Mathematical Modelling of Electrotonic Interaction between Stem Cell-Derived Cardiomyocytes and Fibroblasts

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Abstract

Human embryonic stem cell-derived cardiomyocytes (hES-CMs) represent a promising tool for cell therapy and drug screening. We developed a hES-CM mathematical model based on data acquired with electrophysiological and RT-PCR techniques. Coupling with modelled fibroblasts was assessed too. hES-CM model reproduced satisfactorily most of the action potential (AP) features. Coupling with fibroblasts shows an increment of slope of diastolic depolarization and beating frequency and reduction of the AP peak. These results suggest that our novel mathematical model can serve as a predictive approach to interpret and refine in-vitro experiments on hES-CMs.

1. Introduction

Human embryonic stem cell-derived cardiomyocytes represent an interesting and promising tool for regenerative medicine and drug screening. For these aims in vitro characterization of this lineage is mandatory and useful experimental approaches are represented by different electrophysiological and molecular techniques as current voltage mesurements on single cells and embryonic body and RT-PCR. A complementary approach to analyse hES-CM function resides in developing a mathematical model of their action potential. A better scenario would be also represented letting the single hES-CM interact with one or more human fibroblasts, conveniently modelled: this would allow studying the interaction between these two kinds of cells in terms of electrotonic coupling and modifications of the hES-CM AP shape. This approach has been extensively used for human [1, 2] and rat [3] adult cardiomyocytes. One of the newest aspects of this work is the development of specific AP model for hES evolving into cardiomyocytes, showing a spontaneous beating activity. According to this prospective AP properties of hES-CM model, eventually coupled with one or more in silico fibroblasts, were analysed.

2. Methods

2.1. Experimental data

The hES-CMs in their early developmental stage (15 - 40 days) were characterized with a combination of electrophysiological (single cell patch- clamp and multicellular recordings) and RT-PCR techniques. These experiments led to the characterization of transient outward current I_{to}, delayed rectifier current I_{Kr}, f-current I_f, inward rectifier I_{K1} and L-type current I_{CaL}. More information about the experimental protocol and the single currents characterization can be found in [4, 5]. Data about the other ionic currents were derived from [6–9].

2.2. Modelling the hES-CM

Experimental data were integrated into a modified version of the Ten Tusscher model of human cardiomyocyte (TT04) [10, 11]. Matching the model currents to the experimental data required a refined tuning of parameters since currents are not expressed as in a human adult cardiomyocytes till the development is not terminated. Moreover the hyperpolarization-activated I_f, not present in adult ventricular cardiomyocytes, was incorporated following the Hodking-Huxley formulation with a single activation gate x_f, maximal conductance G_f and Nernst potential $E_f = -17$ mV:

$$I_f = G_f \cdot x_f \cdot (V - E_f) \tag{1}$$

This led to an auto-oscillating cell model with no pacing required, whose AP is expressed by the classical equation:

$$\frac{dV}{dt} = -\frac{I_{ion}(V,t)}{C} \tag{2}$$

where $I_{ion}(V,t)$ represents the sum of the K^+, Na^+ and Ca^{++} currents flowing through the membrane and C the hES-CM capacity. Using this model several AP features were calculated.

Table 1. Maximal conductances and currents incorporated in the model to simulate the action potential of the hES-CM.

$G_{i,max}/I_{i,max}$	hES-CM	Adult	
I_{to}	49.19	294	S/F
I_{Kr}	384	96	S/F
I_f	33.6	–	S/F
I_{K1}	678	5405	S/F
I_{CaL}	0.044	0.175	dm^3/(F·s)
I_{Na}	979	14838	S/F
I_{Ks}	1.57	157	S/F
I_{NaCa}	6000	1000	A/F
I_{NaK}	0.409	1.362	A/F
I_{pK}	–	14.6	S/F
I_{up}	0.013	0.425	mM/s
I_{rel}	12.4	24.7	mM/s
I_{leak}	0.03	0.08	1/s

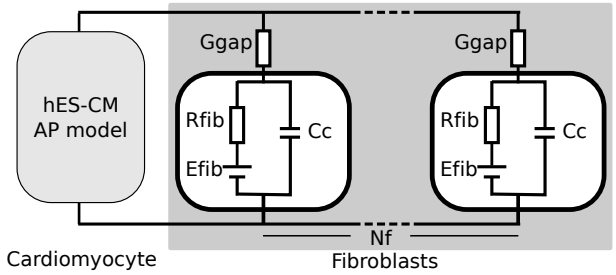

Figure 1. Electrical representation of passive fibroblasts and coupling with the hES-CM.

2.3. Interaction with in silico Human Fibroblast

AP registrations were not performed on single cells but on embryoid bodies, aggregates containing different cell phenotypes among which hES-CMs and fibroblasts. Therefore, an additional human fibroblast model, to couple resistively to the hES-CM, was developed following two different implementations. The aim is testing the interaction between these kinds of cells and trying to fit better the AP features. The electrical representation of the coupled model is shown in figure 1. Equation 2 was therefore modified as follows:

$$\frac{dV}{dt} = -\frac{1}{C} \cdot [I_{ion}(V,t) + N_f \cdot I_{leak}] \quad (3)$$

where

$$I_{leak} = G_{gap} \cdot (V - V_c) \quad (4)$$

V_c is the fibroblast potential, G_{gap} is the conductance of the hES-CM - fibroblast coupling and N_f the number of coupled fibroblasts. The fibroblast AP evolves according to

$$\frac{dVc}{dt} = -\frac{1}{Cc} \cdot [I_c(V_c) - I_{leak}] \quad (5)$$

where C_c represents the fibroblast capacity and I_c the transmembrane current. First, a model of passive fibroblast was developed according to [1–3] with MacCannell's parameters: $G_{gap} = 3$ nS, $C_c = 6.3$ pF, $R_{fib} = 10.7$ GΩ and $E_{fib} = -20$ mV. A second model of active fibroblast was implemented replacing $I_c(V_c)$ with the sum of the four time-voltage dependent currents identified by MacCannell and others (K^+ current I_{Kv}, Inward rectifying K^+ current I_{K1}, Na^+K^+ pump current I_{NaK}, and Background Na^+ current $I_{b,Na}$) [1]. Equations and parameters are the same of the cited article, except for the Background Na^+ current conductance $G_{b,Na}$ changed from 0.0095 nS/pF to 0.0032 nS/pF. We set this parameter in order to equal the integrated Na^+ influx through the leak pathway to the Na^+ efflux through the Na^+K^+ pump. We assume this discrepancy caused by differences in the cardiomyocyte model, since we used a modified version of Ten Tusscher model [11].

3. Results

3.1. hES-CM Model

Table 1 shows the results of hES-CM parameter identification compared to the adult cardiomyocyte ones. Using these parameters we found that APs mimic satisfactorily the recorded ones as shown in figure 2 so AP features were calculated as shown in table 2, column *Exp*.

The proposed model of hES-CM was able to reproduce the experimentally observed AP morphology as shown in figure 2. The AP features measured on the experimental data were recalculated on the hES-CM simulated AP and reported in table 2, column *Simulations - Uncoupled*. Most of the simulated features shows a satisfactory matching to the real ones but MDP, APA and DDR are not good enough. Since the experimental data were acquired from intracellular recordings on embryoid bodies where non-excitable cells are present together with hES-CMs [4], we decided to test our cardiomyocyte model coupled with some fibroblasts, as shown in the next section.

3.2. Coupling with Fibroblasts

Before assessing the results of our model in terms of AP features, some verifications were made in order to test its correctness. First we verified the electrotonic coupling

Table 2. Features of experimental (Exp) and simulated AP. Simulations have been performed using the model of one single hES-CM uncoupled and coupled both with passive and active fibroblasts. N_f, number of coupled fibroblasts; APD, action potential duration; MDP, maximum diastolic potential; APA, action potential amplitude; DDR, diastolic depolarization rate; F, frequency; bpm: beats/min. Bold font reports simulated results not matching the experimental data.

	Exp	Simulations				
		Uncoupled	Passive Fibroblast		Active Fibroblast	
		Nf = 0	Nf = 1	Nf = 2	Nf = 1	Nf = 2
$APD30\%$ (ms)	115±7	110	111	115	110	110
$APD50\%$ (ms)	167±10	166	168	175	167	168
$APD70\%$ (ms)	199±11	202	207	**213**	205	207
$APD90\%$ (ms)	228±11	231	237	**246**	221	237
$Vmax$ (mV/s)	4216 ±611	4778	**4965**	4825	4637	4403
MDP (mV)	-47±7	**-79**	**-79**	**-78**	**-79**	**-79**
APA (mV)	63±5	**92**	**91**	**90**	**91**	**90**
DDR (mV/s)	22±5	**13**	20	27	17	18
F (bpm)	36±6	35	**51**	**61**	38	41

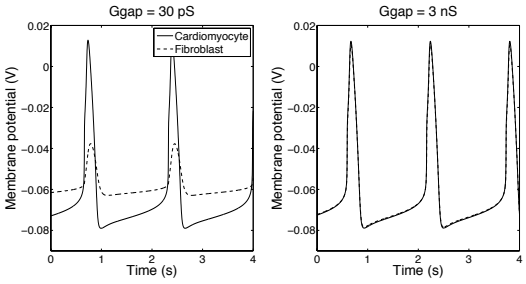

Figure 3. Simulation with different coupling conductance between hES-CM and one single fibroblast: left panel G_{gap} = 30 pS, right panel G_{gap} = 3 nS. Increasing G_{gap} value means a better mimicking of hES-CM AP by fibroblast.

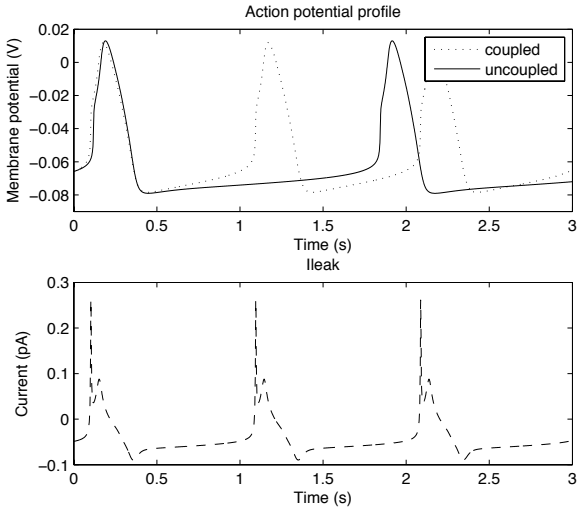

Figure 4. I_{leak} current acts modulating the hES-CM AP.

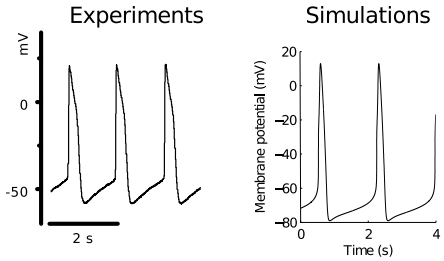

Figure 2. Experimental (left panel) and simulated (right panel) uncoupled hES-CM AP.

between hES-CM and fibroblasts as reported in literature [12]. In figure 3 two different APs arisen in a fibroblast coupled to the cardiomyocyte are shown. Increasing the coupling conductance G_{gap}, the fibroblast AP mimics the cardiomyocyte one in a more refined way, as reported in [1, 2]. A second check concerned about the I_{leak} flowing between cardiomyocyte and fibroblast. As shown in figure 4 it is negative (flowing from fibroblast to cardiomyocyte) during diastolic phase, increasing the cardiomyocyte AP slope, while it is positive (from cardiomyocyte to fibroblast) during the sistolic phase so representing a new repolarizing term. This caused on one side a more steep diastolic phase and an increase of the frequency, on the other a slightly reduced amplitude of the AP peak. After these tests, we proceeded assessing the effect of the passive fibroblast model on the cardiomyocyte AP increasing the N_f term. As shown in figure 5 and table 2 the main effects are: increased F and DDR, reduced MDP, APA and Vmax. Comparing the coupled system results with the electrophysiological data we found that the passive model performs a rude modulation of DDR and frequency. In or-

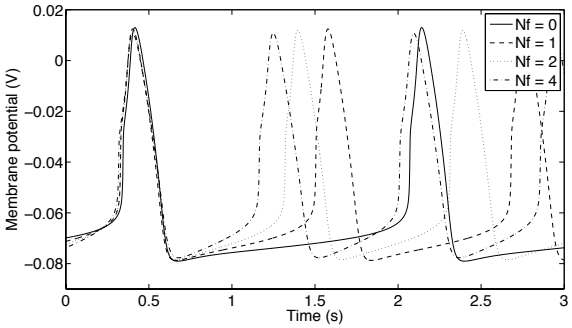

Figure 5. hES-CM coupled with passive fibroblasts.

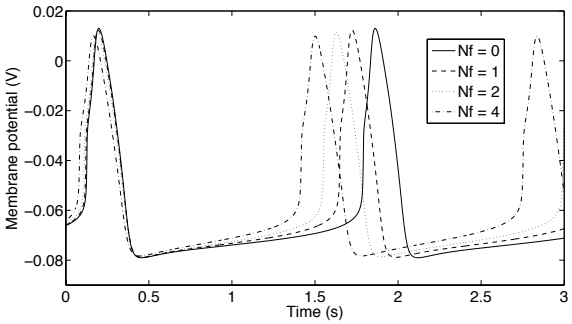

Figure 6. hES-CM coupled with active fibroblasts.

der to test further improvements in MDP, APA, and DDR we used the MacCannell's active fibroblast model, whose results are shown in figure 6 and table 2. Coupling with a small number of fibroblasts, we are able to gain a DDR in good agreement with experimental data, without losing other correct features. Two features (MDP and APA) don't closely match the experimental data yet, but it is interesting to note that increasing N_f they slowly approach the recorded values. In particular with $N_f = 10$ MDP increases of 2.3 mV while APA reduces of 10.5 mV, even if the AP shape is extremely warped (data not shown).

4. Discussion and conclusions

Our mathematical model offers a tool that can serve as a predictive approach to interpret and refine in vitro experiments on hES-CMs. In particular it predicts the effects of electrotonic coupling with fibroblasts detected in the embryoid bodies used for our recordings. A leakage current flows between hES-CM and fibroblasts, representing a depolarizing term during diastole (F and DDR increase) and a repolarizing term during systole (MDP and APA reduction). Our work shows that few fibroblasts can affect DDR while their influence on MDP and APA is relatively small. These model predictions could be validated by comparison with further AP recordings on single hES-CMs.

References

[1] MacCannell KA, Bazzazi H, Chilton L, Shibukawa Y, Clark RB, Giles WR. A mathematical model of electrotonic interactions between ventricular myocytes and fibroblasts. Biophysical Journal Jun 2007;92(11):4121–4132.

[2] Jacquemet V, Henriquez CS. Modelling cardiac fibroblasts: interactions with myocytes and their impact on impulse propagation. Europace Nov 2007;9 Suppl 6:vi29–37.

[3] Kohl P, Kamkin AG, Kiseleva IS, Noble D. Mechanosensitive fibroblasts in the sino-atrial node region of rat heart: interaction with cardiomyocytes and possible role. Exp Physiol Nov 1994;79(6):943–956.

[4] Sartiani L, Bettiol E, Stillitano F, Mugelli A, Cerbai E, Jaconi ME. Developmental changes in cardiomyocytes differentiated from human embryonic stem cells: a molecular and electrophysiological approach. Stem Cells May 2007; 25(5):1136–1144.

[5] Severi S, Sartiani L, Jaconi ME, Mugelli A, Cerbai E. Action potential modelling predicts electrophysiological and pharmacological features of human embryonic stem cell-derived cardiomyocytes. Biophysical Journal 2009;96(3, Supplement 1):664a.

[6] Itoh H, Naito Y, Tomita M. Simulation of developmental changes in action potentials with ventricular cell models. Systems and synthetic biology Mar 2007;1(1):11–23.

[7] Qu Y, Ghatpande A, el Sherif N, Boutjdir M. Gene expression of na+/ca2+ exchanger during development in human heart. Cardiovasc Res Mar 2000;45(4):866–873.

[8] Satin J, Kehat I, Caspi O, Huber I, Arbel G, Itzhaki I, Magyar J, Schroder EA, Perlman I, Gepstein L. Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes. J Physiol Lond Sep 2004;559(Pt 2):479–496.

[9] Otsu K, Kuruma A, Yanagida E, Shoji S, Inoue T, Hirayama Y, Uematsu H, Hara Y, Kawano S. Na+/k+ atpase and its functional coupling with na+/ca2+ exchanger in mouse embryonic stem cells during differentiation into cardiomyocytes. Cell Calcium Feb 2005;37(2):137–151.

[10] ten Tusscher KHWJ, Noble D, Noble PJ, Panfilov AV. A model for human ventricular tissue. Am J Physiol Heart Circ Physiol Apr 2004;286(4):H1573–1589.

[11] Grandi E, Pasqualini FS, Pes C, Corsi C, Zaza A, Severi S. Theoretical investigation of action potential duration dependence on extracellular ca2+ in human cardiomyocytes. J Mol Cell Cardiol Mar 2009;46(3):332–342.

[12] Kohl P, Camelliti P, Burton FL, Smith GL. Electrical coupling of fibroblasts and myocytes: relevance for cardiac propagation. J Electrocardiol Oct 2005;38(4 Suppl):45–50.

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