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Computer Modelling of Cardiac Cells Electrical Activity

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***Abstract*—The electrical restitution property of cardiomyocytes is studied in the paper. The parallel conductance model, improved for the heart cells, is used to simulate the action potential (AP) and ions currents for K^+, Na^+, Ca^{2+} channels. The steep areas of electrical restitution curves for ventricular cardiomyocytes are obtained. The electrophysiological phenomenon of the sudden changes in heart rate at proposed stimulation protocol is demonstrated.**

Keywords — cardiomyocyte; action potential duration; parallel conductance model; electrical restitution curve; sudden change of heart rhythm

I. INTRODUCTION

Cardiac arrhythmias are the most frequent diseases of the cardiovascular system. Therefore, the efforts of many scientists are aimed at developing methods and tools to study myocardial instability, which often leads to potentially life-threatening arrhythmias. The study of the heart electrical activity is performed by using experimental and mathematical models [1, 2].

In the early phase of development of experimental researches, cardiac *in vitro* models possessed several shortcomings such as lack of a 3D biomimetic environment, in which cells can organize and generate 3D constructs, and the use of animal cells, which resulted inadequate to model the human myocardium [3]. Furthermore, experimental research on animals is lengthy, expensive and controversial.

Recent experimental studies [4-6] have shown that the lab-on-chip technology represents an advanced *in vitro* tool to generate more relevant cardiac models. Indeed, these platforms not only allow the precise control of cell environmental conditions, but reduce the reagents and cells. This enabled the manipulation of human-induced pluripotent stem cells (hiPSCs) differentiated into cardiomyocytes (hiPSC-CMs) to perform drug screening and to study tissue regeneration or heart disease development. Through heart-on-chip technology researchers [4] have successfully modeled a human heart on an engineered chip and have measured the effects of drug exposures on cardiac cell functions by measuring cell contraction using microelectrodes.

Authors of the article [5] proposed a simple screening tool for measuring extracellular potentials from cardiomyocytes to identify side effects from drugs on cardiac ion channels in physiological conditions. The study [6] would be helpful in establishing an electrophysiological analysis to evaluate the risk of drug-induced arrhythmia using hiPSC-CMs.

To create native myocardial environment of cells we have recently developed a specific beating heart-on-chip [7] that enable to reproduce cardiac-specific three-dimensional (3D) architecture allowing for complex cell–cell interactions. The proposed device (Fig.1) allows performing cyclic mechanical stretch, generated 3D microtissue, as well as evaluation of the electrophysiological properties of cardiomyocytes. The microfabricated device uses two compartmentalized microchambers, separated by a membrane. The top compartment is subdivided by means of two rows of hanging posts into a central channel and two side channels. Cardiac cells, suspended in a matrix of fibrin gel, fill the central channel and generate a 3D cell construct, while the culture medium is replenished through the side channels.

The lab-on-chip platform provides a quantitative and predictive system to assess cardiac electrical functionality by evaluating the excitation threshold and the maximum capture rate of microtissue, when cultured in different conditions (i.e. static or mechanically stretched). However, experimental studies using hiPSC-CMs are complex and electrophysiological properties of 3D organized cells are difficult to assess. In the case of life-threatening arrhythmias investigation, mathematical modelling could be very useful and preferable method to study tissue electrophysiology, since it can predict possible dangerous behavior of cardiomyocytes.

It is known, that the dynamics of the cardiomyocytes electrical activity is determined by intracellular and extracellular processes of the action potential (AP) generation and its propagation through the heart conduction system. Different researchers have created a wide variety of models that simulate the appearance of the AP in cardiomyocytes. There are complex detailed models for the simulation of action potential, which use a large number of gate variables [1, 8-11]. The model of electrical activity in cardiomyocytes, proposed in [12], simulates the dynamics of action potential and transmembrane currents without unnecessary complications.

Based on this previously mentioned work [12], the present study is devoted to improve methods and tools to study hiPSC-CMs electrical activity by computational modelling of

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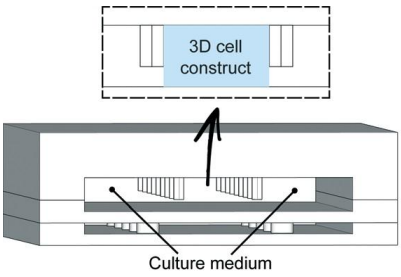
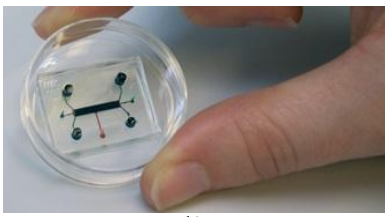
electrophysiological properties of cardiomyocytes that supplements experimental 3D models at the functional level.



a)



b)

Fig. 1. Design of the 3D heart-on-a-chip microdevice: a) the structure of the microfabricated heart-like device; b) the actual 3D heart-on-chip device (view from above). Modified from [7].

II. Computational Modelling

In this research, the parallel conductance model [12] was used to simulate electrophysiological properties of cardiomyocytes at the cellular level. This model takes into account membrane potential and main ionic currents of heart cells. Independent conductance channels were considered for K^+, Na^+, Ca^{2+} and leakage using the approach of Hodgkin and Huxley [1].

Transmembrane potential $V_m(t)$ can be considered as the sum of resting potential V_{m0} and alternative component of membrane potential $v_m(t)$:

$$V_m(t) = V_{m0} + v_m(t),$$

Resting potential V_{m0} is determined from the parallel conductance model as

$$V_{m0} = \frac{-g_{K0}E_K + g_{Na0}E_{Na} + g_{Ca0}E_{Ca} - g_l E_l}{g_{K0} + g_{Na0} + g_{Ca0} + g_l},$$

where g_{K0}, g_{Na0} and g_{Ca0} are the conductances of K^+, Na^+, Ca^{2+} ions at rest; g_l is conductance of the leakage through membrane; the sources E_K, E_{Na}, and E_{Ca} simulate Nernst potential of K^+, Na^+, Ca^{2+}; a source E_l simulates Nernst potential for chlorine ions at rest and leakage.

In accordance with the parallel conductance model, the alternative component of the membrane potential $v_m(t)$ under influence of the depolarizing current I_d can be determined from the differential equation

$$\frac{dv_m}{dt} = \frac{1}{C_m}\left(-I_K(v_m,t) - I_{Na}(v_m,t) - I_{Ca}(v_m,t) - I_l + I_d\right), \quad (1)$$

where currents for K^+, Na^+, Ca^{2+} ions are determined as

$$I_K(v_m,t) = g_K(v_m,t)(V_{m0} + v_m + E_K),$$
$$I_{Na}(v_m,t) = g_{Na}(v_m,t)(V_{m0} + v_m - E_{Na}),$$
$$I_{Ca}(v_m,t) = g_{Ca}(v_m,t)(V_{m0} + v_m - E_{Ca})$$

Current of the leakage through the membrane is defined as

$$I_l = g_l(V_{m0} + v_m + E_l).$$

Depolarizing current has amplitude I_{d0} and duration T_d:

$$I_d = \begin{cases} I_{d0}, & 0 < t < T_d \\ 0, & t \geq T_d \end{cases}$$

This current is applied to the membrane at a frequency of *Fst*, determined by a protocol of electrical stimulation.

Conductances of potassium, sodium and calcium channels under the membrane polarization can be described by the equations:

$$g_K(v_m,t) = g_{K\max} n^4(v_m,t),$$
$$g_{Na}(v_m,t) = g_{Na\max} m^3(v_m,t) h(v_m,t),$$
$$g_{Ca}(v_m,t) = g_{Ca\max} d(v_m,t) f(v_m,t),$$

where $g_{K\max}$, $g_{Na\max}$ and $g_{Ca\max}$ are the membrane conductances for K^+, Na^+, Ca^{2+} ions, when all the channels of the membrane for this type of ions are in the open state; n is activation function of potassium channels; m is activation function of sodium channels; h is inactivation function for sodium channels; d is activation function of calcium channels and f is inactivation function for calcium channels.

Conductances and currents for K^+, Na^+, Ca^{2+} ions are determined by five gating variables *n, m, h, d,* and *f*, which are solutions of the differential equations:

$$\frac{dn}{dt} = \frac{n_\infty - n}{\tau_n}, \quad \frac{dm}{dt} = \frac{m_\infty - m}{\tau_m}, \quad \frac{dh}{dt} = \frac{h_\infty - h}{\tau_h},$$
$$\frac{dd}{dt} = \frac{d_\infty - d}{\tau_d}, \quad \frac{df}{dt} = \frac{f_\infty - f}{\tau_f}, \quad (2)$$

where n_∞, m_∞, and d_∞ are the steady-state values of activation function for K+, Na+, Ca2+ channels respectively;

h_∞ and f_∞ are the steady-state values of inactivation function for sodium and calcium channels; $\tau_n, \tau_m,$ and τ_d are the relaxation periods of activation for potassium, sodium and calcium channels; τ_h and τ_f are the relaxation periods of inactivation for sodium and calcium channels.

Equations (1) and (2) define the Cauchy problem for the system of ordinary differential equations with the initial conditions:

$$v_m(0) = 0\,;\ n(0) = n_0;\ \ m(0) = m_0;$$
$$d(0) = d_0;\ h(0) = h_0;\ f(0) = f_0.$$

where n_0, m_0, d_0 are probabilities that the activation subunits of K^+, Na^+ and Ca^{2+} channels are in the open state at rest of membrane, and h_0, f_0 are probabilities that the inactivation subunits of Na^+, Ca^{2+} channels are in the open state at rest of membrane.

The above system is a set of stiff differential equations. To solve Cauchy problem the implicit method of integration was used. A detailed description of the functions and numerical values for parameters of the proposed model is given in [12].

III. Simulation Results and Discussion

Numerical experiments to model electrical activity in heart cells were performed in Matlab environment by use of the parallel conductance model. The dependences for AP generation, changes of membrane currents and conductances for K+, Na+, Ca2+ channels, as well as property of electrical restitution in ventricular cardiomyocytes were investigated.

The objectives of our study were to research restitution curves and their maximum slopes by means of computational model for cardiomyocytes, to focus on the simulation of dynamics of cardiomyocytes electrical activity during various stimulation protocols and to determine conditions for electrical stimulation in which different heart rhythms in cardiac cells may arise.

It is known that the changes of action potential duration (APD) play an important role in arrhythmogenesis. A lot of research has been carried out to investigate electrical restitution, which is an internal cardiac property of changing APD in accordance with heart rate [13-15]. The large number of investigations have shown the relationship between oscillations in myocardium and the slope of restitution curve ("restitution hypothesis"). It is believed, that under maximum slope of restitution curve dynamic instability of cardiomyocytes may occur.

To investigate the electrical restitution of cardiomyocytes the relationships between APD and stimulation frequency (*Fst*), cycle length (*CL*) or preceding diastolic interval (DI) are determined. Usually, the preceding DI is defined as the residual time interval between *CL* and APD. The APD shortens with decreasing *CL* length and decreasing *DI*. It is thought that restitution takes place because current of Ca^{2+} ions does not restore its properties at short *DI*, which leads to short APD at short *DI* [13-15].

Electrical restitution data were simulated using the dynamic restitution protocol (DYRT) [13], in which stimulation impulses were generated with various stimulation frequencies (*Fst*) or cycle length (*CL*). According to this protocol cardiomyocytes were stimulated in the physiologically determined frequency range with increasing *Fst* (decreasing *CL*) incrementally.

In this work the new protocol of electrical stimulation, consisting from two stages, was proposed. The first stage was designed to obtain the electrical restitution data of cardiomyocytes. The second stage was intended for research of modes, in which the different beating rhythms can occur. Durations of action potentials were measured as the interval from beginning of action potential to the time point of 90% of AP duration (APD90).

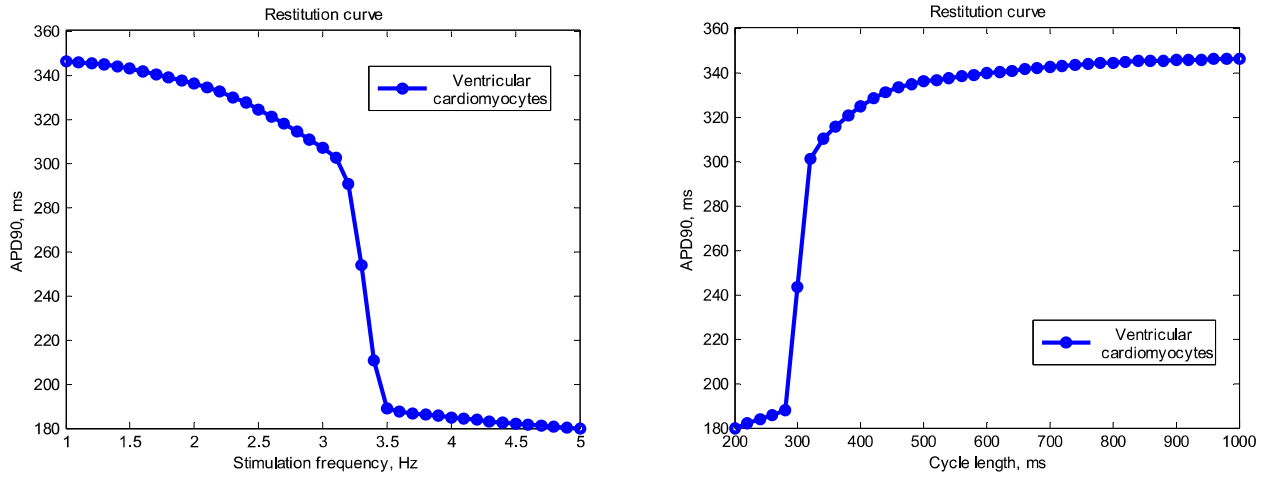
During the first stage the simulation of protocol DYRT was performed by use of the electrical stimulation started at 1 Hz and increased with 1 Hz increments up to 5 Hz. APDs for ventricular cardyomyocytes shortened at increasing *Fst* and decreasing *CL* (Fig. 2). Mean APD90 at each stimulation frequency (cycle length) was obtained by averaging of 20 consecutive steady-state APs. The electrical restitution curves of mean APD90 for ventricular cardyomyocytes are shown as functions of *Fst* (Fig. 2, a) and as functions of *CL*, the inverse of *Fst* (Fig. 2, b). These restitution curves have several phases with the various steepness, at that the maximum slope of the curves arises due to refractoriness of cardiomyocytes.

At the beginning of the second stage of protocol the electrical stimulation was performed at 1 Hz to create the identical initial conditions for the dynamics of the cardiomyocytes electrical activity. Further stimulation was carried out at the constant high stimulation frequency (*Fst*=3.39 Hz) or small cycle length (*CL*=295 ms), which correspond to the areas of the restitution curve with a steep slope. To simulate the change of beating rhythms in cardiomyocytes the different delay time of stimulation pulses (DTSP) was used, which set the various initial conditions.

The set of 10 replies of cardiomyocytes from the 10 stimulus at constant CL=295 ms (beating rhythms 1:1) according to the initial delay time of stimulation pulses (DTSP=280 ms) is demonstrated in Fig. 3 for action potentials (Fig. 3,a); for currents of K^+, Na^+, Ca^{2+} ions (Fig. 3, b); for conductance of Ca^{2+} channels (Fig. 3, c); and for conductance of K^+, Na^+ channels (Fig. 3, d).

Change of beating rhythms (2:1) in cardiomyocytes from the change of the initial delay time of stimulation pulses (DTSP = 310 ms) at constant *CL*=295 ms is demonstrated in Fig. 4 for action potentials (Fig. 4, a); for currents of K^+, Na^+, Ca^{2+} ions (Fig. 4, b); for conductance of Ca^{2+} channels (Fig. 4, c); and for conductance of K^+, Na^+ channels (Fig. 4, d).

Additionally, in this study the dynamics of the cardiomyocytes electrical activity, which determined by intracellular and extracellular processes, was investigated by simulation of several changes in the initial conditions during stimulation (Fig. 5).



a) b)

Fig. 2. Electrical restitution curves for ventricular cardiomyocytes: APD90 against *Fst* (a), APD90 against *CL* (b).

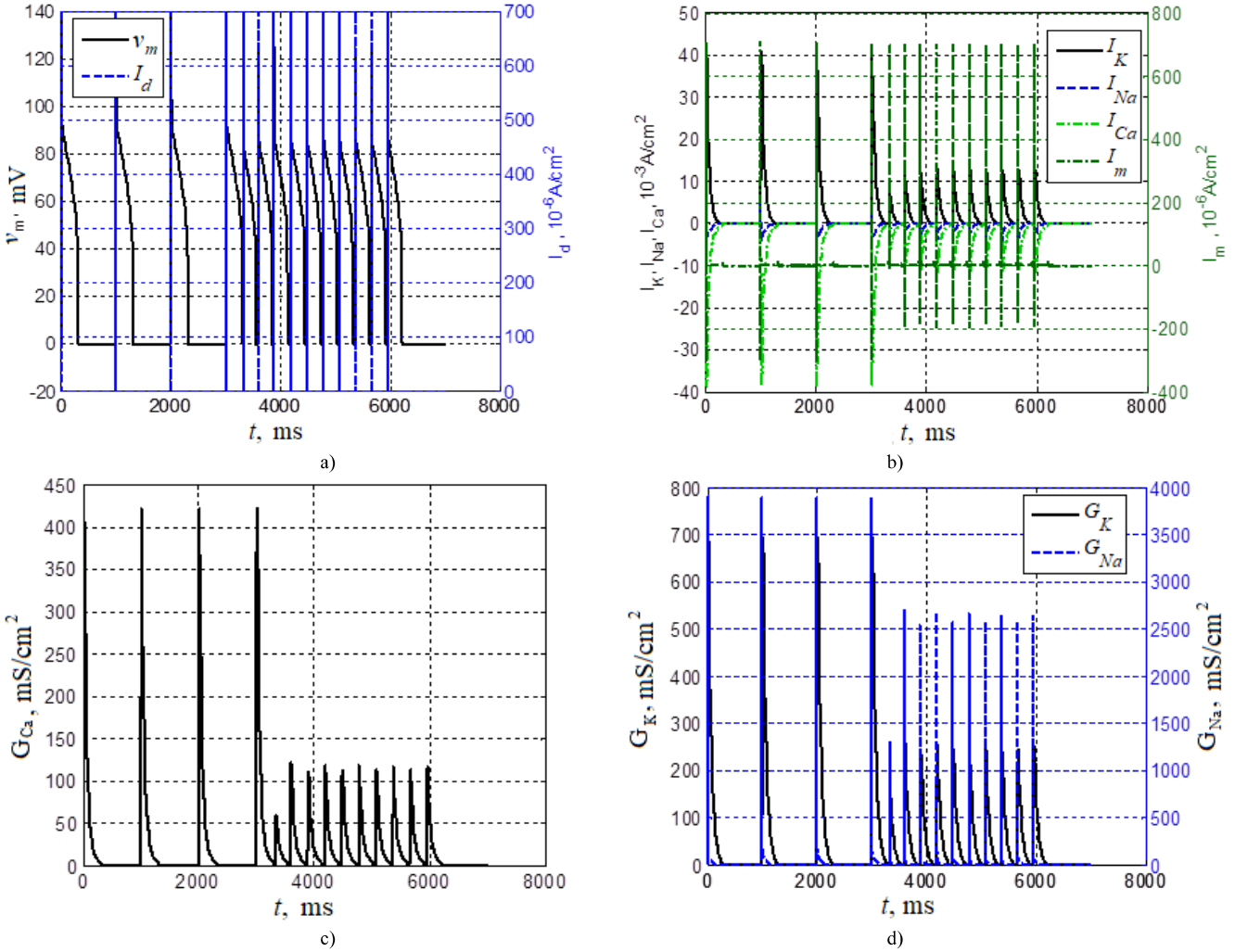


Fig. 3. The set of 4 replies at CL_1= 1000 ms and 10 replies of cardiomyocytes with beating rhythms 1:1 at CL_2=295 ms and the delay of stimulation pulses (*DSP*= 280 ms): action potentials (a); currents of K^+, Na^+, Ca^{2+} ions (b); conductance of Ca^{2+} channels (c); conductance of K^+, Na^+ channels (d).

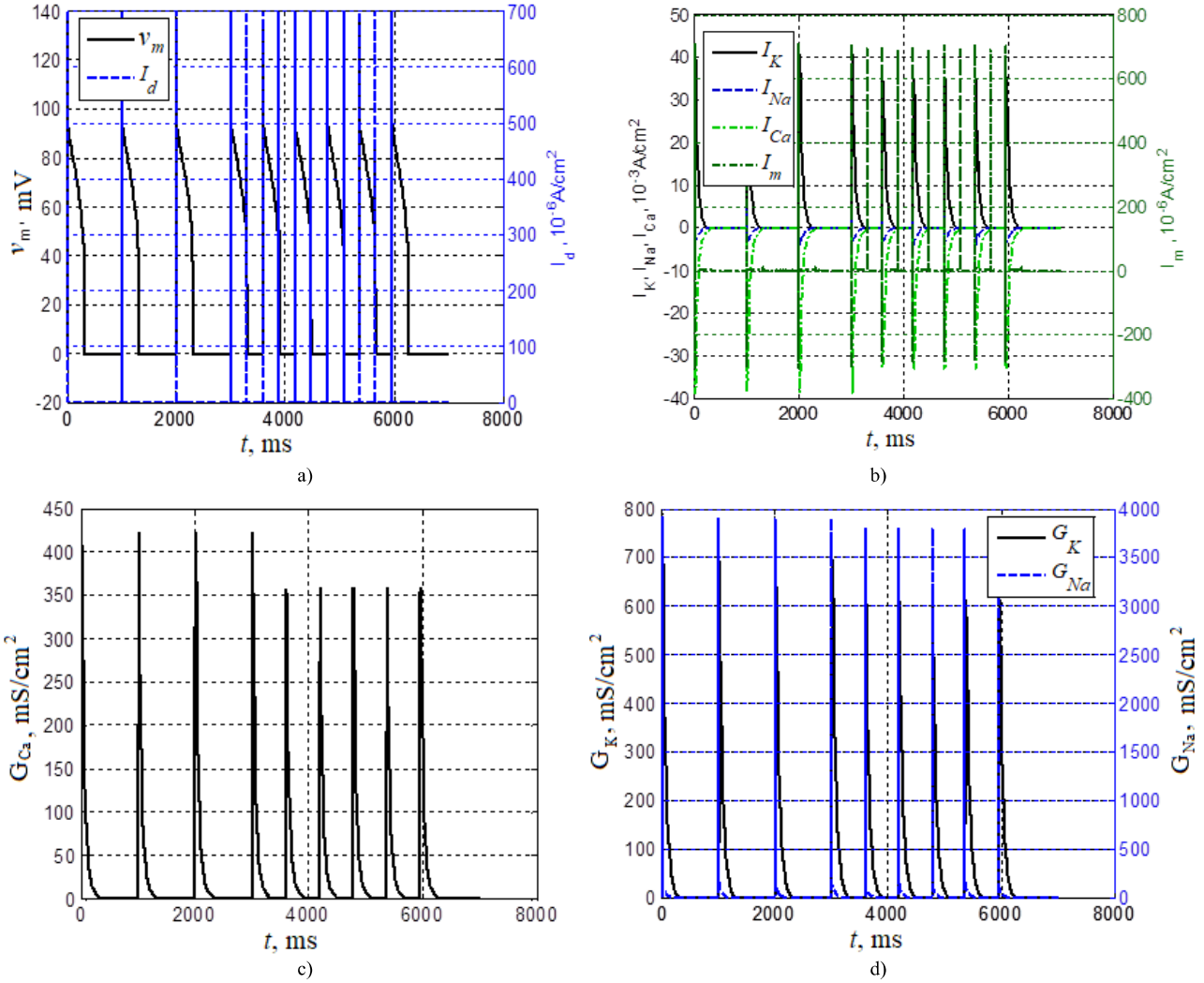


Fig. 4. The set of 4 replies at CL_1= 1000 ms and 5 replies of cardiomyocytes with beating rhythm 2:1 at CL_2=295 ms and delay of stimulation pulses (DSP=310 ms): action potentials (a); currents of K^+, Na^+, Ca^{2+} ions (b); conductance of Ca^{2+} channels (c); conductance of K^+, Na^+ channels (d).

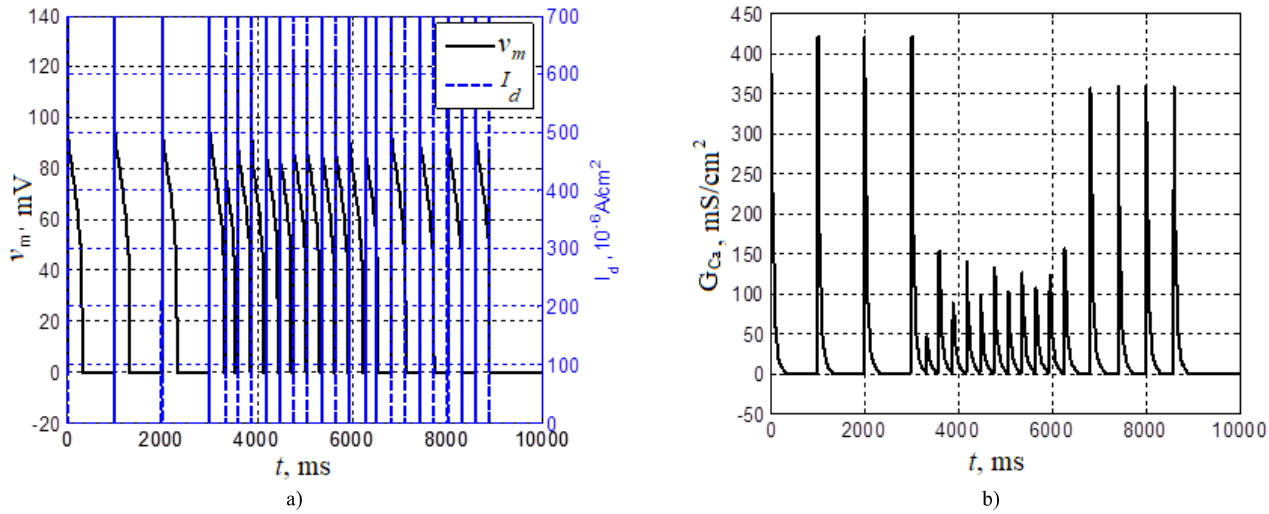


Fig. 5. Fig.5. The switching from heart rhythm 1:1 to heart rhythm 2:1 in cardiomyocytes according to the initial delay of stimulation pulses (at delay DSP_1= 280 ms and delay DSP_2=310 ms): action potentials (a); conductance of Ca^{2+} channels (b).

The obtained results demonstrate bioelectrical processes in cardiomyocytes during second stage of stimulation protocol, which consist in this case of three parts. During the first part of the protocol the electrical stimulation was performed using a constant stimulation frequency (Fst=1 Hz) or cycle length (CL=1000 ms). During the second and the third parts the electrical stimulation was performed at a constant high stimulation frequency (Fst=3.39 Hz) or small cycle length (CL=295 ms), but with different initial delay of stimulation pulses. Fig. 5 demonstrates a phenomenon of switching from heart rhythm 1:1 to rhythm 2:1 in cardiomyocytes according to the initial delay of stimulation pulses (with delay DSP1=280 ms and delay DSP2= 310 ms). Changes of the action potential durations are shown in Fig.5, a, and dependences of the conductance for Ca2+ channels are shown in Fig.5, b.

The set of 4 responses of cardiomyocytes to the impact of stimulus with CL1= 1000 ms, as well as the set of 10 responses to the impact of 10 stimulus with CL2=295 ms, DSP= 280 ms (beating rhythm 1:1) and 5 responses of cardiomyocytes to 10 stimulus with CL2=295 ms, DSP= 310 ms (beating rhythm 2:1) are demonstrated in Fig. 5.

Therefore, the identification of maximum slopes in restitution curves provides an opportunity to define the arrhythmogenic properties of heart cells and to explain the change of cardiomyocytes beating rate.

IV. Conclusion

Computational simulation of the cardiomyocytes electrical activity with use of the improved parallel conductance model allowed us to analyze the electrical restitution curves and detect the regions with elevated slopes. The results of computational experiments demonstrated the regions of electrical restitution curve, where arrhythmogenic properties of cardiomyocytes appear, explaining the changes in cardiomyocytes beating rate.

The new proposed protocol of electrical stimulation can help investigate the changes in AP, calcium current properties of hiPSC-CMs and the causes of the development of arrhythmias in response to application of different stimulation modes. Computational experiments can effectively support the design of new experiments with hiPSC-CM on the lab-on-chip platform.

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