

# ***In Silico* Identification of the Key Ionic Currents Modulating Human Pluripotent Stem Cell-Derived Cardiomyocytes Towards an Adult Phenotype**

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## **Abstract**

*Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) provide a promising resource for regenerative therapies. However, their immature phenotype and heterogeneities raise concern for arrhythmia when hiPSC-CMs are inserted into the native tissue. In silico models can improve understanding of the electrophysiological differences between hiPSC-CMs and adult cardiomyocytes and inform risk predictions.*

*Our aim is to conduct a sensitivity analysis to identify the main ionic currents determining differences in electrophysiological properties between the Paci2020 model (hiPSC-CMs) and the ToR-ORd model (human ventricular cardiomyocytes). Our simulations highlighted the fast  $\text{Na}^+$  current as the key modulator of upstroke velocity, while the inward and rapid delayed rectifier  $\text{K}^+$  currents mainly contributed to a decrease of diastolic potential and action potential duration, respectively.*

*In conclusion, we identified an increase of the conductance of these currents essential to modulate the biomarkers of the hiPSC-CM model towards the adult phenotype.*

view, ventricular-like hiPSC-CMs have an action potential (AP), characterised by slow upstroke velocity, prolonged AP duration (APD), depolarised diastolic potential and spontaneous beating[3]. These properties and heterogeneities between hiPSC-CMs and adult CMs give concern for arrhythmia when introducing hiPSC-CMs into the native tissue.

Computer models (*in silico*) are recognised as a powerful tool for investigations into arrhythmia mechanisms, drug safety studies[4] and to support clinical decisions[5]. They provide a fast and effective alternative to experimental models and present the unique opportunity to test in simulations the effects of cellular changes on heart electrophysiology. Therefore, they constitute the perfect tool to characterise the electrophysiological properties of hiPSC-CMs and their differences with adult CMs, to predict potential arrhythmia caused by their interaction.

In this paper we present a sensitivity analysis of the latest biophysically-detailed hiPSC-CM Paci model[6] (Paci2020), to identify the contribution of the different ionic currents to the modulation of AP biomarkers towards an adult phenotype.

## **1. Introduction**

Cardiovascular diseases are the leading cause of mortality worldwide. Since heart self-regeneration after infarction is limited, efficient alternative strategies to reduce mortality are needed. Therapies with human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) have been explored for cardio-protection, i.e. to prevent remodelling shortly after infarction, as well as for cardio-restoration, i.e. to reverse remodelling and restore cardiac function in the later stages of cardiac injury[1]. Despite their promising regenerative capabilities, hiPSC-CMs are predominately immature in their properties, structure and function compared with adult cardiomyocytes (CMs)[2]. From an electrophysiological point of

## **2. Methods**

Simulations were performed using the Paci2020 model[6] of hiPSC-CMs and the ToR-ORd model[7] representing the adult ventricular phenotype. Both models were paced at 1Hz at 37°C until steady state and the last AP was analysed. We computed the values of four biomarkers: maximum diastolic potential (MDP), peak voltage ( $V_{peak}$ ), maximum upstroke velocity ( $dV/dt_{max}$ ) and APD at 90% of repolarisation (APD<sub>90</sub>).

The sensitivity analysis of Paci2020 focused on the conductances of eleven ionic currents: fast and late  $\text{Na}^+$  currents ( $I_{NaF}$ ,  $I_{NaL}$ ), transient outward  $\text{K}^+$  current ( $I_{to}$ ), rapid and slow delayed outward rectifier  $\text{K}^+$  currents ( $I_{Kr}$ ,  $I_{Ks}$ ), inward rectifier  $\text{K}^+$  current ( $I_{K1}$ ), L-type  $\text{Ca}^{2+}$  cur-

rent ( $I_{CaL}$ ),  $Na^+/Ca^{2+}$  exchanger ( $I_{NaCa}$ ),  $Na^+/K^+$  pump ( $I_{NaK}$ ), SERCA pump ( $I_{up}$ ) and funny current ( $I_f$ ). All these currents are present in both Paci2020 and ToR-ORD, except  $I_f$ , which is not expressed in adult cardiomyocytes. Current conductances (defined as  $G_x$ , for current  $I_x$ ) were scaled by  $\{0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 7, 8, 9, 10\}$  of their baseline values. We included a large range of scaling factors to account for considerable discrepancies in current expression between hiPSC-CMs and adult CMs.

To summarise the results of the sensitivity analysis, we computed the relative sensitivities, as described in [8], using the biomarkers simulated for scaling factors 2 and 0.5, and compared with their baseline values. Relative sensitivities vary between -1 (strong negative correlation) and +1 (strong positive correlation).

### 3. Results

#### 3.1. AP and ionic current comparison of baseline Paci2020 and ToR-ORD

Figure 1 shows the APs and the main ionic currents for the baseline Paci2020 and ToR-ORD models. In the top panel, we can see that compared to ToR-ORD, the AP in Paci2020 displays a slower upstroke ( $dV/dt_{max}$ , 118 vs 349 V/s, Paci2020 vs ToR-ORD), depolarised MDP (-74 vs -89 mV), longer AP ( $APD_{90}$ , 481 vs 273 ms) and higher  $V_{peak}$  (35 vs 33 mV). All current peaks were found lower in Paci2020 compared to ToR-ORD, except for  $I_{Ks}$  which has a similar amplitude (+3%, Paci2020 vs ToR-ORD). The currents whose peaks show the largest differences are shown in the remaining panels of Figure 1:  $I_{NaF}$  (-62%),  $I_{NaL}$  (-80%),  $I_{to}$  (-88%),  $I_{Kr}$  (-59%) and  $I_{up}$  (-95%).  $I_{CaL}$ ,  $I_{NaCa}$ ,  $I_{NaK}$  and  $I_{K1}$  peaks are also lower in Paci2020, but to a lesser extent (-35%, -26%, -32%, -10%, respectively).

#### 3.2. Sensitivity analysis

Figure 2 summarises the sensitivity analysis results of Paci2020, which was performed to identify the ionic currents key in modulating hiPSC-CM biomarkers. The values in Figure 2 correspond to the relative sensitivities, computed as described in the Methods section.

The MDP is very stable to changes in current conductances and mainly modulated by  $G_{K1}$  and  $G_{Kr}$  (10% and 3% MDP increase for two-fold rise of  $G_{K1}$  and  $G_{Kr}$ ).  $dV/dt_{max}$  is mainly modulated by  $G_{NaF}$ . A two-fold increase in  $G_{NaF}$  caused a considerable increase in  $dV/dt_{max}$  (+78%) and  $V_{peak}$  (+42%). This is expected, since  $I_{NaF}$  is the main depolarising current in the upstroke phase. On the contrary, an increase of  $G_{CaL}$  caused a decrease of  $dV/dt_{max}$ . Our investigation concluded this is a consequence of reduced  $I_{Na}$  availability, caused by the

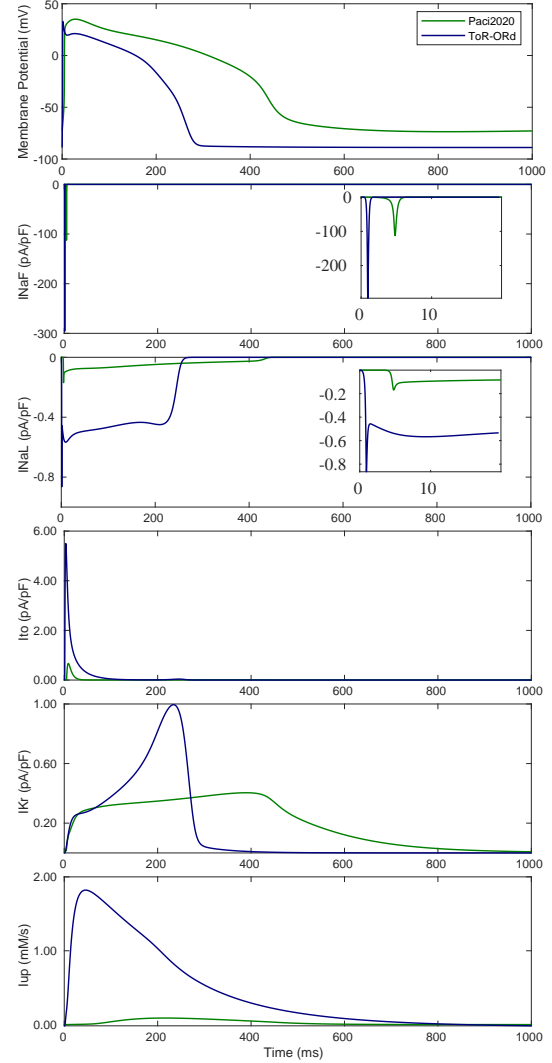


Figure 1. Simulated AP and selection of current traces for Paci2020 (green) and ToR-ORD (blue).

longer APD, which in turn is caused by the increased  $I_{CaL}$ . Indeed, we observed a lower value for the  $I_{NaF}$  recovery gate at the start of the AP, when  $G_{CaL}$  is increased.

$V_{peak}$  is increased when augmenting  $G_{NaF}$ ,  $G_{CaL}$ , and  $G_{NaK}$ . On the contrary, higher  $G_{to}$ ,  $G_{Kr}$ ,  $G_{K1}$ , or  $G_{NaCa}$  reduce  $V_{peak}$ , since they are all outward currents in the initial AP phase. The APD is mainly shortened when increasing the outward  $K^+$  currents, especially  $G_{Kr}$  and  $G_{K1}$  ( $APD_{90}$  -45% and -30% for a two-fold increase in  $G_{Kr}$  and  $G_{K1}$ , respectively).  $I_{Ks}$  does not significantly shorten the APD, since its magnitude is very small.

In summary, the AP biomarkers of the hiPSC-CM Paci2020 model are most sensitive to changes in  $G_{NaF}$ ,  $G_{Kr}$ ,  $G_{K1}$ ,  $G_{CaL}$  and  $G_{NaK}$ . This is reflected in the changes observed in AP morphology when varying these conductances (Figure 3). These results agree with the sen-

	MDP	dV/dt <sub>max</sub>	V <sub>peak</sub>	APD <sub>90</sub>
G <sub>NaF</sub>		1.00	0.59	
G <sub>NaL</sub>				
G <sub>to</sub>			-0.15	
G <sub>Kr</sub>	0.41	0.43	-0.27	-1.00
G <sub>Ks</sub>				
G <sub>K1</sub>	1.00	0.64	-0.16	-0.38
G <sub>CaL</sub>	-0.10	-0.10	0.68	0.63
G <sub>NaCa</sub>	0.13		-0.28	0.21
G <sub>NaK</sub>		0.52	1.00	0.66
G <sub>up</sub>				
G <sub>f</sub>	-0.15	-0.21	-0.19	-0.13

Figure 2. Relative sensitivities computed from the biomarker changes observed in Paci2020 when scaling current conductances between 0.5 and 2. Darker shades of green/red correspond to larger positive/negative relative sensitivity. Values below +/-0.1 are not shown.

sitivity analysis of a previous Paci model[9]. Moreover, our simulation provided additional insights into the role of  $I_{NaK}$ ,  $I_{NaL}$  and  $I_{up}$  in hiPSC-CMs.

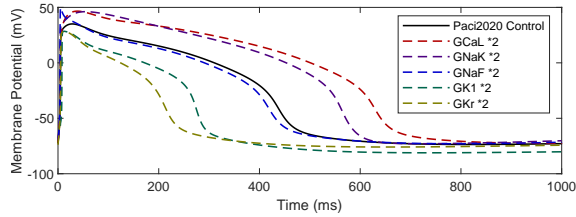


Figure 3. Changes in hiPSC-CM AP when increasing  $G_{CaL}$ ,  $G_{NaK}$ ,  $G_{NaF}$ ,  $G_{K1}$  and  $G_{Kr}$ : baseline (black solid line),  $G_{CaL} * 2$  (dashed red line),  $G_{NaK} * 2$  (dashed purple line),  $G_{NaF} * 2$  (dashed blue line),  $G_{K1} * 2$  (dashed green line) and  $G_{Kr} * 2$  (dashed yellow line).

### 3.3. $I_{NaF}$ , $I_{K1}$ and $I_{Kr}$ as main drivers of hiPSC-CM versus adult cardiomyocyte differences

Based on the simulation results presented in Sections 3.1 and 3.2, we investigated if by scaling the current conductances in Paci2020 to match the current peaks of ToR-ORd we can modulate the hiPSC-CMs biomarkers towards the adult phenotype.

Results are summarised in Figure 4, showing the ratio between Paci2020 biomarkers and peak currents and their corresponding values for ToR-ORd. For each of the currents included in Figure 1 ( $I_{NaF}$ ,  $I_{NaL}$ ,  $I_{to}$ ,  $I_{Kr}$  and  $I_{up}$ ), we chose the scaling factor that brings the current peak closer to the one of ToR-ORd. We also included the results obtained with a two-fold increase in  $I_{K1}$  conductance, due to its strong effect on MDP, and by removing  $I_f$ , which is

not present in ToR-ORd.

Biomarker comparison between the baseline models highlighted that the simulated AP in Paci2020 has a depolarised MDP (-17%), a lower dV/dt<sub>max</sub> (-66%), a slightly higher V<sub>peak</sub> (+7%), and a significantly prolonged APD<sub>90</sub> (+76%), compared to the AP obtained with ToR-ORd. This mostly agrees with experimental results[3].

A three-fold increase in  $G_{NaF}$  in Paci2020 shifts dV/dt<sub>max</sub> closer to the value obtained with the ToR-ORd model, reducing the difference to -30%, Paci2020 vs ToR-ORd. It also slightly decreases the APD<sub>90</sub> (-13%). However, V<sub>peak</sub> moves further away from ToR-ORd (+76%). Increased  $G_{to}$  and  $G_{Kr}$  strongly reduce V<sub>peak</sub>, but also shorten the APD<sub>90</sub>, bringing it closer to the ToR-ORd value (+49 and -3%, respectively).  $G_{NaL}$  and  $G_{up}$  increases bring the  $I_{NaL}$  and  $I_{up}$  current peaks closer to ToR-ORd, but do not significantly affect any biomarker, as expected from the results in Figure 2. Doubling  $G_{K1}$  contributes to depolarise the MDP in Paci2020, decreasing the difference to the MDP in ToR-ORd from -17% to -9%. It also decreases V<sub>peak</sub> to 28.5 mV and moves APD<sub>90</sub> closer to ToR-ORd (from +76% to +24%). Scaling  $G_f$  to 0 had almost no effect on the biomarkers, but we verified that this inhibits spontaneous beating in unpaced conditions. In addition to the changes in biomarkers, Figure 4 also shows the effect on the scaled current peaks and differences to ToR-ORd, which were significantly reduced:  $I_{NaF}$  (from -62% to -19%),  $I_{NaL}$  (from -80% to -18%),  $I_{to}$  (from -88% to -17%),  $I_{Kr}$  (from -59% to -23%) and  $I_{up}$  (from -95% to +4%).

Based on these results, we identified the increase of the following currents as essential in modulating Paci2020 towards the ToR-ORd phenotype:  $I_{NaF}$ , for its effect on dV/dt<sub>max</sub>;  $I_{K1}$ , for its effect on MDP, and  $I_{Kr}$ , for its effect on APD<sub>90</sub>.

	I <sub>peak</sub> Baseline	I <sub>peak</sub> Scaled	MDP	dV/dt <sub>max</sub>	V <sub>peak</sub>	APD <sub>90</sub>
Baseline			0.83	0.34	1.07	1.76
G <sub>NaF</sub> *3	0.38	0.81	0.81	0.70	1.76	1.54
G <sub>NaL</sub> *5	0.20	0.92	0.82	0.33	1.22	2.06
G <sub>to</sub> *8	0.12	0.83	0.85	0.34	0.68	1.49
G <sub>Kr</sub> *2	0.41	0.77	0.85	0.34	0.85	0.97
G <sub>up</sub> *6	0.05	1.04	0.84	0.35	1.16	1.86
G <sub>K1</sub> *2	0.90	1.79	0.91	0.36	0.86	1.24
G <sub>f</sub> *0	N/A	N/A	0.86	0.40	1.17	1.97

Figure 4. Ratio between Paci2020 and ToR-ORd ionic current peaks and AP biomarkers for selected current conductance scalings. Shades of blue indicate how close the values are to an adult phenotype (ratio equal to 1). The first 2 columns show the current peak ratios with the baseline values on the left, while the last 4 columns show the AP biomarker ratios, with the baseline values at the top.

## 4. Discussion

In this study, we compared AP biomarkers and currents in the hiPSC-CM Paci2020 model with the ones of the human adult CM ToR-ORd model. Next, we performed a sensitivity analysis of Paci2020 to identify the main ionic currents determining its modulation from hiPSC-CM to adult CM, considering ToR-ORd as benchmark.

We showed that  $I_{NaF}$ ,  $I_{NaL}$ ,  $I_{to}$ ,  $I_{Kr}$  and  $I_{up}$  current peaks are significantly lower in Paci2020 than in ToR-ORd. In partial agreement with this, lower expression of  $I_{Na}$ ,  $I_{to}$  and  $I_{Kr}$  has been reported experimentally [10, 11].

Studies hypothesised that the main cause for the more depolarised MDP in hiPSC-CMs is a significant under-expression of  $I_{K1}$ [12]. Our *in silico* comparison was not able to confirm this and instead found comparable current peaks in Paci2020 and ToR-ORd. However, a recent experimental study indicated that  $I_{K1}$  in hiPSC-CMs is not necessarily lower than in human adult CMs[13].

Our investigation concluded that increased  $I_{NaF}$  was the main driver to increase  $dV/dt_{max}$  towards the ToR-ORd phenotype. We also showed that increased  $I_{K1}$  and  $I_{Kr}$  contribute to a lower MDP and shorter  $APD_{90}$ , respectively. This agrees with [12] where it was hypothesised that increasing  $I_{K1}$  could improve MDP and reduce pro-arrhythmia of hiPSC-CMs.

Based on these results, future work may investigate the combinations of different scaling factors to modulate Paci2020 from hiPSC-CM to an adult phenotype, through automated optimisation methods.

## 5. Conclusion

In this study, we performed a sensitivity analysis of an *in silico* model of hiPSC-CM and highlighted the contribution of the single ionic currents to AP biomarker changes. We concluded that increased  $I_{NaF}$ ,  $I_{K1}$  and  $I_{Kr}$  are essential to modulate the hiPSC-CM model towards an adult phenotype.

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