Simulation of the Pacemaker Created from the Cardiomyocytes by Reducing Inward-Rectifier K+ Current

Yue Zhang[1], Kuanquan Wang[1], Henggui Zhang[1,2], Qince Li[1], Yongfeng Yuan[1]

1 School of Computer Science and Technology, Harbin Institute of Technology, Harbin, China
[2] School of Physics and Astronomy, University of Manchester, Manchester, UK

Abstract

Cardiac conduction disorders are common diseases, to treat which, the best way by now is to implant electronic pacemakers; nevertheless, there are many disadvantages, such as limited battery life, infection and so on. Bio-pacemaker has been being expected to replace the electronic devices. This study aimed to develop a 2D model of human bio-pacemaker created from the ventricular endo-myocytes. First, the TNNP06 human ventricular model was used to investigate the automaticity of the created single pacing cell. Then the 2D model was developed by incorporating the automatic cells into the ventricular sheet in three different ways. For single pacing cell, the action potentials were stable with the period around 852ms. For 2D tissue, the pacemaker in the first way could not drive the tissue at all, while the whole ventricular sheet could be driven in the other two ways. What's more, the pacemaker paced at the same rhythm to the single auto-cell when it worked. This demonstrated that the driving force of pacemaker closely related to the conduction mode in addition to the size.

1. Introduction

The sinoatrial node (SAN) cells are the source of the normal excitation, initiating the heartbeat and control the rhythm [1]. However, the genuine pacemaker cells, containing no more than 10,000, drive the whole heart which contains about 10 billion cells [2]. The failure of the SAN cells causes cardiac conduction disorders, leading to syncope, easy fatigability or circulatory collapse.

The best way to treat the diseases is to implant electronic pacemakers. Nevertheless, there are still many disadvantages, such as, the limited battery life, infection, and the setting rate not able to respond to emotion [3]. Therefore, bio-pacemaker has been being expected to recapitulate the main aspects of endogenous SAN.

Three general approaches have been focused on to create bio-pacemakers: (a) Introduce special ion channels into cardiomyocytes by gene transfer. (b) Express ion channels in non-cardiomyocytes and then sent them to native myocytes in situ by cell fusion. (c) Use embryonic stem cells grown along a cardiac lineage and manifesting the electrophysiologic properties of SAN cells [4].

Miake et al. dominant-negatively inhibited the inward-rectifier potassium channels, turning the non-pacemaking ventricular cells into automatic pace-making cells [5]. Cell fusion strategies were used by Plotnikov et al. [6] and Cho et al. [7] to create bio-pacemakers, unfortunately, which didn't resemble the natural pace-making cells. Xue et al. first differentiated the embryonic stem cells into cardiomyocytes with pacemaker activity and then introduced directly into the heart of pigs or guinea pigs and obtained successful results [8]. Nonetheless, the teratogenic potential and heterogeneity [9] influenced the clinical translation.

A temporary bio-pacemaker is the preliminary target. Once infections occur, it always needs a complete removal of all the electronic hardware [10]. When the device is removed, the bio-pacemaker could provide hardware-free chronotropic support during the antibiotic treatment to clear the infection, which typically required about 2 weeks [11]. Accordingly, most subsequent experiments took an at least 14-day observation and record. In fact, short-term bio-pacemakers have been successfully created. Plotnikov et al. produced bio-pacemakers in the canine hearts by open-chest [12] and transarterial left-sided [13] approaches, however, in which the delivery methods were extremely invasive.

Recent years, bio-pacemaker technology has developed fast. Cingolani et al. [14] first applied the venous catheters to create bio-pacemaker. The adenoviral vector cocktail (K_{AAA} + H2), expressing dominant-negative I_{K1} (Kir2.1AAA) and hyperpolarization-activated cation channel (HCN2) genes, was injected into the atrioventricular junctional region through the femoral vein. The suppressed I_{K1} unleashed the automaticity of ventricular myocytes (VMs) while the exogenous I_f simultaneously increased the automatic depolarization in phase 4. Under the dual-gene approach, the significant pacemaker activity was observed over a 14-day period. They concluded that the delivery of K_{AAA} + H2 into atrioventricular junction region induced bio-pacemaker activity.

Transcription factors, such as Shox2, Tbx3, Tbx5 and Tbx18 are known as the obvious candidates of embryonic

SAN development [15]. Tbx18 was chosen by Kapoor et al. to induce bio-pacemaker in neonatal rat ventricular cells [16]. It was the first time applying only one single gene to directly convert cardiomyocytes to pacemaker cells. The converted ventricular myocytes, called induced SAN, became smaller, thin, and tapered, acquiring the exact morphological characteristics of SAN cells. What's more, the automatic electrical phenotype was also similar to that of SAN pacemaker cells.

Hu et al. created a bio-pacemaker in large animal heart [17], which was effective for up to 14 days. They transduced the gene encoding human Tbx18 into porcine ventricular cardiomyocytes by adenovirus vector. The pacemaker activity, emanating from the injection site, was observed even when the heart was completely blocked, indicating that the induced SAN was successfully working. Furthermore, the increase of arrhythmias was not observed in the bio-pacemaker heart. The results suggested that somatic reprogramming might be a viable strategy to create bio-pacemaker, increasing the possibility of clinical translation.

The temporary bio-pacemaker activity has been observed in bio-experiments. In this study, we aimed to create a 2D pacemaker computer model in the human ventricle to simulate the bio-pacemaker. The reduction of I_{K1} could induce automaticity of ventricular cells. Therefore, we achieved the single bio-pacemaker cell by depressing the I_{K1} of human ventricular endo-myocyte. The automaticity and correlative currents had been analyzed [18]. Then the working 2D pacemaker model was developed. Finally, the pseudo electrocardiogram (ECG) was computed to evaluate the function of the induced bio-pacemakers.

2. Methods

2.1. Models of ventricular myocytes

To simulate the human ventricular single cell and tissue, TNNP 2006 model [19] was used. The single cell model were given by the following equations.

$$\frac{dV}{dt} = -\frac{I_{ion} + I_{stim}}{C_m} \quad (1)$$

In the equation,

$$I_{ion} = I_{Na} + I_{K1} + I_{to} + I_{Kr} + I_{Ks} + I_{CaL} + I_{NaCa} + I_{NaK} + I_{pK} + I_{pCa} + I_{bCa} + I_{bNa}$$

where, V is the transmembrane potential; I_{ion} is the sum of all the transmembrane ion currents; I_{stim} is the external stimulus current; C_m is membrane capacitance per unit surface area; I_x are the corresponding currents in the original model. Except G_{K1}, all the other parameters keep the same as in original paper.

2.2. 2D tissue model

The 2D model was described in the following:

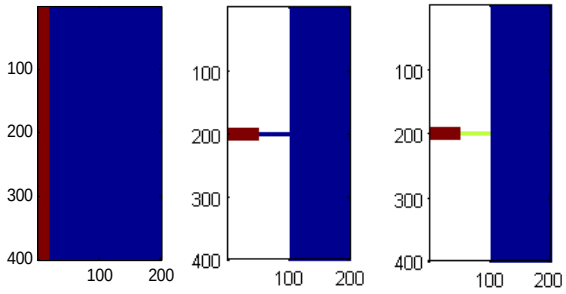
$$\frac{\partial V_{i,j}}{\partial t} = -\frac{I_{ion} + I_{stim}}{C_m} + D_{i-1}(V_{i-1,j} - V_{i,j}) + D_{i+1}(V_{i+1,j} - V_{i,j}) + D_{j-1}(V_{i,j-1} - V_{i,j}) + D_{j+1}(V_{i,j+1} - V_{i,j}) \quad (2)$$

Where, $V_{i,j}$ was the transmembrane potential of the cell at coordinate (i,j); D_k $(k = i-1,\ i+1, j-1, j+1)$ was the coupling conductance. The other parameters were the same to those in equation (1).

2.3. Tissue setting

First, the basic virtual human ventricular tissue size was set 100 cells in length and 400 cells in width, which from left to right was divided into endocardial region, mid-myocardial part and epicardial layer with the proportion of 25%, 35% and 40%.

Then, the pacemaker region was incorporated into the ventricular sheet in three different ways as shown in Figure 1: (a) the first ten volumes of endo-myocytes were replaced by automatic cells; (b) the pacemaker tissue was connected with the ventricular sheet by a bridge consisted of endo-myocytes; (c) the bridge was consisted of Purkinje fiber cells. In the last two ways, the pacemaker was set 50 cells in length and 20 cells in width. In the Figure, the red region was the pacemaker, and the blue area was the ventricular tissue and the green part was the Purkinje fiber.



(a) (b) (c)

Figure 1. Three different ways the pace-maker incorporated into the tissue.

Simulation time for both single cell and 2D model was more than 600,000ms. The space step was 0.15mm and the time step was 0.02ms.

3. Results

3.1. Action potential single automatic cell

Depressing I_{K1} by modulating the maximal conductance G_{K1} could induce automaticity of ventricular myocytes. The more the I_{K1} was suppressed, the stronger the autorhythmicity the myocytes displayed. From Figure 2, we could clearly see that the periods of automatic AP for G_{K1} = 0.1 nS/pF, G_{K1} = 0.05 nS/pF and G_{K1} = 0 nS/pF were

obviously decreasing, which indicated the enhancing of automaticity with the decline of I_{K1}.

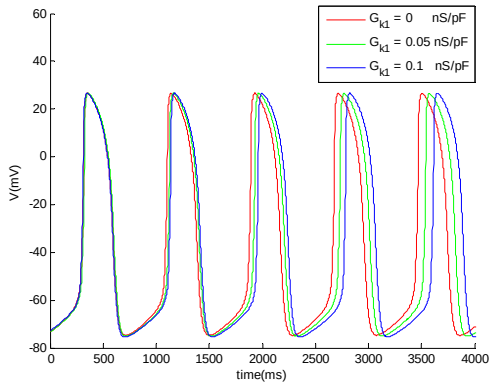


Figure 2. Effects of depressing I_{K1} on automaticity of VMs. The red curve described the APs for G_{K1} = 0 nS/pF, which meant that I_{K1} was completely blocked. The green was AP curve for G_{K1} = 0.05 nS/pF and the blue for G_{K1} = 0.1 nS/pF.

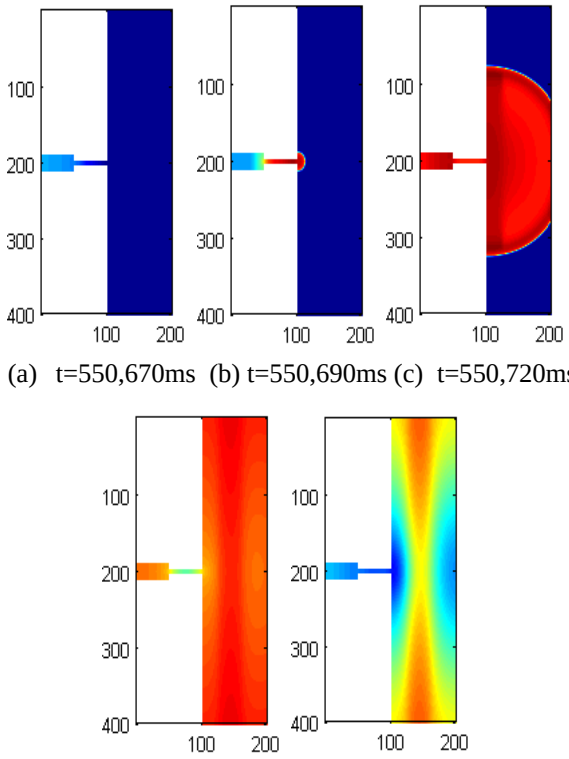
In our study, we chose G_{K1} = 0.05 nS/pF for simulation. Simulated more than 500,000ms, finding that the single automatic cell (AC) keeping in stable state with a period around 852ms. However, when simulated for 80,000ms, the average period between 70,000ms and 80,000ms was about 740ms. The difference was more than 100ms. Therefore, the simulation time for 2D slice was set no less than 600,000ms to obtain a stable state.

3.2. Propagation of automatic excitation

In the 2D model, the ACs were firstly directly connected to ventricular slice as shown in Figure 1(a). We set out to study the relationship between driving capability and the quantity of ACs, finding that autorhythmic pacemaker activity of the ACs was depressed by the surrounding VMs even under the condition that there were more than 20 volumes of myocytes changed into ACs, which might already lose the biological meaning.

The effective and reasonable pacemaker might not be created by simply increasing the number of the ACs. The ACs failed to pace because of the suppression from the adjacent VMs. In fact, for native SAN, the salient gap junction proteins made a lower single –channel conductance [20]; moreover, the excitation from SAN could only conduct to myocardium through several critical pathways [21, 22]. These shielded the SAN from the hyperpolarization environment of atrial tissue. Accordingly, in the simulation, the electric excitation of ACs was set not directly electric to the ventricular slice, only through the path from Purkinje fiber as shown in Figure 1(c). In this way, the created pacemaker could be protected from the serious depression of ventricular quiescent cells and obtained a stronger driving capability.

Figure 3 showed the propagation of the excitation generated from the pacemaker. We could find that the pacemaker worked successfully driving the whole tissue.



(a) t=550,670ms (b) t=550,690ms (c) t=550,720ms

(d) t=550,930ms (e) t=551,000ms

Figure 3. Snapshots of excitation wave propagating in the 2D tissue model.

In order to study the global function of the pacemaker for the ventricular tissue, a pseudo ECG was calculated. Result was shown in Figure 4.

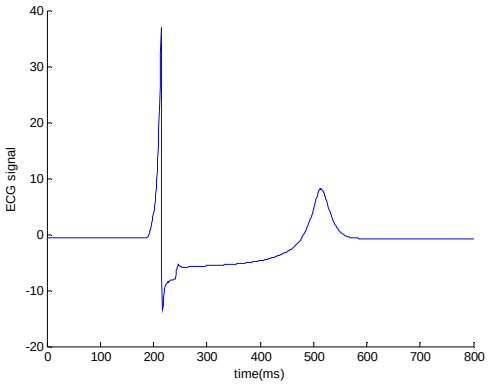


Figure 4. Simulated time course of pseudo ECG in response to the conduction of excitation wave in the ventricular tissue.

The simulated ECG showed typical features of normal ECG with positive QRS and T waves, which inferred that the created pacemaker might play important role in the

ventricular slice as the native SAN. However, the it remained negative after S wave. This may be attributable to the more positive diastolic potential of the automatic cells (around -50mV) as compared to the resting potential of other ventricular myocytes (about -85mV).

In an extra simulation, we found that the pacemaker created in the second way shown in Figure 1 (b) could also drive the ventricular tissue successfully.

4. Discussion and conclusion

In this study, we first analyzed the effects of I_{K1} on the automaticity of ventricular cells, finding that the autorhythmicity increased with the depression of I_{K1}. Then we incorporated the automatic cells into the 2D idealized ventricular tissue to investigate its pacemaker activity. When the ACs were set directly connected to the slice, the tissue could not be driven even though there were more than 8000 automatic cells which already lose the bio-physiological meaning.

When the created pacemaker conducted to the slice only through the Purkinje fiber, the tissue was successfully driven even though there were only 1000 computing units. The pseudo ECG was also calculated, showing the typical features of the normal ECG, which demonstrated the effective function of the crated pacemaker. The average periods were computed every 10,000ms, which were around 852ms similar to that of the corresponding single automatic cell.

This study demonstrated that driving force of a pacemaker closely related to the conduction way in addition to the size. Insulation from the ventricular working cells except for several conduction ways played a major part in the function of pacemaker. The results could provide guidance and advice for the bio-experiments.

Acknowledgements

This work is supported by the National Natural Science Foundation of China (NSFC) under Grant No. 61571165 and NO. 61572152.

References

[1] Bouman LN and Jongsma HJ. Structure and function of the sino-atrial node: a review. European heart journal. 1986;7(2):94-104.

[2] Bleeker WK, Mackaay AJ, Masson-Pevet M *et al.* Functional and morphological organization of the rabbit sinus node. Circulation research. 1980;46(1):11-22.

[3] Khafaji HAH. Biologic Pacemaker-Role of Gene and Cell Therapy in Cardiac Arrhythmias. INTECH Open Access Publisher; 2011.

[4] Munshi NV and Olson EN. Translational medicine. Improving cardiac rhythm with a biological pacemaker. Science. 2014;345(6194):268-9.

[5] Miake J, Marban E and Nuss HB. Biological pacemaker created by gene transfer. Nature. 2002;419(6903):132-3.

[6] Plotnikov AN, Shlapakova I, Szabolcs MJ *et al.* Xenografted adult human mesenchymal stem cells provide a platform for sustained biological pacemaker function in canine heart. Circulation. 2007;116(7):706-13.

[7] Cho HC, Kashiwakura Y and Marban E. Creation of a biological pacemaker by cell fusion. Circulation research. 2007;100(8):1112-5.

[8] Xue T, Cho HC, Akar FG *et al.* Functional integration of electrically active cardiac derivatives from genetically engineered human embryonic stem cells with quiescent recipient ventricular cardiomyocytes: insights into the development of cell-based pacemakers. Circulation. 2005;111(1):11-20.

[9] Ripplinger CM and Bers DM. Human biological pacemakers: intrinsic variability and stability. Circulation. 2012;125(7):856-8.

[10] Henrikson CA and Brinker JA. How to prevent, recognize, and manage complications of lead extraction. Part I: Avoiding lead extraction—Infectious issues. Heart rhythm : the official journal of the Heart Rhythm Society. 2008;5(7):1083-7.

[11] Betts T. Regional survey of temporary transvenous pacing procedures and complications. Postgraduate medical journal. 2003;79(934):463-5.

[12] Qu J, Plotnikov AN, Danilo P *et al.* Expression and function of a biological pacemaker in canine heart. Circulation. 2003;107(8):1106-9.

[13] Plotnikov AN, Sosunov EA, Qu J *et al.* Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. Circulation. 2004;109(4):506-12.

[14] Cingolani E, Yee K, Shehata M *et al.* Biological pacemaker created by percutaneous gene delivery via venous catheters in a porcine model of complete heart block. Heart rhythm : the official journal of the Heart Rhythm Society. 2012;9(8):1310-8.

[15] Christoffels VMs, Smits GJ, Kispert A *et al.* Development of the pacemaker tissues of the heart. Circulation research. 2010;106(2):240-54.

[16] Kapoor N, Liang W, Marban E *et al.* Direct conversion of quiescent cardiomyocytes to pacemaker cells by expression of Tbx18. Nature biotechnology. 2013;31(1):54-62.

[17] Hu YF, Dawkins JF, Cho HC *et al.* Biological pacemaker created by minimally invasive somatic reprogramming in pigs with complete heart block. Science translational medicine. 2014;6(245):245ra94.

[18] Zhang Y, Wang K, Zhang H *et al.* Simulation of ventricular automaticity induced by reducing inward-rectifier K+ current. 2014 IEEE International Conference on Bioinformatics and Biomedicine (BIBM); 2014: IEEE.

[19] ten Tusscher KH and Panfilov AV. Alternans and spiral breakup in a human ventricular tissue model. American journal of physiology Heart and circulatory physiology. 2006;291(3):H1088-100.

[20] Valiunas V, Weingart R and Brink PR. Formation of heterotypic gap junction channels by connexins 40 and 43. Circulation research. 2000;86(2):E42-9.

[21] Fedorov VV, Chang R, Glukhov AV *et al.* Complex interactions between the sinoatrial node and atrium during reentrant arrhythmias in the canine heart. Circulation. 2010;122(8):782-9.

[22] Fedorov VV, Glukhov AV and Chang R. Conduction barriers and pathways of the sinoatrial pacemaker complex: their role in normal rhythm and atrial arrhythmias. American journal of physiology Heart and circulatory physiology. 2012;302(9):H1773-83.

Address for correspondence.

Kuanquan Wang
Mailbox 332, Harbin Institute of Technology
Harbin 150001, China
wangkq@hit.edu.cn