Journal of Zhejiang University-SCIENCE B (Biomedicine & Biotechnology)
ISSN 1673-1581 (Print); ISSN 1862-1783 (Online)
www.jzus.zju.edu.cn; www.springerlink.com
E-mail: jzus@zju.edu.cn

Review:

Biological pacemaker: from biological experiments to computational simulation*

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Received Nov. 5, 2019; Revision accepted Mar. 11, 2020; Crosschecked June 5, 2020

Abstract: Pacemaking dysfunction has become a significant disease that may contribute to heart rhythm disorders, syncope, and even death. Up to now, the best way to treat it is to implant electronic pacemakers. However, these have many disadvantages such as limited battery life, infection, and fixed pacing rate. There is an urgent need for a biological pacemaker (bio-pacemaker). This is expected to replace electronic devices because of its low risk of complications and the ability to respond to emotion. Here we survey the contemporary development of the bio-pacemaker by both experimental and computational approaches. The former mainly includes gene therapy and cell therapy, whilst the latter involves the use of multi-scale computer models of the heart, ranging from the single cell to the tissue slice. Up to now, a bio-pacemaker has been successfully applied in big mammals, but it still has a long way from clinical uses for the treatment of human heart diseases. It is hoped that the use of the computational model of a bio-pacemaker may accelerate this process. Finally, we propose potential research directions for generating a bio-pacemaker based on cardiac computational modeling.

Key words: Biological pacemaker; Gene therapy; Cell therapy; Cardiac simulation; Computational modeling
https://doi.org/10.1631/jzus.B1900632 **CLC number:** Q81

1 Introduction

Cardiovascular disease has become a severe health problem all over the world. In China, cardiac diseases account for about 40% of total mortality (Chen et al., 2018). Particularly, the pacemaking dysfunction, such as sick sinus syndrome and atrioventricular heart-block, is one of the most serious cardiac diseases, and may result in heart failure and even death. Currently, the electronic pacemaker is the only treatment for pacing dysfunction. Since the clinical use of the electronic pacemaker from 1958, it has saved more than three million lives all over the world. However, there are still several shortcomings of the device. For instance, the batteries and leads have limited lives and therefore need to be changed periodically; the device may suffer interference from electromagnetics which may obstruct the normal life of the patients; open-chest surgery during implanting a pacemaker may induce infection; an electronic pacemaker cannot response to emotion; the device must be changed with the growth of a pediatric patient (Cohen et al., 2005; Rosen et al., 2011). What is more, the long-term use of electronic pacemakers will increase the risk of heart failure (Freudenberger et al., 2005). Therefore, a biological pacemaker (bio-pacemaker) was

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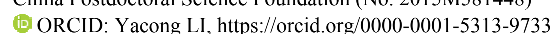
* Project supported by the National Natural Science Foundation of China (Nos. 61572152, 61601143, and 81770328), the Science Technology and Innovation Commission of Shenzhen Municipality (Nos. JCYJ20151029173639477 and JSGG20160229125049615), and the China Postdoctoral Science Foundation (No. 2015M581448)

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proposed as a substitute for the electrical pacemaker (Starzl et al., 1963; Mayer et al., 1967; Morishita et al., 1981).

A bio-pacemaker is potentially a new treatment for pacing dysfunction. It is developed to conquer the defects of electronic pacemakers, sustaining a robust pacing activity in the whole heart. Bio-pacemaker implementation can be performed by injecting pacemaker cells into the heart, which then initiates and controls the spontaneous rhythms in the whole heart. This approach avoids the risk of repeated surgery and the rhythm can respond to emotion (Shlapakova et al., 2010). We compared the performances of electronic pacemakers and bio-pacemakers (Table 1). Actually, several biological experiments have provided some promising evidence for the practicability of the bio-pacemaker. Quiescent heart-muscle cells have been successfully converted into pacemaker cells using viral gene transfer (Miake et al., 2002). Also, human embryonic stem cells (hESCs) have been successfully differentiated into cardiomyocytes (CMs) which have the ability to activate quiescent ventricular myocytes (VMs) (Xue et al., 2005). Indeed, human embryonic stem-derived CMs were transplanted to the hearts of swine with complete atrioventricular block, and successfully drove the whole heart (Kehat et al., 2004). These approaches provide an encouraging perspective on arrhythmia treatment.

Theoretically, the construction of a bio-pacemaker is based on the differences in cellular electrophysiology between pacemaking sinoatrial node (SAN) cells and non-autorhythmic cardiac cells. SAN is the origin of intrinsic automaticity. The pacing ability of SAN cells results from a low expression of the inward rectifier potassium channel current (I_{K1}) and a high expression of hyperpolarization-activated channel current (funny current (I_f)) as compared with atrial myocytes (AMs) and VMs. I_{K1} is an outward current, repolarizing cellular action potential (AP) during the terminal phase of the AP. Thus, the low expression of I_{K1} could reduce the level of hyperpolarization and elevate the maximum diastolic potential, which makes depolarization easier. I_f is generally regarded as the "pacemaker current," playing a dominant role in initiating the diastolic depolarization and pacing activity (Ravagli et al., 2016). In non-autorhythmic cells, I_{K1} is highly expressed (Miake et al., 2002; Azene et al., 2005; Cho et al., 2007), but I_f expression is low or even absent (Yu et al., 1993). The high expression of I_{K1} and absence of I_f jointly result in the loss of auto-rhythm in non-autorhythmic cells. In contrast, overexpressing I_f and inhibiting I_{K1} in non-autorhythmic cells are possible approaches to turn non-autorhythmic cells into autorhythmic cells, creating a bio-pacemaker. At the high-dimensional level, the intercellular coupling between SAN cells and neighboring cells is weak, which helps to conduct the spontaneous electrical signal to the neighboring cells. When producing the bio-pacemaker at the two-dimensional or three-dimensional level, reducing the intercellular coupling should be taken into consideration.

After over five decades' studies of bio-pacemaker, there has been considerable progress. However, limitations such as funding and moral issues are limiting experimental research. Thus, the computational simulation of a bio-pacemaker based on the cardiac computational model is a necessity.

In this review, we first introduce the progress in the development of a bio-pacemaker aiming for gene therapy and cell therapy. Then, we summarize current progress of autologous therapy and highlight the obstacles in producing a bio-pacemaker. Finally, we propose possible future trends in bio-pacemaker research—computational simulation. This review provides a systematic view on generating a bio-pacemaker from biological experiment to computational simulation. This may be helpful for the better design of a future generation of stable and robust bio-pacemakers.

2 Biological experiments for producing biological pacemakers

In the heart, there are three aspects to causing auto-rhythmicity: trigger cells with spontaneous beats, substrate cells with the function of delivering signal,

Table 1 Comparison between electronic pacemaker and bio-pacemaker

Pacemaker	Extra power	Fixed size	Electromagnetic interference	Transplant surgery	Emotion response	Risk of heart failure
Electronic pacemaker	Yes	Yes	Yes	Yes	No	Yes
Biological pacemaker	No	No	No	No	Yes	Maybe

and trigger–substrate connection (Rosen et al., 2011). In the cases of SAN cell failure, a possible treatment could be inducing rhythm in other positions, such as AMs or VMs, and making them take over the function of SAN. To realize this scenario, first, autonomic pacemaker cells should be developed. Generally, some methods have been used to create pacemaker cells: expressing a specific gene which controls pacemaking-related ionic channels by cell fusion (called gene therapy), and differentiating ESCs into pacemaker cells (called cell therapy) (Munshi and Olson, 2014; Rosen, 2014). Cell therapy is always accompanied by gene therapy so that cardiac myocytes transformed from stem cells could produce a pacemaking function, which is called hybrid therapy. The experiments by hybrid therapy are described in the section of "Cell therapy." Then, a certain amount of pacemaker cells should be injected into specific locations of the heart in vivo to induce heartbeats. The number and location of pacemaker cells should ensure the ability and stability of pacing activity.

2.1 Gene therapy

Up to now, bio-pacemaker gene therapy usually controls the expression of hyperpolarization-activated cyclic nucleotide-gated (*HCN*) gene family (Santoro and Tibbs, 1999), *Kir2.1* and *Kir2.2* genes (Zaritsky et al., 2001), and transcription factor T-box 18 (*TBX18*) (Kapoor et al., 2013). The AP characteristics and I_f density of bio-pacemaker cells created by gene therapy are shown in Table 2.

HCN gene family controls the expression of I_f. Overexpressing the *HCN* gene into non-autorhythmic cells could promote the expression of I_f in these cells, promoting depolarization during the diastolic interval, hence inducing spontaneous beats. In the mammalian heart, injecting *HCN* channel 2 (*HCN2*) into the canine left atrium (LA) (Qu et al., 2003) and the left bundle-branch (LBB) (Plotnikov et al., 2004) via adenoviral constructs has successfully produced escape beats. That experiment also indicated that the LBB was a preferable location when creating pacemaker cells because it was proximal to the ventricular conducting system and easy to conduct the electrical signal into the ventricle (Plotnikov et al., 2004). As for rodent animals, highly expressing *HCN2* in neonatal rat VMs caused spontaneous beats. However, in adult rat VMs, it did not work, because the I_{K1} density was higher in adult rat VMs than in neonatal rat cells (Qu et al., 2001), which inhibited the depolarization. In addition to single gene expression, a dual gene therapy, which co-expressed skeletal muscle sodium channel 1 (*SkM1*) with *HCN2* in the canine left bundle branch, showed a more efficient pacemaking activity

Table 2 Review of gene therapy experiments

Reference	Gene type	Subject	CL (ms)	APD_{50} (ms)	APD_{90} (ms)	MDP (mV)	$V_{1/2}$ (mV)	I_f density at −70 mV (pA/pF)
Qu et al., 2003	*HCN2*	Canine left atrium					−94.00	−3.2
Plotnikov et al., 2004	*HCN2*	Canine left bundle-branch	340	100	180	−74.7		−31.0
Qu et al., 2001	*HCN2*	Neonatal rat VMs	681	170	255	−42.5±1.6$^{\#}$ (n=11)	−78.00	−41.3
Boink et al., 2013	*HCN2*/*SkM1*	Canine left bundle-branch	625	195±5$^{\#}$ (n=6)	213±5$^{\#}$ (n=6)	−85.0±2.0$^{\#}$ (n=6)		
Valiunas et al., 2009	Mouse *HCN2*	Canine VMs	648	266	479	−66.0		
Cho et al., 2007	*HCN1*	Guinea pig VMs	279	113	155	−70.0		−12.5
Miake et al., 2002	*Kir2.1*-AAA	Guinea pig VMs	598	230	295	−63.0		
Zaritsky et al., 2001	*Kir2.1*$^{−/−}$	Neonatal mouse VMs	Non*					−11.7±1.2$^{\#}$ (n=8)
Kapoor et al., 2013	*TBX18*	Neonatal rat VMs	631	242	320	−47.0±10.0$^{\#}$ (n=6)	−3.46	−8.0
		Guinea pig VMs	443	137	189	−55.8±6.0$^{\#}$ (n=5)		
Hu et al., 2014	*TBX18*	Porcine VMs	730					
Bakker et al., 2012	*TBX3*	Neonatal rat VMs	580					

HCN: hyperpolarization-activated cyclic nucleotide-gated; *HCN1*/*2*: *HCN* channel 1/2; *SkM1*: skeletal muscle sodium channel 1; *TBX18*: transcription factor T-box 18; VMs: ventricular myocytes; CL: cycle length; APD_{50}: action potential duration at 50% repolarization; APD_{90}: action potential duration at 90% repolarization; MDP: maximum diastolic potential; $V_{1/2}$: average half-maximal activation voltage of funny current's activation gate; I_f: funny current; pA/pF: unit of current density, $(10^{-12}$ A$)/(10^{-12}$ F$)$. * No auto-rhythm was observed; $^{\#}$ Data are expressed as mean±standard error of the mean (SEM)

than single gene expression. *SkM1* could maximize the opening of a sodium current channel, which led to a quick depolarization and a more positive AP (Boink et al., 2013). This is the first report that cardiac autorhythms were induced by bio-pacemaker in large animals. An experiment in a two-cell system verified that an *HCN2*-expressing cell could propagate spontaneous electrical signals to a quiescent VM cell (Valiunas et al., 2009). This provides evidence for the possibility of creating a bio-pacemaker by overexpressing *HCN2*. *HCN1* was also used to induce spontaneous rhythms. Spontaneous oscillations could be initiated in guinea pig VMs fused with *HCN1*-expressing fibroblasts under inhibition of I_{K1} (Cho et al., 2007). *HCN1* currents needed a long period (approximately 3000 ms) to reach equilibrium (Azene et al., 2005), but the pacing cycle is only 250 ms in guinea pigs and 1000 ms in humans, which would be a potential problem for the stability of *HCN1*-induced bio-pacemaker cells. Also, some experiments indicated that acute expression of *HCN* gene suppresses cardiac automaticity (Azene et al., 2005; Cho et al., 2007; Lieu et al., 2008; Sun et al., 2017) and the expression of the *HCN* gene in VMs may raise the risk of arrhythmicity (Kuwabara et al., 2013). Thus, the stability and robustness of *HCN* gene therapy need to be proved.

Kir2.1 and *Kir2.2* encode I_{K1} in many cell types, including CMs. Knocking out the *Kir2.1* and *Kir2.2* could depress I_{K1} and initiate spontaneous beats in isolated CMs. Removing *Kir2.1* and *Kir2.2* in newborn murine VMs could elevate the resting potential and promote spontaneous rhythms (Zaritsky et al., 2001). Replacing three amino-acid residues in the pore structure of *Kir2.1* produced a dominant negative construct called *Kir2.1*-AAA, which could inhibit I_{K1}. Miake et al. (2002) transduced *Kir2.1*-AAA in the left ventricular cavity of guinea-pigs, discovering that destroying *Kir2.1* caused an 80% decline of I_{K1}. They first verified that infecting *Kir2.1*-AAA in VMs produced more rapid beats than native pacing activity. After this study, they analyzed the role of I_{K1} in the excitability of VM via adenoviral gene transfection (Miake et al., 2003). They reported that *Kir2.1*-AAA caused a 50%–90% decline of I_{K1} and only a greater than 80% suppression of I_{K1} resulted in pacemaking activity, contributing to a longer AP duration, a slow phase 3 repolarization, and a more positive resting membrane potential.

This experiment provided a possible direction for pacemaker creation. However, an unfavorable finding was that decreased I_{K1} caused a long-QT phenotype and produced arrhythmia (Miake et al., 2003). Also, *Kir2.1*-knockout was readily reported to cause death in the adult animals, so that the experimental subjects were limited to the newborn animals (Zaritsky et al., 2001; Miake et al., 2003). As a result, developing a pacemaker by I_{K1}-knocking-out seems not to be an excellent approach.

Sun et al. (2017) showed that neither I_f overexpression nor I_{K1} suppression alone could initiate automaticity rhythm in CMs. As a result, the gene that codes I_{K1} and I_f at the same time may be a promising candidate of bio-pacemaker gene therapy. The embryonic transcription factor *TBX18* has effects on the expression of several pacemaking-related genes such as the *HCN* gene family and *Cx43* gene family as well as encodes the activation of I_{K1} (Choudhury et al., 2018). *Cx43* encodes electrical propagation between cells and downregulating intercellular coupling facilitates the transmission of automatic rhythms between cardiac cells. All these facts imply that *TBX18* has tremendous potential for producing a bio-pacemaker. In rodent animals, VMs, which were infected with *TBX18* via adenoviral vectors, showed pacemaker cell morphology and appropriate autonomic responses in the induced SAN (iSAN) (Kapoor et al., 2013). The expression of *TBX18* caused a 78% reduction in the density of I_{K1}, a 1.4-fold increase in the *HCN4* expression level as well as the downregulation of *Cx43*, which produced a reduction of coupling between cells (Kapoor et al., 2013). These factors caused the autorhythmicity jointly. Experiment in large animals exhibited similar results. Hu et al. (2014) created iSAN cells in porcine VMs by reprogramming *TBX18*, which was the second report of a bio-pacemaker created in the large animals. Indeed, the injected site showed a downregulated *Kir2.1*, a promoted *HCN4*, and a sparse *Cx43*, which had the result that the iSAN cells had the ability to support both heart rate and physical activity. Although this was just a transient bio-pacemaker, an excellent result was that *TBX18* expression did not increase the risk of arrhythmia. Apart from *TBX18*, *TBX3* could also reduce I_{K1} and intercellular coupling in the mice atrium (Bakker et al., 2012). In the rat right atrium, overexpressing *TBX3* caused upregulation of *HCN1*,

HCN2, and *RYR2*, but downregulation of *HCN4* was witnessed (Bakker et al., 2012). There was no evidence that overexpressing *TBX3* could create a bio-pacemaker.

2.2 Cell therapy

Generally, four kinds of cells were used to differentiate into bio-pacemakers: ESCs, bone marrow stem cells (BMSCs), adipose-derived stem cells (ADSCs) and induced pluripotent stem cells (iPSCs). Other types of cells were also deemed as candidates in cell therapy, such as SAN (Zhang et al., 2008) and CM progenitor cells (Végh et al., 2019). To create preferable autonomic pacing CMs, pacing-related genes are usually expressed into induced CMs. This is a hybrid therapy. In this paper, we consider hybrid therapy as a kind of cell therapy, which is introduced in this section. The cycle length (CL) and I_f density of bio-pacemaker cells created by cell therapy as well as the CL of co-cultured cells are presented in Table 3.

Kehat et al. (2001) reported that hESCs could differentiate into CMs. Then, they verified that rat CMs derived from hESCs could generate embryonic-like AP and pacing activity. They first demonstrated that hESCs-induced CMs (hESC-CMs) could successfully create electromechanical integration and drive the heart in the pig with complete atrioventricular block (Kehat et al., 2004). Then, researchers began focusing on the pacing function of CMs differentiated from ESCs. In vivo, under atrioventricular nodal cryoablation condition, the hESC-CMs-injected adult guinea pig heart presented induced automaticity, and no evidence showed that hESC-CMs led to tumor or arrhythmias (Xue et al., 2005). To enhance the ability of pacing, pacing-related genes were injected into the ESCs in hybrid therapy. Overexpressing *HCN4* in mouse ESCs (mESCs) generated considerably larger I_f currents, and hence a faster spontaneous rate of (87.4±11.9) beats/min (bpm) than the control (Saito et al., 2015). Actually, in human iPSCs (hiPSCs)

Table 3 Review of cell therapy experiments

Reference	Cell type	Gene type	CL[1] (ms)	I_f density at −70 mV (pA/pF)	Co-cultured subject	CL[2] (ms)
Kehat et al., 2004	Human ESCs		1351			
Xue et al., 2005	Human ESCs				Neonatal rat VMs	1224
					Adult guinea pig heart	740
Saito et al., 2015	Mouse ESCs	*HCN4*	724			
Ionta et al., 2015	Mouse ESCs	*Shox2*	192	−1.80	Rat heart with complete heart block	1416
Potapova et al., 2004	Human MSCs	Mouse *HCN2*		−7.90	Canine VMs	983
					Neonatal rat VMs	372
Plotnikov et al., 2007	Human MSCs	Mouse *HCN2*		−7.12	Canine heart	1153
Bruzauskaite et al., 2016	Human BMSCs	Mouse *HCN2*	Non*	−3.15		
Zhou et al., 2007	Rabbit BMSCs	Human *HCN2*			Cardiac myocytes	402
Zhou et al., 2013	Rabbit MSCs	Human *HCN1*			Neonatal rabbit VMs	465
Zhang et al., 2013	Porcine BMSCs	Human *HCN4*		−1.26	Porcine VMs	1224
Planat-Benard et al., 2004	Mouse ADSCs		909			
Yang et al., 2016	ADSCs	*TBX18*	Non*	−5.30	Neonatal rat VMs	800
Chen et al., 2017	ADSCs	*TBX18*	181			
Ruhparwar et al., 2002	Fetal atrial CMs				Canine left VMs	710±30# (*n*=2)
Zhang et al., 2011	Sino atrial nodes				Canine right ventricle	1200
Chauveau et al., 2017	Human iPSCs		435	−24.50	Canine VMs	1395

ESCs: embryonic stem cells; MSCs: mesenchymal stem cells; BMSCs: bone marrow stem cells; ADSCs: adipose-derived stem cells; CMs: cardiac myocytes; iPSCs: induced pluripotent stem cells; *HCN*: hyperpolarization-activated cyclic nucleotide-gated; *HCN1*/*2*/*4*: *HCN* channel 1/2/4; *TBX18*: transcription factor T-box 18; CL: cycle length; I_f: funny current; pA/pF: unit of current density, $(10^{-12}$ A)/$(10^{-12}$ F); VMs: ventricular myocytes. [1] CL of pacemaker cell; [2] CL of co-cultured cell. * No auto-rhythm was observed on that occasion; # Data are expressed as mean±standard error of the mean (SEM)

co-cultured with *HCN4*-overexpressing mESC-CMs, there was a higher beating rate at (82.8±4.8) bpm than that in the control condition (hiPSCs co-cultured with enhanced green fluorescent protein with a beating rate of (55.2±13.8) bpm) (Saito et al., 2015). It meant that overexpressing *HCN4* promoted the pacing ability of mESC-CMs in co-culture system with other excitable cells in vitro. *Shox2* is a homeodomain transcription factor that controls the development and function of the native cardiac pacemaker, specifically expressing in the SAN but not in the atrium or ventricle. In the single cell, expressing *Shox2* caused a lager I_f, a shorter AP duration, and a faster spontaneous pacing rate (Ionta et al., 2015). In vitro, under the overexpression of *Shox2*, the proportion of mESCs with spontaneous beats increased and its beating rate was considerably faster than that under the control condition (Ionta et al., 2015). Meanwhile, in vivo, injecting *Shox2*-mESCs into the left ventricular apex of the rat heart contributed to a higher rate (at (44±3) bpm) than the control (Ionta et al., 2015). After that, this team established an ESC-based cardiac differentiation model using *Shox2* as a molecular tool (Hoffmann et al., 2017), which provides a fundamental basis for the investigation of molecular pathways. These studies indicate that ESCs have great potential to differentiate into sinoatrial-like cells.

Compared with ESCs, mesenchymal stem cells (MSCs) are easier to achieve and culture. Some experiments verified that implanting MSCs loaded with the pacemaker gene in the myocardium could induce pacemaker function. For instance, the expression of mouse *HCN2* (*mHCN2*) in human MSCs (hMSCs) could create I_f-like current and initiate spontaneous rates in rat VMs at (93±16) bpm (Potapova et al., 2004). *mHCN2*-induced hMSCs could also generate electrical coupling in canine VMs and drive the canine heart with idioventricular rates at (61±5) bpm during sinus arrest (Potapova et al., 2004). This team also investigated the influence of the size and location of *mHCN2*-hMSCs on the pacing function, concluding that injecting 70000 hMSCs into the conducting system could drive the ventricle (Plotnikov et al., 2007). The MSCs are usually extracted from bone marrow and adipose tissues; these are called BMSCs and ADSCs, respectively. Both BMSCs (Zhou et al., 2007, 2013; Huang et al., 2010, 2012; Zhang et al., 2013; Bruzauskaite et al., 2016) and ADSCs (Rangappa et al., 2003; Planat-Benard et al., 2004; Choi et al., 2010; Yang J et al., 2012; Zhao et al., 2012; Yang M et al., 2016; Chen et al., 2017) show the ability to differentiate into CMs. This provides another option for creating a bio-pacemaker by cell therapy.

Experiments showed that fluid shear stress (Huang et al., 2010) and cyclic strain (Huang et al., 2012) could transdifferentiate BMSCs into CMs. Except for physiotherapy, BMSCs are ideal candidates in hybrid therapy. Transfecting human *HCN2* (*hHCN2*) into rabbit BMSCs could express I_f current (Zhou et al., 2007). Rabbit CMs co-cultured with *hHCN2*-transfected MSCs showed spontaneous beats at (149±14) bpm, which was significantly higher than the control at (87±11) bpm (Zhou et al., 2007). High cell viability and spontaneous beating activity were witnessed in *mHCN2*-transfected hMSCs, but the proliferative activity was somewhat low (Bruzauskaite et al., 2016). In terms of other kinds of *HCN* gene, injecting *HCN1* into BMSCs can initiate spontaneous automaticity in co-cultured neonatal rabbit VMs (Zhou et al., 2013). Also, *HCN4*-expressed BMSCs can drive the porcine ventricle (Zhang et al., 2013). In addition, infecting *TBX18* into rat BMSCs can make them differentiate into a phenotypic and functional pacemaker. More specifically, the *TBX18*-BMSCs expressed structural proteins—α-actin, Ca^{2+} dynamic proteins—cTnI, *HCN4* gene and repressed *Cx43* (Li et al., 2018). The vector used to transform the pacemaking-related gene is also a vital factor in long-term gene expression. Adenovirus is the most frequently used vector in gene transfection, but the gene expression by adenovirus vector showed a decline in one month (Zhang et al., 2013) and the adenovirus vector easily induced an immune reaction (Li et al., 2018). To create a durable pacemaker, lentiviral vector (LentiV) was used to transfect *hHCN2* into rabbit BMSCs because of its function of generating persistent transgene expression. Indeed, the rabbit CMs co-cultured with LentiV-transfected MSCs produced a persistent gene expression (Zhou et al., 2007).

Compared with BMSCs, ADSCs appear to be a superior source to be used to differentiate into a sinus node-like cell, because the sample is available, easy to culture and reproduce. Also, ADSCs present more attractive pacing properties than BMSCs, such as a superior ability to differentiate into CMs as well as readily expressing the *HCN* gene (Yang et al., 2012).

In the rat, 5-azacytidine (5-aza) chemical treatment can cause spontaneous beats in rabbit ADSCs at three weeks' culture (Rangappa et al., 2003). Also, adult mouse ADSCs can be induced into pacemaker cells with CM-like morphology and AP with a pacing rate of 66 bpm (Planat-Benard et al., 2004). However, 5-aza could not differentiate human ADSCs into pacemaker cells (Choi et al., 2010). Except for chemical substances like 5-aza, some genes could also influence the differentiation of ADSCs into CMs. For example, the *TBX18* gene is helpful in differentiating ADSCs into a pacemaker-like cell. An experiment indicated that silencing *TBX18* inhibited the expression of *TBX3* and *Shox2*, and terminated the differentiation of ADSCs into a pacemaker phenotype (Chen et al., 2017). Not surprisingly, expressing *TBX18* into ADSCs promoted the expression of *HCN4* and hence increased the expression of I_f (Yang et al., 2016). *TBX18*-ADSC showed a similar morphology to pacemaker cells (Zhang and Huang, 2019). The neonatal rat VMs co-cultured with *TBX18*-transfected ADSCs presented autonomous synchronized beatings (Yang et al., 2016). *Nkx2.5* is an essential transcription factor that regulates CM differentiation. It has been used to affect the differentiation of ADSCs into a pacemaker, although the results were not satisfactory. In mouse ADSCs, the overexpression of *Nkx2.5* and cardiac α-actin can increase the expression of cardiac-related proteins but inhibit beating function (Zhao et al., 2012). Dramatically, an experiment successfully differentiated ADSCs into beating CMs by direct cell–cell interaction with rat CMs (Choi et al., 2010), but the reason for this is not clear. Although further experiments are required to ensure the safety of ADSCs-induced bio-pacemakers, the work provides a new research direction for clinical bio-pacemakers.

Another candidate for creating CMs is iPSC. Human iPSCs can be generated from various types of somatic cells, particularly fibroblasts (Novak et al., 2010; Chauveau et al., 2017) and can be induced into CMs by the LentiV (Novak et al., 2010) and chemical means (Burridge et al., 2014). Zhang et al. (2009) indicated that hiPSC could differentiate into nodal, atrial, and ventricular phenotypes. iPSC from human hair follicle keratinocytes also had potential to differentiate into functional CMs and this kind of iPSC-CMs showed intracellular calcium concentration ($[Ca^{2+}]_i$) transient and contraction amplitudes under the action of β-adrenergic (Novak et al., 2010). In addition, iPSC from human foreskin fibroblast presented $[Ca^{2+}]_i$ handling and excitation–contraction coupling (Germanguz et al., 2011). Moreover, the iPSC-CMs can respond to ivabradine and iPSC-CMs-injected canine heart can display rhythms with the infusion of epinephrine (Chauveau et al., 2017). These experiments suggest the possibility of creating a biological pacemaker cell based on iPSC. Based on these facts, it is hoped that the iPSC-CM can produce automaticity. Gorabi et al. (2019b) used a hybrid therapy to produce a pacemaker cell based on iPSC. They successfully transfected *TBX18* into hiPSC-CMs by a LentiV and *TBX18*-hiPSC-CMs appeared to present more pacemaker-like traits than the control (8% vs. 2%). A further study, which transferred *TBX18* lentiviruses or *TBX18*-hiPSC-pacemakers into the rat ventricle, indicated that the function of *TBX18*-transduced VMs (cell therapy) was better than that of *TBX18*-hiPSC-pacemakers (hybrid therapy) on some measures, such as heart rate (Gorabi et al., 2019a). The *TBX18*-hiPSC-pacemaker injection seemed to be a safer therapy because the coupling between pacemaker cells and native myocytes could be controlled and optimized.

2.3 Autologous therapy

Up to now, most bio-pacemaker experiments are xenograft, which has the risk of delayed rejection and ethical issues. Also the function of pacemakers may be damaged by their low durability (Gorabi et al., 2019a). Zhang et al. (2013) showed that autograft *HCN4*-transferred porcine BMSCs could create a pacemaking function in the porcine heart with complete heart blocks. Moreover, 5-aza chemical treatment is potentially an autologous therapy for a bio-pacemaker (Rangappa et al., 2003). In addition, human somatic cells such as hair follicle keratinocytes or skin cells have been verified to be reprogrammed to iPSC and differentiated into CMs (Zhang et al., 2009), and this provides a possible approach for creating an autograft human pacemaker. Apart from autograft stem cell therapy and chemical treatment, autologous-SAN-injection is also a hopeful therapy for a bio-pacemaker. Fetal canine atrial CMs were implanted into the adult canine left ventricle, and an escape rhythm was observed in the site where the atrial CMs were injected (Ruhparwar et al., 2002). Zhang et al. (2008) showed that injecting autologous

SAN cells in the canine right ventricle could create spontaneous beats with a rate of approximately 50 bpm. However, its biological pacing properties are distinct from native SAN. This may relate to the difference of substrate (Zhang et al., 2011). The mortality of the SAN cells in the injected site is somewhat high, and stable pacing is difficult to ensure. Therefore, the dose of SAN cells for injection is a research interest for the future. It is hoped that these approaches will eliminate the immunosuppression of the xenograft.

2.4 Obstacles to producing biological pacemakers

Research on creating a bio-pacemaker by gene and cell therapies has provided many useful results. However, biological experiments have to overcome several obstacles in producing a bio-pacemaker. For instance, it is not easy to explain the synchronous mechanism of bio-pacemaker by biological experiment. The molecular mechanisms of how a bio-pacemaker works in healthy and diseased conditions are barely understood. In addition, experiments are limited by errors caused by experimental condition and by species difference. The current–voltage (*I–V*) curves of I_f in different bio-pacemaker experiments are shown in Fig. 1. The current density shows a great difference because it is not easy to control in experiments. In addition, considering the great effects of the location and size of bio-pacemaker cells on the cardiac electrical and mechanical dynamics, pacemaker cells should be injected into a certain position and in a certain pattern, which is not easy to accurately control. Also, culturing materials and subjects needs a lot of time and expenditure. Last but not least, biological experiments have ethical and moral issues, such as with the xenograft (Zhang et al., 2008). All these shortcomings prompt us to build a computational model of a bio-pacemaker to simulate the process and results of bio-experiments. This is a more feasible approach for studying the bio-pacemaker mechanism.

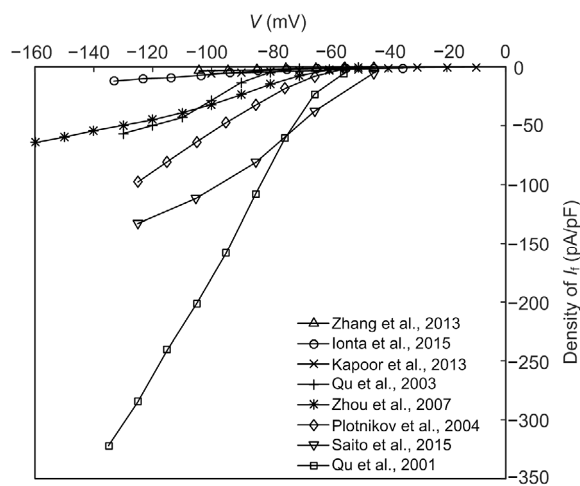

Fig. 1 *I–V* curves of I_f measured in biological pacemaker experiments
I–V: current–voltage; I_f: funny current; pA/pF: unit of current density, $(10^{-12}\ A)/(10^{-12}\ F)$

3 Computational simulations of biological pacemaker

Although there have been plenty of experiments on the bio-pacemaker, up to now, few bio-pacemaker computational models have been published. To build a bio-pacemaker computational model, first, an appropriate chamber of the heart should be chosen as the initiation of heartbeats. It has been verified that VMs can be induced as pacemaker cells via adenoviral gene transfection (Hu et al., 2014), which makes VMs a candidate for a pacemaking origin. A series of simulations have been done to examine the effect of different ion currents on the ventricular automaticity. Kurata et al. (2005) elucidated the roles of individual ionic currents in pacemaking by bifurcation analyses based on the first human VM model (PB model (Priebe and Beuckelmann, 1998)). They claimed that L-type calcium current (I_{CaL}) is responsible for equilibrium point instability, whereas the deactivation of Na^+/Ca^{2+} exchanger current (I_{NaCa}) and rapid delayed rectifier current (I_{Kr}) contributes to phase 4 depolarization. Another simulation (Zhang et al., 2014) demonstrated that I_{CaL} and I_{NaCa} play a positive role in the initiation of automatic depolarization, while I_{Kr} and Na^+/K^+ pump current (I_{NaK}) have a contrary effect.

Although these ion channels show a degree of influence on cardiac pacing, I_{K1}, I_f, and cell coupling are still the crucial factors that induce automatic depolarization. Inhibiting I_{K1}, overexpressing I_f, and downregulating the diffusion constant are the main approaches to causing automatic activity in the VM single cell and tissue model. There has been some research on the role of I_{K1} in induced pacing. For example, Silva and Rudy (2003) clarified the mechanism of pacing activity in I_{K1}-downregulated VMs by adjusting conductance of I_{K1} (G_{K1}) in a guinea

pig VM model (Luo-Rudy model (Luo and Rudy, 1991)). They verified that it was I_{NaCa} that carried the pacemaking current, and this process depended on the oscillation of $[Ca^{2+}]_i$. Then, Tong and Holden (2005) used a continuation algorithm to analyze the stability of induced pacemaker activity by downregulating G_{K1} in a human VM model (TP04 model (ten Tusscher et al., 2004)) and found that the stable periodic oscillations appeared when G_{K1} was 0.02–0.05 times the value of original G_{K1}. Based on the result of the single cell model, the number of bio-pacemaker cells, which are needed for successful pacing, was investigated in a two-dimensional (2D) model. The result was that adjacent VMs suppressed the depolarization of automatic cells. Therefore, Purkinje fiber was added as a medium for propagating electric excitation from pacemakers to VMs. In this model, only 500 automatic cells were needed to drive the tissue slice (Zhang et al., 2016). As for cell coupling, biological experiments showed that overexpressing *TBX18* in the rat (Cho et al., 2007) and the pig (Azene et al., 2005) VMs could downregulate *Cx43*, and then diminish intercellular coupling, thus inducing auto-rhythms in VMs. A simulation verified that decreasing coupling conductance could increase ventricular pacing activity (Zhang et al., 2015), which was consistent with the experimental result. According to the experiment by Qu et al. (2001), increasing the density of I_f is helpful to enhance the CL and give rise to pacemaker activity. It is hoped that computational simulation will reveal the effect of I_f on pacing activity in a non-autorhythmic CM model. In addition, an experiment has preliminarily explored the synergetic actions of I_{K1} and I_f. A simulation (Sun et al., 2017) showed that it was the ratio of I_{K1} and I_f that regulated the frequency of rhythmic oscillation. However, this model included I_{K1} and I_f only, which was not a whole CM model.

Computational study based on CM model provides a fundamental basis for the investigation of molecular pathways for creating a bio-pacemaker under physiological conditions. Some publications (Azene et al., 2005; Sun et al., 2017) have combined the biological experiments and computational approaches, setting an example for guiding biological research by computational simulation. The conclusions of simulation could be verified by biological experiments, i.e., establishing the feasibility of model forecasts. The computational approach will be a trend for bio-pacemaker research.

4 Conclusions

The bio-pacemaker is an attractive therapy for cardiac rhythm disturbances. Because of its biological properties, it has shown many advantages over electrical pacemakers. For example, the bio-pacemaker needs no open-chest surgery, and this could reduce the risk of complications. Also, the pacing frequency of a bio-pacemaker can respond to emotion rather than remaining a fixed rhythm. However, some problems still need to be solved, such as the approaches of producing pacemaker cells, the magnitude of pacing cells, as well as the location for injection of the pacing cells. Fortunately, the computational model could be used to simulate the production of pacemaker cells and its effect on the cardiac pacemaking activity. The computational approach could avoid the high cost and moral constraints of clinical experiments. The development of bio-pacemaker simulation began from modifying I_{K1} and I_f channel formulations at the subcellular level and should end at establishing pacemaker–CM-coupling tissue models at the multi-dimensional level. Many problems in bio-pacemaker simulation still need to be solved, such as the interaction mechanism between ionic channels across the cell membrane, the space structures of the pacemaker, and so on.

Although further experiments are required to assess safety and efficacy before implementation in clinical practice, the bio-pacemaker is still a discernible overall trend for the clinical therapy of arrhythmia and computational simulation would accelerate its development.

Contributors

Yacong LI performed the literature research, wrote and edited the manuscript. Kuanquan WANG participated in the conceptualization, and reviewed and edited the manuscript. Qince LI investigated and collected the experiment data, and wrote the manuscript. Henggui ZHANG participated in the conceptualization, and writing and editing of the manuscript. All authors have read and approved the final manuscript.

Compliance with ethics guidelines

Yacong LI, Kuanquan WANG, Qince LI, and Henggui ZHANG declare that they have no conflict of interest.

This article does not contain any studies with human or animal subjects performed by any of the authors.

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中文概要

题 目：生物起搏器：从生物实验到计算机模拟

概 要：起搏功能障碍已成为威胁人类健康的一种重大疾病，严重时可能导致心律失常、晕厥，甚至死亡。到目前为止，治疗起搏功能障碍的最佳方案是植入电子起搏器。但是它存在一些缺点，例如电池寿命有限，手术过程具有感染的风险，起搏频率单一等。因此，对生物起搏器的研究显得尤为迫切。生物起搏器不但引起并发症的风险较低，而且能够对生理情绪做出反应，从而有望替 代电子起搏器，进行心脏起搏障碍治疗。本文从生物实验和计算机模拟两方面对生物起搏器的发展进行综述。前者主要包括基因疗法和细胞疗法的实验成果，而后者介绍了多尺度的心脏建模从单个细胞到组织切片进行起搏器研究的进展。迄今为止，生物起搏器已被应用于大型哺乳动物实验，但将其应用于临床心脏病治疗，仍有很长的路要走。利用计算机模型对生物起搏器诱发过程进行建模，有望加速研究进程。在本文中，我们首先回顾了生物起搏器实验研究的发展，然后介绍了生物起搏器计算机模型的目前的相关工作。最后，我们提出了基于心脏计算机模型研究生物起搏器的潜在研究方向。

关键词：生物起搏器；基因治疗；细胞治疗；心脏模拟；计算机模型