart Circ. Physiol.*, vol. 286, no. 4, pp. H1573-89, Apr. 2004.

[35] C. Shah, S. Jiwani, B. Limbu, S. Weinberg, and M. Deo, “Delayed afterdepolarization-induced triggered activity in cardiac purkinje cells mediated through cytosolic calcium diffusion waves,” *Physiol. Rep.*, vol. 7, no. 24, Dec. 2019.

[36] R. Storn, “On the usage of differential evolution for function optimization,” in *Biennial Conference of the North American Fuzzy Information Processing Society - NAFIPS*, 1996, pp. 519–523.

[37] O. S. Center, “Ohio Supercomputer Center,” *Ohio Supercomputer Center*. p. Columbus, OH, 1987.

[38] J. Towns *et al.*, “XSEDE: Accelerating scientific discovery,” *Comput. Sci. Eng.*, vol. 16, no. 5, pp. 62–74, Sep. 2014.

[39] N. Guglielmi and E. Hairer, “Implementing Radau IIA methods for stiff delay differential equations,” *Comput. (Vienna/New York)*, vol. 67, no. 1, pp. 1–12, 2001.

[40] J. R. Dormand and P. J. Prince, “A family of embedded Runge-Kutta formulae,” *J. Comput. Appl. Math.*, vol. 6, no. 1, pp. 19–26, Mar. 1980.

[41] L. Petzold, “Automatic Selection of Methods for Solving Stiff and Nonstiff Systems of Ordinary Differential Equations,” *SIAM J. Sci. Stat. Comput.*, vol. 4, no. 1, pp. 136–148, Mar. 1983.

[42] A. M. Lodrini, L. Barile, M. Rocchetti, and C. Altomare, “Human induced pluripotent stem cells derived from a cardiac somatic source: insights for an in-vitro cardiomyocyte platform,” *International Journal of Molecular Sciences*, vol. 21, no. 2. MDPI AG, 02-Jan-2020.

[43] H. D. Devalla *et al.*, “Atrial‐like cardiomyocytes from human pluripotent stem cells are a robust preclinical model for assessing atrial‐selective pharmacology,” *EMBO Mol. Med.*, vol. 7, no. 4, pp. 394–410, Apr. 2015.

[44] U. Hoppe C and D. J. Beuckelmann, “Characterization of the hyperpolarization-activated inward current in isolated human atrial myocytes,” *Cardiovasc. Res.*, vol. 38, no. 3, pp. 788–801, Jun. 1998.

[45] A. M. Frace, F. Maruoka, and A. Noma, “External K+ increases Na+ conductance of the hyperpolarization-activated current in rabbit cardiac pacemaker cells,” *Pflügers Arch. Eur. J. Physiol.*, vol. 421, no. 1, pp. 94–96, May 1992.

[46] D. DiFrancesco, A. Ferroni, M. Mazzanti, and C. Tromba, “Properties of the hyperpolarizing‐activated current (if) in cells isolated from the rabbit sino‐atrial node.,” *J. Physiol.*, vol. 377, no. 1, pp. 61–88, Aug. 1986.

[47] S. M. Biendarra-Tiegs, F. J. Secreto, and T. J. Nelson, “Addressing Variability and Heterogeneity of Induced Pluripotent Stem Cell-Derived Cardiomyocytes,” in *Advances in Experimental Medicine and Biology*, vol. 1212, Springer, 2020, pp. 1–29.

[48] D. Smirnov *et al.*, “Genetic algorithm-based personalized models of human cardiac action potential,” *PLoS One*, vol. 15, no. 5, p. e0231695, May 2020.

[49] J. Tomek *et al.*, “Development, calibration, and validation of a novel human ventricular myocyte model in health, disease, and drug block,” *Elife*, vol. 8, Dec. 2019.

[50] M. Paci *et al.*, “All-Optical Electrophysiology Refines Populations of In Silico Human iPSC-CMs for Drug Evaluation,” *Biophys. J.*, vol. 118, no. 10, pp. 2596–2611, May 2020.

[51] J. Ma *et al.*, “High purity human-induced pluripotent stem cell-derived cardiomyocytes: Electrophysiological properties of action potentials and ionic currents,” *Am. J. Physiol. - Hear. Circ. Physiol.*, vol. 301, no. 5, pp. 2006–2017, 2011.

[52] Z. Zhao *et al.*, “Ion Channel Expression and Characterization in Human Induced Pluripotent Stem Cell-Derived Cardiomyocytes,” *Stem Cells Int.*, vol. 2018, 2018.

[53] D. Dobrev *et al.*, “The G protein-gated potassium current IK,ACh is constitutively active in patients with chronic atrial fibrillation,” *Circulation*, vol. 112, no. 24, pp. 3697–3706, Dec. 2005.

[54] M. Argenziano *et al.*, “Electrophysiologic Characterization of Calcium Handling in Human Induced Pluripotent Stem Cell-Derived Atrial Cardiomyocytes,” *Stem Cell Reports*, vol. 10, no. 6, pp. 1867–1878, Jun. 2018.

[55] K. Blinova *et al.*, “International Multisite Study of Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes for Drug Proarrhythmic Potential Assessment,” *Cell Rep.*, vol. 24, no. 13, pp. 3582–3592, Sep. 2018.

**Figure Captions**

**Figure 1.** Experimental data IV plots - A: fast sodium current (INa), B: transient outward potassium current (Ito), C: delayed rectifier potassium current (IKr), D: L-type calcium current (ICaL), E: Hyperpolarization-activated current (If). The errors bars represent experimental standard deviations from multiple cell recordings.

**Figure 2**. Schematic of the hiPSC-CM model depicting major ionic currents and fluxes.

**Figure 3**. Algorithmic description of the genetic algorithm (GA) protocol used in fitting the ionic currents. : original model chromosome, : original model gene, : chromosomal length/ number of genes, : population size, : offspring proportion (parent-offspring ratio = ), : loss function (SSE or RMSE), : initial population constraint, : mutation constraint, : an iterable comprising lists of crossover point(s). . All intervals (i.e. [a, b], (a, b] or (a, b)) are uniformly distributed. Parameters belonging these intervals were randomly drawn

**Figure 4.** GA-based parameter fitting of ionic currents. The left plots show initial and final fitted I-V plots for each current compared with corresponding experimental values. Middle plots show simulated time course of current activations based on corresponding voltage clamp protocols (insets). Right plots show loss (RMSE or SSE) over 100 generations showing convergence during the GA fitting process.

**Figure 5.** Comparison of simulated action potential morphology (blue) to experimentally recorded (orange).

**Figure 6.** Model AP (top) and corresponding ionic current transients (bottom) during spontaneous AP generation. Some currents are scaled to fit.

**Figure 7.** Sensitivity analysis of model showing color-coded correlation coefficients corresponding to the ionic current variations and their effects on AP parameters.

**Figure 8.** Effect of blocking various ionic currents on AP characteristics in the hiPSC-CM model such as AP morphology (Panels A-F), AP durations (Panels G-I), cycle length (Panels J-L), and MDP (Panels M-O).

Figure 9.

Figure 10.

Figure 11.

Figure 12.

**Tables**

**Table 1.** Summary of Sources (References) to Adopted Model Formulations

|  |  |
| --- | --- |
| **Ionic Current(s)** | **Formulation Source** |
| IKur , INaCa , INaK , IpCa , IbNa , IbCa , IKs | Courtemanche et al. [13] |
| INa | Luo-Rudy [11] |
| IKr, IKACh | Kurata et al. [12] |
| ICaL | Kurata et al. [12], Ten-Tusscher et al. [34] |
| Ito, IK1 | Grandi et al. [30] |
| If | Stewart et al. [31] |

**Table 2.** Summary of Genetic Algorithm Protocols for Ionic Currents.

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
|  | **INa** | **Ito** | **IKr** | **ICaL** | **If** |
| **# Parameters** | 20 | 20 | 18 | 6 | 7 |
| **Population size** | 27 | 80 | 25 | 54 | 80 |
| **Crossover** | 4-point | 2-point | 2-point | 1-point | 2-point |
|  | 0.8 | 0.8 | 0.2 | 0.5 | 0.5 |
| **Mutation** | All genes | All genes | All genes | All genes | All genes |
|  | 0.2 | 0.5 | 0.1 | 0.1 | 0.2 |
| **Fitness** | RMSE | SSE | SSE | SSE | SSE |

**Table 3.** Loss and Fitness Values of the GA-based Optimization of Currents

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Ionic Current** | **Initial loss** | **Final loss** | **Initial *R2***  **(initial model)** | **Final *R2***  **(fitted model)** |
| INa | 107.449 2.76 | 81.661 0.56 | 0.519 | 0.8412.18e-3 |
| Ito | 288.313 44.792 | 0.1050.038 | -0.165 | 0.9991.19e-4 |
| IKr | 1.4030.215 | 0.004675.15e-4 | -1.558 | 0.9955.16e-4 |
| If | 0.7080.076 | 1.8450.186 | -0.201 | 0.9861.49e-3 |
| ICaL | 57.89210.048 | 7.4350.224 | 0.358 | 0.94051.8e-3 |

**Table 4.** Comparison of Action Potential Parameters.

|  |  |  |
| --- | --- | --- |
| **Parameter** | **Model** | **Experiments\*** |
| AP duration, APD50 (ms) | 104.93 | 102.596 2.615 |
| AP duration, APD75 (ms) | 126.26 | 126.104 2.667 |
| AP duration, APD90 (ms) | 142.86 | 141.169 3.231 |
| Cycle Length, CL (ms) | 470.23 | 482.918 21.572 |
| Maximum diastolic potential, MDP (mV) | -75.90 | -74.751 0.368 |
| Beats per Minute, BPM | 126.0 | 122.147.73 |

\*n = 62 APs over 29,700ms. For BPM, 2000 ms time window were slided over 28000 ms total time span (i.e., n = 14), to obtain information on variance.

Table 5.