In Silico Human Induced Pluripotent Stem Cell Derived Cardiomyocyte Electro-Mechanical Modelling and Simulation

Milda Folkmanaite, Xin Zhou, Francesca Margara, Manuela Zaccolo, Blanca Rodriguez

University of Oxford, Oxford, United Kingdom

Abstract

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CM) enable accessible human-based cardiology studies. However, an immature hiPSC-CM electrophysiological and contractile phenotype hinders data translation to adult cardiomyocytes. In silico hiPSC-CM investigations could aid in hiPSC-CM data translation but most hiPSC-CM models do not feature a contractile element which limits their application for such studies. To address this issue, we have developed an electromechanical hiPSC-CM computer model by coupling an electrophysiological hiPSC-CM model and a human contractile machinery model. The newly established model has been calibrated using experimental hiPSC-CM data. We demonstrate that the computed active tension, calcium transient and action potential biomarkers agree with the experimental ranges. The peak twitch tension generated at 1 Hz pacing was 0.44 kPa which is in range with experimentally observed values (0.21-6.5 kPa). Comparisons with the adult myocyte electromechanical model demonstrate the potential usability of the hiPSC-CM model in the future data translation. Altogether, we present a new electromechanical hiPSC-CM model for comprehensive in silico hiPSC-CM-based studies.

1. Introduction

Human induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) are now widely used in *in vitro* experiments ranging from drug safety assessments to disease modelling [1]. *In silico* hiPSC-CM studies have been successfully used to expand our understanding of hiPSC-CM pharmacological and physiological properties, and aided data translation to adult cells [2]. This is pivotal since hiPSC-CMs possess a specific electrophysiology and contractile phenotype which differs from adult cells. Computer models have been widely used to investigate the differences in electrophysiology, but the contractility is rarely assessed. It is known that hiPSC-CM have a lower peak twitch tension, and a longer time to peak tension (TP), and time to 50% relaxation (RT50) than adult cells [3]. As the number of hiPSC-CM-based studies is growing, it is vital to enable investigations of hiPSC-CM-specific contractile features to aid in mechanistic investigations and data translation.

The goal of our study is to develop and evaluate an electromechanical hiPSC-CM model with the aim to facilitate the investigations of hiPSC-CM-specific electrophysiological and contractile phenotype. Finally, a comparison between hiPSC-CM and human adult cell simulations demonstrate that the relative differences between hiPSC-CM and adult cardiomyocytes can be captured by the models, providing confidence for future investigations.

2. Methods

2.1. Experimental data

The experimental data for model calibration was based on hiPSC-CM studies [4,5] conducted at 1 Hz pacing, 37^{o}C, using tissue strips from engineered heart tissue grown from hiPSC-CMs day 15-23 post-differentiation. Reported active tension biomarkers TP, RT50, minimal and maximal active tension were used for calibration. After the coupling of the models, peak twitch tension, calcium transient (CaT) and action potential (AP) biomarkers from multiple studies were used for model evaluation [2,6-8]. Adult cardiomyocyte experimental data was used for comparisons with hiPSC-CM data [9].

2.2. Electro-mechanical coupling of hiPSC-CM electrophysiology and human-based contractile machinery models

The previously published hiPSC-CM electrophysiology model by Paci et al. [2] was coupled with the Land-Niederer human tension model [10] to generate the new Paci-Land model for hiPSC-CM electromechanics. The Paci model reflects the action potential and calcium dynamics observed in hiPSC-CM. The Land-Niederer

model is based on adult cardiomyocyte measurements and includes troponin (TRPN), tropomyosin, and a three-state crossbridge model. Given the differences in the active tension between hiPSC-CM and adult cardiomyocyte, the parameters in the tension model were recalibrated to create the hiPSC-CM tension model.

Coupling of the models was implemented by using intracellular calcium concentration from the electrophysiology model as an input for the tension model and using the amount of calcium bound to TRPN in the tension model for electrophysiology model, as in [9] (Fig.1 A). The model was built on the assumption that the ratio of calmodulin to TRPN is the same as in adult cells.

The biomarkers computed from simulations included TP, RT50, time to 90% decay (RT90) for active tension; CaT time to peak (CTTP), duration to 50% (CTD50) and 90% (CTD50) decay; and AP time to peak (APTP), duration to 50% (APD50) and 90% (APD90) repolarisation (Fig.1. B-D).

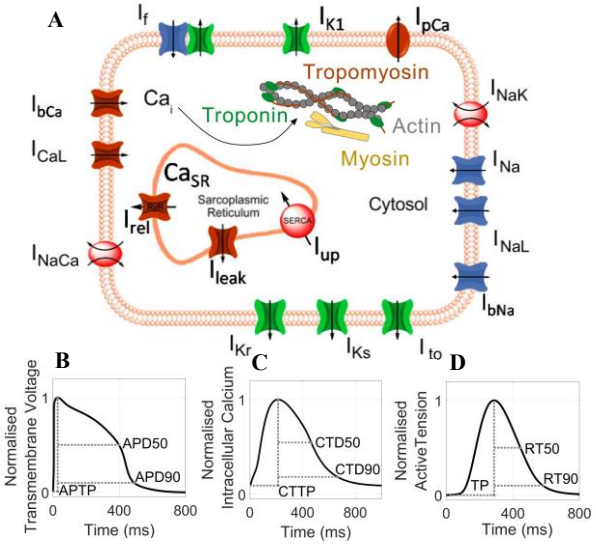

Figure 1. A. Schematic representation of Paci-Land hiPSC-CM model. The image is adapted from Paci et al. [11]. B. Representative AP waveform and biomarkers computed which include APTP, APD50 and APD90. C. Representative CaT waveform and biomarkers computed which include CTTP, CTD50 and CTD90. D. Representative active tension waveform and biomarkers computed which include TP, RT50 and RT90.

2.3. Calibration of the model

The Paci-Land model was calibrated using the experimental hiPSC-CM data. This was needed to create an active tension model which was in agreement with hiPSC-CM experimental values since the original Land-Niederer model used was based on adult cardiomyocyte data. To select parameters for recalibration, a sensitivity analysis on active tension biomarkers was performed by varying parameter values by ±10%. Two parameters were selected for fitting: *nTm* and *ku*, representing the Hill coefficient of cooperative activation between calcium-bound TRPN and the fraction of unblocked myosin binding sites, and the tropomyosin rate constant, respectively. These parameters were fitted using the MatLab function *ga* which finds the minimum of a [cost] function using a genetic algorithm. The cost function here included TP, RT50, maximum and minimum active tension (Ta) values; and the one used here was an altered formula of the cost function used by Land et al. [10]:

$$d_t = d(tp, [169,215]) + d(rt50, [141,178]) + 10d(max(Ta), [0.21,6.5]) + 25min(Ta)$$

where the distances d between a biomarker and the experimental ranges were summed to obtain the total cost d_t which was then minimized.

2.4. Simulation protocols

Simulations were conducted using MatLab (Mathworks Inc. Natwick, MA, USA) using the ordinary differential equation solver ode15s. Stimulus currents of 5 ms and 550 pA were used. The cell length was kept unchanged unless stated otherwise. Biomarkers were computed in the steady state (after 800 s).

3. Results

3.1. Evaluation of electro-mechanical coupling

To examine whether the physiological hiPSC-CM contractile phenotype can be reproduced by the Paci-Land model, we performed simulations with the model and evaluated the active tension biomarker values.

Comparison of the simulated and experimental active tension biomarkers shows that they are in agreement, confirming that the Paci-Land model can produce physiological hiPSC-CM active tension biomarker values (Fig. 2 B, Table 1). The differences in the active tension waveforms between calibrated and non-calibrated Paci-Land models highlights the initial need for recalibration of the Land-Niederer model parameters in order to obtain active tensions biomarker values that are physiological for hiPSC-CM (Fig. 2 A-B, Table 1).

To test if electromechanical coupling affects cellular electrophysiology, we evaluated AP and CaT biomarkers after simulations with the Paci-Land hiPSC-CM model.

Comparison of AP and CaT features between the Paci and the Paci-Land models reveals minimal differences between the simulated biomarker values in paced, and slightly greater differences in the spontaneously beating mode. CaT and AP biomarker values remain within the experimental ranges demonstrating that the electromechanical coupling does not affect the normal CaT and AP behavior (Fig. 2 C-F, Table 1).

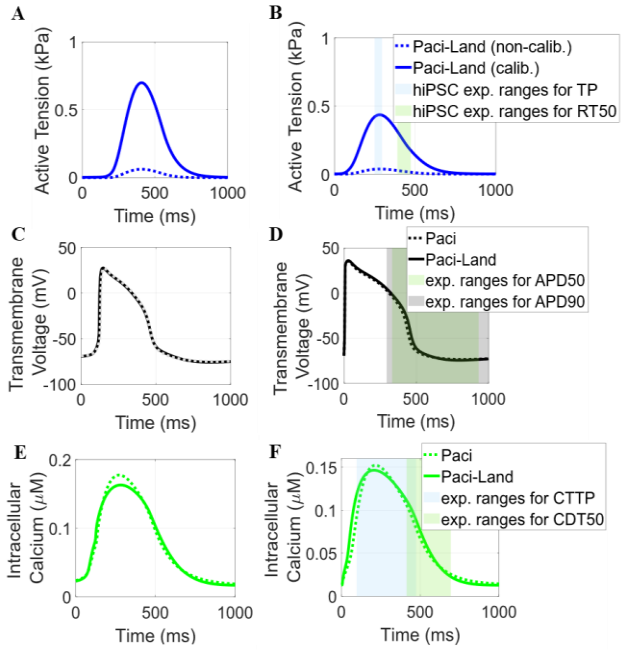

Figure 2. Simulated active tension changes after calibration (Paci-Land (calib.)) in spontaneously beating (A) and paced (B) modes. Biomarkers simulated from the calibrated model (Paci-Land (calib.)) are within experimental data ranges (B). Electro-mechanical coupling induces minimal changes to AP (C) and CaT (E) initially described by the Paci model in spontaneously beating and paced (D and F) simulation modes. AP and CaT biomarkers simulated from the calibrated Paci-Land model are within the experimental ranges.

Table 1. Experimental hiPSC-CM and simulated biomarker values including CaT and AP amplitudes (ampl.) using calibrated and non-calibrated Paci-Land models at 1 Hz pacing.

Biomarkers	Paci-Land (non-calib.)	Paci-Land (calib.)	Exp. value ranges
TP (ms)	188	201	169-215
RT50 (ms)	157	164	141-178
RT90 (ms)	284	329	
Peak tension (kPa)	0.04	0.44	0.21-6.5
CTTP (ms)	208	208	162-378
CTD50 (ms)	494	495	511-1168
CTD90 (ms)	669	670	310-1803
CaT ampl. (μM)	0.147	0.147	0.16
APTP (ms)	30	30.8	
APD50 (ms)	416	416.5	333-932
APD90 (ms)	517	516.5	296-1398
AP ampl. (mV)	110.99	110.99	98-118

3.2. HiPSC-CM and adult cardiomyocyte contractility comparisons *in silico*

We then performed a comparison of active tension biomarkers simulated using the Paci-Land hiPSC-CM model versus the human adult cardiomyocyte models for active tension (Land-Niederer [10]), and electromechanical function (ToR-ORd-Land [9]). These adult cell models have different active tension and CaT due to the different experimental data used in their calibration.

Figure 3 shows the differences observed in active tension and CaT between the models. This reflects the differences observed experimentally between human adult cardiomyocytes and hiPSC-CM. We aimed to quantitatively assess these differences between hiPSC-CM and human adult cardiomyocyte active tension to aid in data translation from hiPSC-CM to adult cardiomyocytes.

To make this assessment, we compared the simulated active tension biomarkers with experimental data [9]. We calculated the fold changes in experimental and simulated biomarker values by dividing adult cardiomyocyte biomarker values by hiPSC-CM values. Comparison of experimental and simulated fold changes in TP and RT50 shows that the relative differences in hiPSC-CM and adult myocyte models are in agreement with experiments (Fig.3 C, D). The relative difference in active tension amplitudes between the Paci-Land and ToR-ORd-Land models are in agreement with the experiments, while the Land model produced a higher amplitude than the adult experimental ranges used in Figure 3D [9]. Overall, this suggests that the Paci-Land model in combination with adult myocyte *in silico* models can be used for hiPSC-CM data translation to adult cells in most cases.

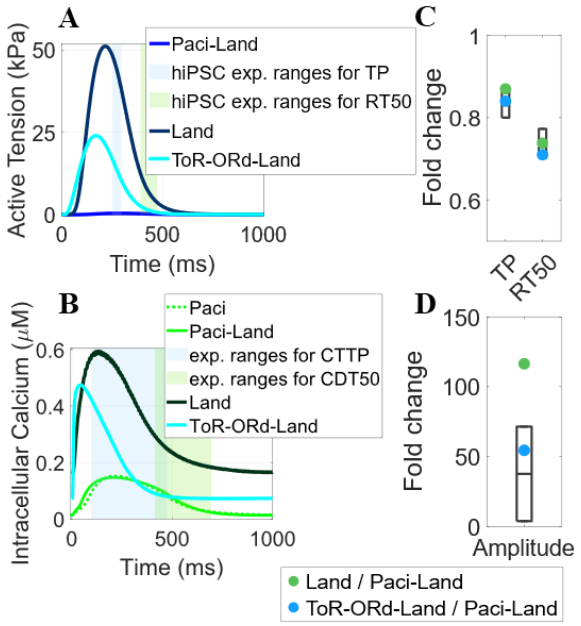

Figure 3. HiPSC-CM and adult cardiomyocytes show different active tension (A) and CaT (B) *in silico*. Fold change in biomarker values of TP, RT50 (C) and peak twitch tension (D) between simulated adult models and hiPSC-CM model are in agreement with experimental

ranges represented by box plots in most cases.

4. Conclusions

Computational hiPSC-CM studies have been useful for studying stem cell electrophysiology. However, currently available models rarely feature a contractile element. In this study, we developed a new hiPSC-CM model which features active tension generation to address this issue and facilitate comprehensive hiPSC-CM *in silico* electromechanical studies. We designed the model by coupling an established electrophysiological hiPSC-CM model with a human adult cardiomyocyte contractility model. We then recalibrate the active tension parameters used to describe adult cell contractility to induce hiPSC-CM-like active tension generation.

Simulated active tension generated by the calibrated Paci-Land hiPSC-CM model show a peak at 0.44 kPa and demonstrate TP and RT50 values that are all in agreement with experimental hiPSC-CM data. Non-calibrated model biomarkers are not within the experimental ranges signifying the need for model recalibration.

Simulations with the Paci-Land model reveal that the electromechanical coupling inflicts minimal changes to CaT and AP which remain in agreement with experimental data in paced and spontaneously beating cell simulation modes.

Aiming to test if the established model can be used for hiPSC-CM contractile data translation to adult cells, we compare the hiPSC-CM Paci-Land and the human adult cardiomyocyte models. The quantitated differences in active tension biomarkers between the hiPSC-CM and human adult cell models mirror the differences observed between the two cell types experimentally. This demonstrates that the Paci-Land model can be used together with adult cell *in silico* models to help translate experimental contractility data obtained with hiPSC-CM to human adult cells phenotypes in most cases.

Overall, we present a calibrated and validated hiPSC-CM electromechanical model with simulated active contraction properties that are physiological. This model provides a strong foundation for future hiPSC-CM-based electromechanical *in silico* studies.

Acknowledgments

MF was funded by the British Heart Foundation Studentship (FS/20/34132). XZ was funded by Oxford-Bristol Myers Squibb Fellowship. FM was supported by Personalised In-Silico Cardiology project, European Union's Horizon 2020 research programme under the Marie Sklodowska Curie grant agreement 764738. MZ was supported by the British Heart Foundation (PG/15/5/31110, RG/17/6/32944). BR was funded by a Wellcome Trust Fellowship in Basic Biomedical Sciences (214290/Z/18/Z) and an NC3Rs Infrastructure for Impact Award (NC/P001076/1).

References

[1] Li J, Hua Y, Miyagawa S, Zhang J, Li L, Liu L, et al. hiPSC-Derived Cardiac Tissue for Disease Modeling and Drug Discovery. International Journal of Molecular Sciences. 2020;21(23).

[2] Paci M, Passini E, Klimas A, Severi S, Hyttinen J, Rodriguez B, et al. All-Optical Electrophysiology Refines Populations of In Silico Human iPSC-CMs for Drug Evaluation. Biophysical Journal. 2020;118(10):2596-611.

[3] Schwan J, Campbell SG. Prospects for In Vitro Myofilament Maturation in Stem Cell-Derived Cardiac Myocytes. Biomarker insights. 2015;10(Suppl 1):91-103.

[4] Schwan J, Kwaczala AT, Ryan TJ, Bartulos O, Ren Y, Sewanan LR, et al. Anisotropic Engineered Heart Tissue Made From Laser-Cut Decellularized Myocardium. Scientific Reports. 2016;6:32068-.

[5] Sewanan LR, Schwan J, Kluger J, Park J, Jacoby DL, Qyang Y, et al. Extracellular Matrix From Hypertrophic Myocardium Provokes Impaired Twitch Dynamics in Healthy Cardiomyocytes. JACC Basic Transl Sci. 2019;4(4):495-505.

[6] Ruan J-L, Tulloch Nathaniel L, Razumova Maria V, Saiget M, Muskheli V, Pabon L, et al. Mechanical Stress Conditioning and Electrical Stimulation Promote Contractility and Force Maturation of Induced Pluripotent Stem Cell-Derived Human Cardiac Tissue. Circulation. 2016;134(20):1557-67.

[7] Ma J, Guo L, Fiene SJ, Anson BD, Thomson JA, Kamp TJ, et al. High Purity Human-Induced Pluripotent Stem Cell-Derived Cardiomyocytes: Electrophysiological Properties Of Action Potentials And Ionic Currents. American Journal Of Physiology-Heart And Circulatory Physiology. 2011;301(5):H2006-H17.

[8] Takaki T, Inagaki A, Chonabayashi K, Inoue K, Miki K, Ohno S, et al. Optical Recording Of Action Potentials In Human Induced Pluripotent Stem Cell-Derived Cardiac Single Cells And Monolayers Generated From Long QT Syndrome Type 1 Patients. Stem Cells International. 2019;2019:7532657-.

[9] Margara F, Wang ZJ, Levrero-Florencio F, Santiago A, Vázquez M, Bueno-Orovio A, et al. In-Silico Human Electro-Mechanical Ventricular Modelling And Simulation For Drug-Induced Pro-Arrhythmia And Inotropic Risk Assessment. Progress In Biophysics And Molecular Biology. 2020.

[10] Land S, Park-Holohan S-J, Smith NP, dos Remedios CG, Kentish JC, Niederer SA. A Model Of Cardiac Contraction Based On Novel Measurements Of Tension Development In Human Cardiomyocytes. Journal Of Molecular And Cellular Cardiology. 2017;106:68-83.

[11] Paci M, Hyttinen J, Aalto-Setälä K, Severi S. Computational Models Of Ventricular- And Atrial-Like Human Induced Pluripotent Stem Cell Derived Cardiomyocytes. Annals Of Biomedical Engineering. 2013;41(11):2334-48.

Address for correspondence:

Milda Folkmanaite.
Department of Computer Science, University of Oxford,
Wolfson Building.
milda.folkmanaite@msdtc.ox.ac.uk