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Biological Pacemaker – Main Ideas and Optimization

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1. Introduction

Electronic pacemaker, since its invention over five decades ago, has saved numerous lives and improved the life quality of patients suffering from cardiac arrhythmias. However, it has its own limitations. Over the past decade, rapid progress in the molecular studies of cardiac ion channels and stem cell biology has led to efforts for creating a biological pacemaker to supplement the widely used electronic pacemaker. We focus on the development of the ideas for creating a working biological pacemaker. The gene-based and cell-based approaches to meet the requirements of a working biological pacemaker will be reviewed. The important roles of the hyperpolarization-activated cyclic, nucleotide-modulated (HCN) channels, the inward rectifier Kir2.1 potassium channels, and the gap junctions in the biological pacemaker system will be discussed. Finally, recent development of cell-based strategy and precautions for creation of an effective biological pacemaker superior to the electronic counterpart will also be discussed.

2. Hierarchic organization of cardiac pacemakers

Throughout life, the heart beats close to 3 billion times (assuming 70 beats per minute with a mean life span of 80 years). The ability of the heart to beat spontaneously and continuously relies on its highly organized pacemaker system.

The cardiac pacemaker activity is originated in the sinoatrial node (SAN) located in the right atrium. The electrical impulses are transmitted to the atria, then the atria-ventricular (AV) node to the His/Purkinje conducting system, and to the working ventricles.

Pacemaker activity has also been found in regions outside the SA node, including atria, AV node, and Purkinje fibers. Under physiologic conditions, these regions have much slower intrinsic pacing rates, and thus are overdriven by the SA node. Pacemaker activity is absent in the adult mammalian ventricle, but is present in the cultured newborn ventricle. Abnormal pacemaker activity also appears in cardiomyopathies such as heart failure and hypertrophy.

Heart rate is subject to autonomic regulation. Sympathetic stimulation accelerates heart rate by activating β adrenergic receptors, whereas parasympathetic stimulation slows heart rate by activating muscarinic acetylcholine receptors. Through the G-protein/adenylate cyclase

pathway, autonomic stimulation alters the intracellular cAMP levels, which in turn, changes the heart rate by altering the gating properties of membrane transporters including ion channels, exchangers and pumps. Increased cAMP levels enhance, while decreased cAMP levels reduce, the heart rate.

2.1 Cardiac pacemaker activity generation

Cardiac pacemaker activity begins with the diastolic (phase-4) depolarization, which is a slow time-dependent depolarizing process different from the rapid action potential firing (Figure 1). The major ionic mechanisms for the initiation of this diastolic depolarization have

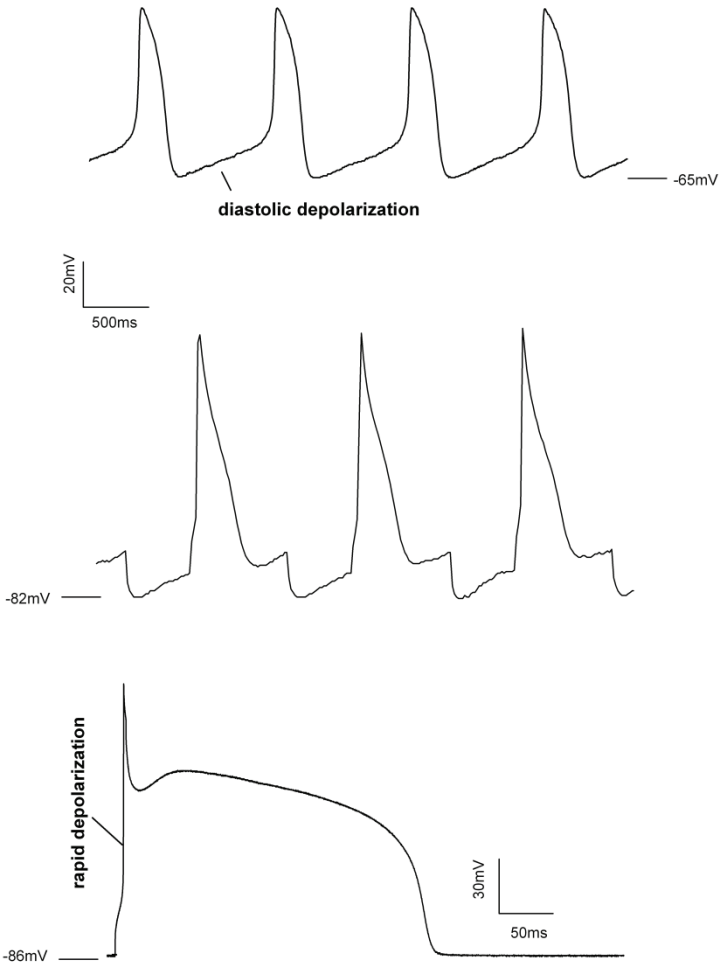


Fig. 1. Action potentials recorded from single myocytes isolated from rabbit SA node (A), canine Purkinje fiber (B), and canine ventricle. Current stimulus was used to generate action potentials in Purkinje and ventricular myocytes.

been debated for nearly three decades (Brown *et al.*, 1979; DiFrancesco, 1995; Vassalle, 1995; Liu *et al.*, 1998; Lipsius & Bers, 2003; Bers, 2006; Sanders *et al.*, 2006; Lakatta & DiFrancesco, 2009; DiFrancesco, 2010; Lakatta, 2010), reflecting the complex nature of this essential biological function. Despite its complicated mechanism, there is a widely accepted agreement that a *net* inwardly increasing current (net positive ions entering the cell) must contribute to this process. The *minimal* requirement for producing such a net inward current could be a combination of either a time-dependent outward current and a constant inward background current, or a time-dependent inward current and a time independent outward background current (Vassalle *et al.*, 1999). With regard to the biological pacemaker, we will mainly focus on the discussion of the latter combination. This, by no means, undermines other mechanisms such as Ca^{2+} clocks that also play important roles in cardiac pacemaker activity (Lakatta *et al.*, 2008; Lakatta & DiFrancesco, 2009; Lakatta *et al.*, 2010).

2.2 Action potentials and membrane currents of cardiac myocytes

Cardiac pacemaker activity is tissue-specific. Spontaneous action potentials can be readily recorded in single myocytes isolated from SA node (Figure 1, upper panel), but harder from isolated Purkinje myocytes. However, with current stimulus, phase-4 depolarization can be elicited in isolated Purkinje cells (Figure 1, middle panel). In isolated ventricular myocytes, phase-4 depolarization cannot be induced with stimulus under physiological conditions (Figure 1, lower panel). According to Ohm's law, these differential action potentials in different regions of the heart indicate different underlying ionic mechanisms. Figure 2 shows the membrane currents near the end of repolarization that are different in primary pacemaker tissue (SA node), conducting system (Purkinje fibers), and working ventricles. In response to a hyperpolarizing pulse to the indicated voltages, in the SA node, there is a strong time-dependent inward current, but little background potassium outward current, indicated by the lack of an instant jump at the beginning of the hyperpolarizing pulse (upper panel); in the Purkinje cells, in additional to the time-dependent inward current, there is a considerable amount of background potassium current (middle panel); in the ventricular myocytes, the background outward current is dominant with little time-dependent inward current (lower panel).

It is noted that if the time independent outward current is so small that it can be negligible, such as in the SA node, inducing diastolic depolarization requires only a few pA of time-dependent inward current (Vassalle *et al.*, 1999). On the other hand, if the time-dependent background outward current is large, a much larger time-dependent inward current is needed to initiate diastolic depolarization, such as in the Purkinje fibers (Figure 2, middle panel). Once the time independent outward current dominates over the time dependent inward current, diastolic depolarization cannot be elicited, which is the case in the ventricle (Figure 2, lower panel). Thus, generation and inhibition of diastolic depolarization can be achieved by adjusting the expression levels of the time independent outward current and/or the time dependent inward current.

3. Hyperpolarization-activated HCN, inward rectifier Kir2.x potassium channels, and gap junction channels in the sinus node and in the ventricles

Action potential is an electrical process that reflects the time dependent changes in charges across the cell membrane. Due to uneven distribution of ions inside and outside of the cell,

ions crossing the cell membrane via ion channels alter the membrane potential in a dynamic manner. The time- and voltage-dependent inward current (e.g., Na^+ current) makes the membrane more positive (or less negative), whereas the time- and voltage-dependent outward current (e.g., K^+ current) drives the membrane potential toward more negative values. The delicate balance of inward and outward currents initiates and maintains the rhythmic heart beat under physiological conditions. Disruption of this ionic balance predisposes the heart to arrhythmias (irregular heart beat).

In this chapter, we focus on the properties of two ionic currents, the hyperpolarization-activated cation current, I_f, and the inward rectifier potassium current, I_{K1}. We also describe the gap junction channels in the heart due to their critical roles in electrically connecting the myocytes to produce synchronous cardiac contraction.

3.1 I_f and HCN channels

I_f is an important contributor to the cardiac pacemaker activity. Opened by hyperpolarization near the end of repolarization in the sinus node, I_f contributes significantly to the diastolic (phase-4) depolarization, which leads to the threshold of Ca-channel activation and firing of an action potential.

Unlike most voltage-gated ion channels that are activated by membrane depolarization, I_f is activated by membrane hyperpolarization at the end of repolarization (Figure 2, Figure 4B). I_f channels pass Na^+ and K^+ ions (DiFrancesco, 1993) and a tiny amount of Ca^{2+} ions (Yu *et al.*, 2004; Yu *et al.*, 2007). Under physiological conditions, mainly Na^+ ions go in and much less K^+ ions come out through the channel near maximum diastolic potential (MDP) (e.g., -60mV to -90mV), generating a time dependent inward current with a reversal potential around –20mV (DiFrancesco, 1993).

The threshold activation of I_f is closely associated with the tissue pacemaker activity. In the SA node, I_f activates near -50mV and the MDP is around -65mV (upper panels in Figures 1&2). In Purkinje fibers, I_f activates around -70mV and the MDP is near -85mV (middle panels in Figures 1&2). In the ventricle, I_f activates at potentials more negative than -100mV (Yu *et al.*, 1993, 1995; Robinson *et al.*, 1997), and the MDP is around -90mV (lower panels in Figures 1&2).

In adult mammalian ventricles, the threshold activation of I_f in ventricular myocytes is also species specific. It is extremely negative in guinea pig ventricles (more negative than -140mV) and a little more positive in canine ventricles (around -120mV) (Yu *et al.*, 1993, 1995). In rat ventricles, there are varying results with regard to I_f activation. Early studies showed a -110mV threshold activation of I_f in myocytes isolated from 3-month old rat ventricles (Robinson *et al.*, 1997). However, more positive threshold activation of I_f in rat ventricles within the physiological voltages has been reported in several other studies (Cerbai *et al.*, 1994, 1996; Ranjan *et al.*, 1998; Xiao *et al.*, 2007). It has been hypothesized that the potential contribution of I_f to the diastolic depolarization is masked by a large I_{K1} in the ventricular myocytes (see below).

In the newborn ventricles, I_f activates around -70mV (Robinson *et al.*, 1997). Diseased adult ventricles also increases I_f and shifts its threshold activation into the physiological voltages, near -55mV (Cerbai *et al.*, 1997; Cerbai & Mugelli, 2006). The molecular basis of this developmental and disease modulated voltage dependent activation remains unclear.

I_f is generated by HCN channels. Among the four isoforms that have been identified, three of them (HCN1, HCN2, HCN4) are differentially expressed in various regions of the heart.

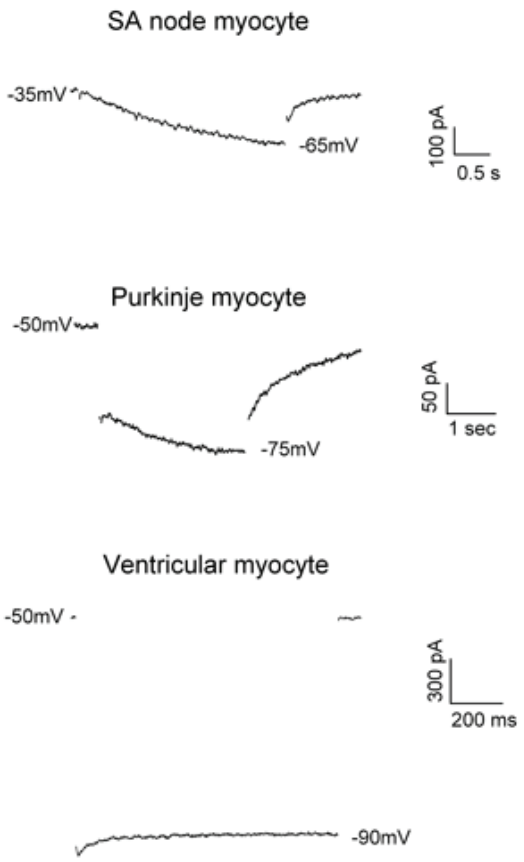


Fig. 2. Membrane currents recorded in single myocytes isolated from rabbit SA node (upper panel), canine Purkinje fibers (middle panel), and canine ventricle (lower panel). The holding potential was -35mV for SA node myocyte, and -50mV for Purkinje and ventricular myocytes. The hyperpolarizing pulse was applied to -65mV (SA node), -75mV (Purkinje), and -90mV (ventricle), respectively.

HCN total transcripts levels are the highest in the SA node, intermediate in the Purkinje fibers, and the lowest in the working ventricles (Shi *et al.*, 1999; Shi *et al.*, 2000). HCN4 is the dominant isoform in the SA node, representing about 80% of the total HCN transcripts. Equal amounts of HCN1 and HCN4 are present in the Purkinje fibers. In the ventricles, HCN2 expression levels are higher than HCN4, and no HCN1 has been detected (Shi *et al.*, 1999; Shi *et al.*, 2000; Han *et al.*, 2002).

When expressed in the heterologous expression systems (*Xenopus* oocytes, mammalian culture cells such as HEK and COS cells), HCN1, HCN2, and HCN4 channels displayed distinct properties. HCN1 activates at the least negative potential (near -50mV) associated with the fastest activation kinetics, mimicking the SA node I_f. HCN2 and HCN4 are activated at more negative potentials (-60mV to -80 mV) with much slower activation kinetics (Robinson & Siegelbaum, 2003). In addition, HCN1 is nearly insensitive to

intracellular cAMP, while HCN2 and HCN4 activation curves can be shifted to depolarizing potentials by cAMP for more than 10mV. Binding of cAMP to the cyclic nucleotide binding domain in the C-terminus facilitates the gating of HCN channels (Wainger *et al.*, 2001).

3.2 I_{K1} and Kir2.1 channels

The inwardly rectifying potassium current, I_{K1}, is essential in the resting phase of ventricular action potential. Its reversal potential is close to the resting membrane potential. I_{K1} channels pass more inward current at potentials more negative than the potassium reversal potential (E_K), but much fewer outward current at potentials more positive than E_K; in the range of -40mV to 0mV, I_{K1} is negligibly small and close to zero (Dhamoon & Jalife, 2005).

I_{K1} is nearly absent in the sinus node, making the resting potential more positive (around -65mV) in comparison to that in the ventricular myocytes (around -86mV) (Fig. 1). The relatively depolarized membrane potential in the sinus node allows small but significant I_f inward current to slowly depolarize the membrane during diastole, which is critical in initiating the pacemaker activity (Figure 1, upper panel). In the ventricular myocytes, due to the presence of a large I_{K1} and low expression I_f, there is no diastolic depolarization under physiological conditions (Figure 1, lower panel).

I_{K1} is nearly undetectable in neonatal rat ventricular myocytes that have spontaneous pacemaker activity (Sekar *et al.*, 2009). Similarly, in failing ventricles I_{K1} is reduced (Beuckelmann *et al.*, 1993). Meantime, I_f activity is increased in neonatal (Robinson *et al.*, 1997) and in failing ventricles (Cerbai *et al.*, 1997; Hoppe *et al.*, 1998). A combination of reduced I_{K1} and an increased I_f predisposes the ventricle to generate unwanted diastolic depolarization (Cerbai & Mugelli, 2006).

I_{K1} is mainly produced by inward rectifier potassium Kir2.x (x=1,2,3,4) channels in the heart. In ventricular myocytes Kir2.1 is the predominant isoform, although Kir2.2 may also contribute to I_{K1} to some extent (Dhamoon & Jalife, 2005). Kir2.1 transcripts have been reported to be at the lower levels in the SA node and atria, but much higher in the ventricles (Gaborit *et al.*, 2007). During cardiac development, Kir2.1 is also found at the lowest levels in the mice SA node (Schweizer *et al.*, 2009). In neonatal rat ventricular myocytes, Kir2.1 protein expression is barely detected (Sekar *et al.*, 2009).

For both HCN and Kir2.1 channels, tetramers are formed as the functional channels. Different HCN or Kir2.x channel isoforms may form the functional heteromeric channels generating native I_f or I_{K1} in the heart.

3.3 Cardiac gap junction channels

Gap junctions allow direct passage of ions and small signaling molecules with molecular weight up to ~1000 Daltons between adjacent cells. Gap junctions are required in the orchestrated electrical activation of the heart. Spontaneous action potentials generated from the SA node are efficiently transmitted to the working ventricles due to electrical coupling among the myocytes in different regions of the heart. The coordinated muscle contraction depends upon normal gating of gap junction channels between myocytes.

Gap junctions are formed by two hemi-channels (connexons) with each expressed on the plasma membrane of adjacent myocytes. Each hemi-channel is assembled by six connexins (Cx) spanning the plasma membrane (Yeager, 1998). Their extracellular domains form the gap junction with their N- and C-termini in the intracellular space for channel regulation.

There are three main connexins identified in the heart: Cx40, Cx43, and Cx45. They can form either homomeric or heteromeric connexins (Yeager, 1998). When expressed in the

heterologous expressing systems, they exhibit distinct single channel conductance that determines the propagation speed of electrical signals. Single channel conductance is large for Cx43 and Cx40, about 100pS and 158-198 pS, respectively, and small for Cx45, about 30pS (Gonzalez *et al.*, 2007).
Gap junctions with large conductance facilitate fast transmission of electrical signals such as Purkinje fibers and working ventricles. Gap junctions with small conductance are mainly restricted in the pacemaker tissues such as the SA node and AV node in which the electrical transmission is slow.
Cx43 is the dominant connexin in the working ventricles, co-expressed with Cx40 in atria, absent in the SA node and AV node. Cx45 is present in the atria including the SA node, AV node, His bundle and ventricles (Saez *et al.*, 2003; Severs *et al.*, 2008). All three connexins are present in Purkinje fibers (Severs *et al.*, 2008). In the slow conducting tissues, such as the SA node and AV node, only Cx45 is expressed. Redistribution and reduction in cell surface expression of gap junctions have been linked to severe arrhythmias associated with ischemia and failing heart (Severs *et al.*, 2008).

4. Need for biological pacemaker

When dysfunction of the SA node or blockade of the AV node occurs, generation of normal action potentials or the propagation of the normal electrical signals to the working ventricle is disrupted. The latent pacemakers in atria and Purkinje fibers will pace at their own rates (slower than the sinus rate). This creates an asynchronous rhythmic contraction of the heart muscle leading to a decreased blood pumping efficiency. It also predisposes the heart to more dangerous arrhythmias. To preserve the normal muscle contraction under SA node dysfunction, the standard surgical intervention is to implant an electronic pacemaker, which has become the industrial standard since its invention.
While extremely successful and widely used as a life-saving device, the electronic pacemaker has its own limitations. They include 1) battery life; it usually needs to be replaced every 5-7 years; 2) implantation sites of the leads which may affect the cardiac output; 3) in growing children, pacemaker lead length is problematic requiring multiple replacements in pediatric patients; 4) lack of physiological response to autonomic stimulations in exercise, although software has been developed to improve responses to varying heart rate in exercise; 5) potentially increased risk of heart failure for long-term use; 6) a separation of wires connecting the lead to the battery. In responding to these challenges, creating a biological pacemaker has been emerged as a promising supplement.

5. Implementation of biological pacemaker

Cardiac action potential is finely maintained by a number of channels, pumps, and exchangers (Figure 3). For discussion of biological pacemaker, we focus on the roles of I_{K1} and I_f currents that contribute to the membrane stability and diastolic depolarization.
Differential expression of HCN and Kir2.x channels and distinct properties of I_f and I_{K1} contribute to the different resting and action potentials in the sinus node and ventricular myocytes. As depicted in Figure 3, one of the characteristics in an electrically quiescent myocyte (such as a ventricular myocyte) is the abundant expression of I_{K1} and low expression of I_f. On the other hand, spontaneous myocytes (i.e., SA node cells) express high levels of I_f and no I_{K1}. Gap junction channels are needed to connect these two different types of myocytes for pacing at the same rate.

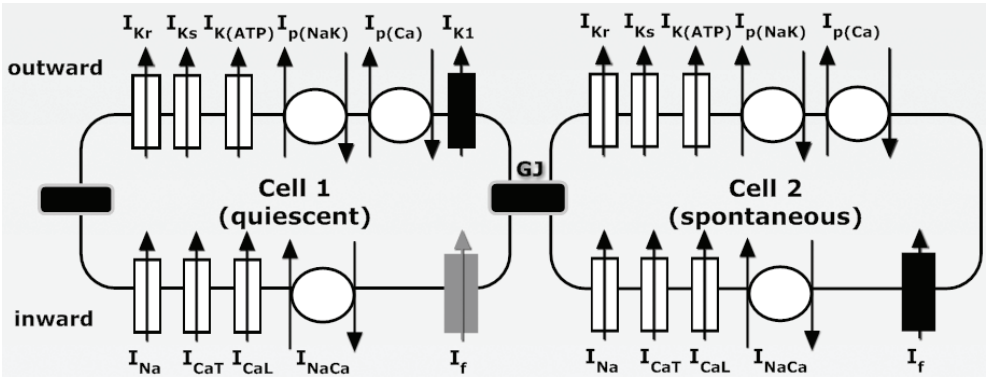


Fig. 3. Schematic illustration of major ion channels in quiescent and spontaneous cardiac myocytes. GJ= gap junction. Dark filled channels, I_{K1} and I_f, are the focuses in quiescent and spontaneous myocytes. I_f, I_{K1}, and gap junctions are essential requirements (among others) for enhancing, suppressing, and propagating the pacing activity throughout the entire heart. I_f in grey indicates much less physiological role in quiescent myocytes than in spontaneous myocytes.

A combination of an absent I_{K1} and an activated I_f at diastolic potential sets up the stage for the cardiac pacemaker activity in the sinus node (Fig. 4B). On the other hand, large I_{K1} and small I_f with negative activation mask the spontaneous pacemaker activity in the ventricle (Fig. 4A). These principles have been used as a working guidance in the development of the biological pacemaker. Therefore, enhancing HCN channel activity or inhibiting Kir2.1 channel activity or a combination of both should increase the spontaneous pacemaker activity in the ventricle.

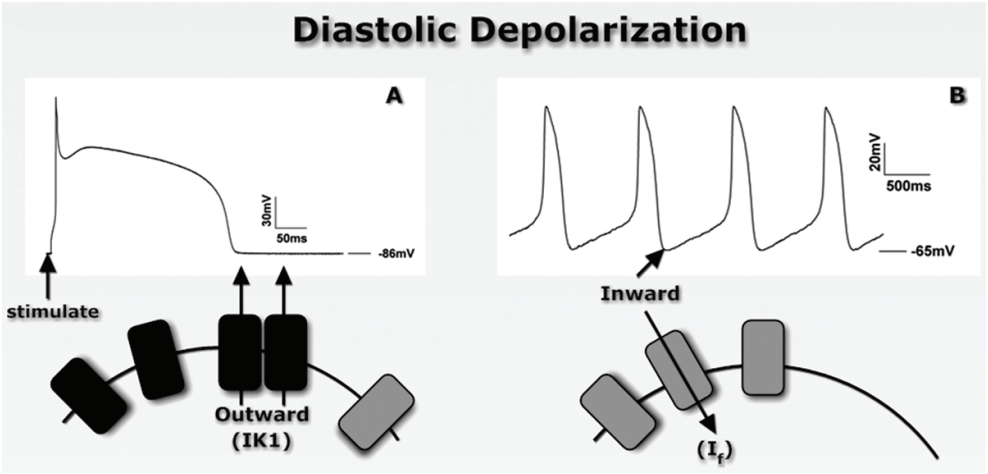


Fig. 4. Schematic illustration of roles for I_{K1} (dark) and I_f (grey) at diastolic depolarization of action potentials in a canine ventricular myocyte and in a spontaneous myocyte (e.g., rabbit sinus node cell).

Using a computer simulation of ventricular action potential (Figure 5), we had predicted that if I_{K1} conductance is reduced by 40% and the maximal conductance of I_f is increased by three-fold associated with a 40mV depolarized shift of the activation curve into the physiological voltages, the spontaneous pacemaker activity can be induced in the ventricles. These represent the minimal conditions to induce a ventricular spontaneous diastolic depolarization. In the past ten years the overexpression of either a dominant negative Kir2.1 or HCN channels have successfully induced spontaneous activity in the adult ventricular myocytes and in the hearts of intact animal models. The altered biophysical properties for both I_{K1} and I_f have far exceeded the simulation conditions shown in Figure 5.

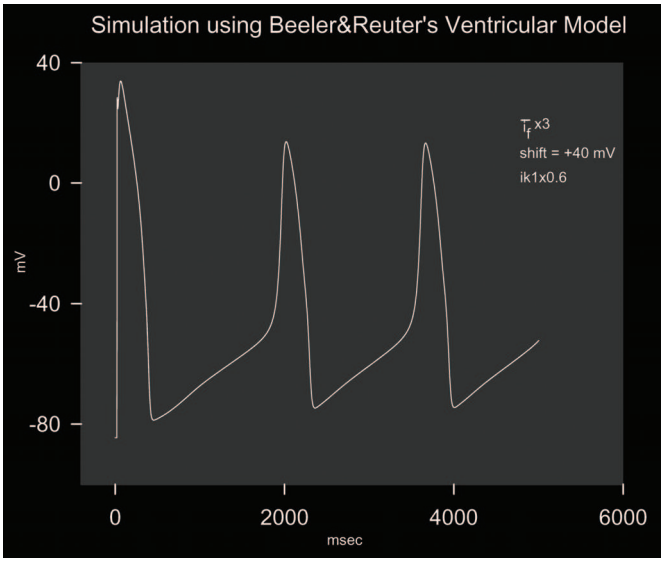


Fig. 5. Computer simulation of ventricular action potential using a Beeler and Reuter ventricular model (Beeler & Reuter, 1977). The parameters for I_{K1} and I_f were adjusted for minimal requirements to induce a ventricular pacing. I_f: maximal conductance. Shift: the voltage shift of the threshold activation.

Manipulation of HCN and Kir2.1 gene expression is a gene-based strategy for creation of a biological pacemaker. An alternative approach, a cell-based strategy, is to use the cells that have already had the desired ion channels for pacing (e.g., sinus node cells) or cells that are able to differentiate into the desired myocytes (e.g., stem cells). The advantages and disadvantages of these two approaches will be discussed below.

5.1 Gene-based approach

5.1.1 Overexpression of β-adrenergic receptors

The direct consequence of a sinus node dysfunction is the slow heart rate. Stimulation of β-adrenergic receptor can increase heart rate by increasing I_f (Brown *et al.*, 1979; DiFrancesco, 1986). Early studies in late 1990s demonstrated that overexpression of the β-adrenergic receptors in mouse embryonic and neonatal ventricular myocytes increased pacing rate (Edelberg *et al.*, 1998). Similar observation was made in a 6-week old intact mouse heart after

the right atria were injected with full-length β-adrenergic receptors plasmid (Edelberg *et al.*, 1998).

The cellular and ionic mechanisms that mediate the positive chronotropic effect of β-adrenergic receptors have been well documented. Stimulation of β-adrenergic receptors activates adenylate cyclase via G-proteins. Adenylate cyclase catalyzes ATP to cAMP which enhances I_f activity (DiFrancesco, 1993; Vassalle *et al.*, 1999). It is thus not surprising that overexpression of adenylate cyclase may also be able to pace the quiescent ventricle.

5.1.2 Overexpression of adenylate cyclase

In a pig AV-block model, Ruhparwar et al successfully converted the quiescent ventricular myocytes into the pacing cells near the injection site by overexpressing adenylate cyclase type VI (AC-VI) in the left ventricles (Ruhparwar *et al.*, 2010). The main idea was to boost the intracellular levels of cAMP that significantly increases the gating of HCN channels. The AC-VI in adenoviral vector was delivered into the left ventricle of a porcine heart. After 12 days, complete AV-block was induced by catheter ablation. After rapid ventricular pacing, an escape rhythm in the injection area was detected (Ruhparwar *et al.*, 2010).

Lack of normal ventricular diastolic depolarization is a result of large I_{K1} and small I_f. Although cAMP has been reported to cause a depolarizing shift of the I_f/HCN channel activation curves (DiFrancesco, 1986, 1993), it is unclear whether the dramatic increase of cAMP beyond the physiological level might also enhance the protein expression of HCN channels in the pig's ventricles. Another possibility is that rapid ventricular pacing may up-regulate HCN channel expression in the ventricle. A third possibility is that the basal level of intracellular cAMP is dramatically increased in AC-VI over-expressed ventricular myocytes (Ruhparwar *et al.*, 2010). Increased cAMP enhances the opening probability of HCN channels (Wainger *et al.*, 2001). However, the expression levels of both HCN2 and HCN4 are low in the ventricles under physiological conditions. Lack of diastolic depolarization of ventricular myocytes is well explained by a large I_{K1} and non-physiological threshold activation of I_f which are encoded by HCN2/HCN4 in the ventricles. Currently, it is unknown whether the dramatic increase in intracellular cAMP levels might also increase the functional HCN channels at the plasma membrane of the ventricular myocytes, which would make sense of converting quiescent ventricular myocytes into pacing myocytes by overexpression of AC-VI. Thus, how AC-VI overexpression is able to transform quiescent ventricular myocytes into spontaneously pacing myocytes remains an open question.

Adult cardiac myocytes are well differentiated and difficult in taking up exogenous proteins. One common method is to use virus-mediated infection. The gene of interest (HCN or Kir2.1) is inserted into a viral vector and viral particles containing the gene of interest are generated. Infected ventricular myocytes exhibit the spontaneous pacemaker activity under the condition in which either the Kir2.1 channel activity is inhibited or HCN channel activity is enhanced.

5.1.3 Inhibition of I_{K1}

The first evidence to demonstrate the feasibility of creating a biological pacemaker was to inhibit I_{K1} by overexpression of a dominant negative mutant of Kir2.1 (Kir2.1AAA) in guinea pig ventricular myocytes (Miake *et al.*, 2002). I_{K1} was inhibited by nearly 80%, leading to an increase in the resting membrane potential, action potential duration, and an induction of phase-4 depolarization (Miake *et al.*, 2002, 2003).

The idea of this approach is to remove I_{K1} suppression of the latent pacemaker activity for creation of a biological pacemaker in the quiescent adult ventricles. Later, studies have shown that strong inhibition of I_{K1} significantly reduced the membrane stability, and thus, can create large I_{K1} heterogeneity, which is pro-arrhythmogenic (Sekar *et al.*, 2009).

5.1.4 Enhancement of I_f

In both human atrial and ventricular myocytes, I_f has been detected at the physiological voltages (Hoppe & Beuckelmann, 1998; Hoppe *et al.*, 1998). Large I_{K1} suppresses the potential contribution of I_f to the pacemaker activity in these tissues. If reduced I_{K1} can unmask the pacemaker activity in ventricular myocytes as elegantly illustrated in the Kir2.1-AAA work, enhanced I_f should also be able to achieve the same goal.

HCN2 was first to be chosen to test an idea that overexpression of HCN2 in the adult ventricles can increase I_f activity leading to the spontaneous pacemaker activity. The reasons for choosing HCN2 among other HCN isoforms were HCN2's intermediate activation kinetics and its strong response to cAMP (Robinson *et al.*, 2006). HCN2 was overexpressed in the canine left atrium mediated by adenovirus (Qu *et al.*, 2003). After sinus arrest was achieved by vagal stimulation, spontaneous rhythm was detected in the left atrium. I_f amplitude was found more than 100-fold larger in HCN2-overexpressed atrial myocytes compared to native atrial myocytes or green fluorescent protein infected atrial myocytes by immunohistochemistry and Western blots. This work proved that it is feasible to create spontaneous pacemaker activity by increasing I_f current even in the presence of a large I_{K1}.

In a later study adenovirus containing HCN2 was injected to canine left bundle-branch. After two days, ventricular escape rhythms were recorded by 24-hour ECG monitoring during vagal stimulation (Plotnikov *et al.*, 2004). HCN2 overexpression was verified in the dissected tissues. The work provided strong evidence for a HCN2-based gene approach to induce a biological pacemaker within the left bundle-branch in the dog heart.

HCN1 has also been tested for creation of a biological pacemaker. A HCN1 mutant in which the S3-S4 linker was shortened by deleting three residues (HCN1- ΔΔΔ) in favor of channel gating was used to create an artificial SA node in a porcine model (Tse *et al.*, 2006). HCN1-ΔΔΔ was first overexpressed in the left ventricle of guinea pig to demonstrate its ability to pace the otherwise quiescent ventricular myocytes. Large I_f with physiological activation and fast activation kinetics, mimicking the SA node I_f, were detected in the adult guinea pig ventricular myocytes. This was to compare with the small I_f with very negative threshold activation (Yu *et al.*, 1993). The group then produced sinus node dysfunction by radiofrequency ablation to generate a sick sinus syndrome pig model (Tse *et al.*, 2006). At 10 to 14 days after injection of adenovirus containing HCN1-ΔΔΔ construct into the left atrial appendage, spontaneous atrial rhythm at a rate of 64 bpm was recorded. The advantage of using this HCN1 mutant is its fast activation kinetics similar to that of the native SA node I_f.

A recent study used HCN4 to create a biological pacemaker in the left ventricles of the pigs via an adenoviral gene transfer method (Cai *et al.*, 2007). In this research, one-month old pigs underwent catheter ablation for inducing a complete AV block after 3-4 days of adenoviral gene transfer of HCN4. ECG recordings detected idioventricular rhythm. HCN4 transcripts were not detected in non-infected ventricles, but abundantly expressed in Ad-HCN4 infected ventricles. Accordingly, large I_f-like current was recorded in the myocytes isolated from the Ad-HCN4 infected ventricles. The threshold activation was around -60mV with

midpoint activation near -90mV. The I_f-like current activation curve was shifted to a more positive potential in response to 1μM isoproterenol, which was proposed as the cellular basis for the accelerated idioventricular rhythm (Cai *et al.*, 2007).

It is unclear why small I_f-like currents were also recorded in myocytes from saline treated ventricles of pigs with similar gating properties, presumably due to the presence of HCN2. I_f is encoded by both HCN2 and HCN4 in the ventricles (Shi *et al.*, 1999; Stillitano *et al.*, 2008). Recent evidence has shown that they can form heteromeric channels *in vitro* and *in vivo* producing an I_f with physiological activation (Much *et al.*, 2003; Whitaker *et al.*, 2007; Zhang *et al.*, 2009).

The respective roles for I_f and I_{K1} in the induced ventricular pacemaker activity have recently been evaluated in the guinea pig left ventricular myocytes overexpressing either HCN1-ΔΔΔ or Kir2.1 (Chan *et al.*, 2009). It was found that I_f activity is correlated with the slope of phase-4 depolarization and the firing frequency as well as the APD_{90}, whereas I_{K1} is correlated with APD90, but not the phase-4 slope and firing frequency (Chan *et al.*, 2009). The I_f functioning as a determinant for the basal firing frequency has also been observed in the neonatal rat ventricular myocytes (Chan *et al.*, 2010).

5.1.5 Creation of biological pacemaker by channel engineering

Recently, an alternative strategy has been used to create a biological pacemaker. Kv1.4 is a voltage dependent K^+ channel which is not present in the ventricle. It was converted into a hyperpolarization-activated, cation non-selective channel using site-directed mutagenesis (R447N, L448A, R431I in the S4 segment, and G528S in the pore region) (Kashiwakura *et al.*, 2006). Adenovirus-mediated gene transfer of this mutant channel into the left ventricle of guinea pig induced ectopic pacemaker activity in otherwise quiescent ventricle. This work provided additional supporting evidence for the requirement of time-dependent inward current, not necessarily from HCN channels, for the diastolic depolarization leading to the induction of pacemaker activity in adult heart ventricles.

5.2 Cell-based approach

5.2.1 Naturally pacing myocytes

Sinus node cells are natural cardiac pacemakers. The electrical impulses generated from the sinus node are transmitted to the neighboring atrial myocytes through gap junctions (Figure 6, upper panel). A fundamental question is how a SA node cell can pace its neighboring atrial myocytes, despite of the less negative MDP in SA node than in atrial myocytes? The upper panel in Figure 6 depicts a working principle (Robinson *et al.*, 2006), assuming the coupled SA node and atrial myocyte pair is at rest. The more negative membrane potential of the atrial myocyte hyperpolarizes the coupled SA node cell, leading to activation of HCN channels. The HCN inward current is a depolarizing current, which can pass through the gap junction and depolarize the atrial myocyte. When the HCN current is large enough to depolarize the membrane toward a threshold for further activation of the Na^+ channels, an action potential is fired in the atrial myocyte. After the SA node cell is depolarized, HCN channels will be deactivated. Thus, this working mechanism guarantees a depolarizing current only at diastole (Robinson *et al.*, 2006). This working principle also outlines the minimal requirements for creating a cell-base biological pacemaker. First, the implanted cells should be able to produce depolarizing current at the end of repolarization (i.e., at diastole). It does not matter whether the depolarizing current is a result of an enhanced

HCN current or a reduced I_{K1} current, or some other currents. Second, the implanted cells must be electrically coupled with the host cell via gap junctions. Third, the depolarizing current should not be needed after the action potential is fired in both the implanted and the host cells.

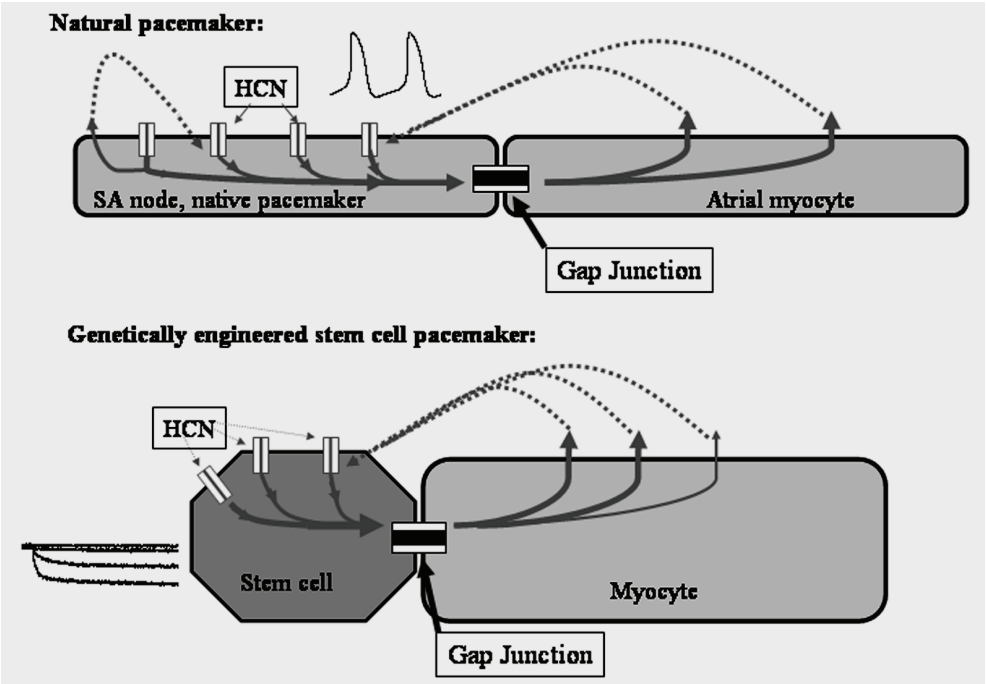


Fig. 6. Schematic illustration of using cell-based approach to deliver HCN channel genes into a ventricular myocyte. Upper panel: the native pacemaker cell (SA node cell) coupled to its adjacent myocyte (e.g., an atrial myocyte) for propagation of spontaneous action potentials via gap junction. Lower panel: engineered stem cell containing HCN channels to deliver HCN genes to a ventricular myocyte via formed gap junction for over-expression of HCN channels in the targeted myocyte. Reprinted with permission from (Rosen *et al.*, 2004).

In early 1990s, effort was made to first remove the SA node cells from a subject (the patient), then culture to generate a sufficient amount of healthy SA node cells, and finally implant these cells back to the right ventricle to provide pacing (King, 1992). This idea was to recreate a new working SA node in a new site. Although a great idea, the approach itself faced several challenges such as technical feasibility and working principles. First, growing adult human SA node cells to a critical mass represents a technical difficulty, although newborn rat SA node cells have been reported to culture for up to 4-5 days (Marvin *et al.*, 1984). Thus, feasibility of this approach is in question. Second, whether the reproduced SA nodal cells were able to form gap junctions with the ventricular myocytes was unclear. The native SA node cells are coupled with surrounding atrial cells through gap junctions. Without effective coupling between a spontaneous cell and a quiescent cell, the diastolic depolarization in the spontaneous cell cannot be transmitted to the quiescent cell for

triggering spontaneous pacing. Third, implantation in the right ventricle could represent a latent ectopic pacemaker that may trigger arrhythmia.

The proof-of-concept for creating a cell-based working biological pacemaker was first reported by transplanting the isolated fetal canine atrial myocytes including sinus node cells into the adult canine left ventricles (Ruhparwar *et al.*, 2002). After 3-4 weeks, atrial-ventricular node was ablated by catheter and a ventricular escape rhythm from the transplantation site was recorded, driving the pace of the heart. The functional coupling between the host myocytes (adult canine left ventricular myocytes) and the donor myocytes (fetal canine atrial myocytes) was evident by expression of connexin 43 between the injected and recipient myocytes (Ruhparwar *et al.*, 2002).

Using pacing fetal myocytes as a potential biological pacemaker has been further tested in the porcine model (Lin *et al.*, 2005). Human atrial myocytes containing sinus node were isolated from the aborted fetuses (14-19 weeks of gestation). These cells were injected into the left ventricles of pigs. After complete AV block was induced by catheter ablation, the idioventricular beat was detected near the injection site. Connexin 43 formed between the donor and recipient cells was identified, suggesting that the fetal myocytes are able to form gap junctions with the adult myocytes. Further, isoprenalin significantly increased the idioventricular rate (Lin *et al.*, 2005). However, the limited source of pacing fetal myocytes and strong ethical concerns have hampered further development of the biological pacemaker using this approach for possible clinical application.

5.2.2 Stem cell derived cardiomyocytes

Rapid progress on recent studies of embryonic stem cell differentiation has prompted an idea of creating a biological pacemaker using human embryonic stem cells (hESC) - derived myocytes. Human embryonic stem cells have the ability of proliferation in culture and are able to differentiate into different cell types. Under certain conditions, hESC can be induced to differentiate into spontaneously beating cardiac myocytes expressing HCN channels but not Kir2.1 channels. Therefore, the hESC-converted-beating-myocytes are capable of creating a biological pacemaker within the ventricles.

Human embryonic stem cells have been shown to differentiate into cardiac myocytes (He *et al.*, 2003). Various action potentials similar to those recorded in the SA node, atrial, and ventricular myocytes have been observed (He *et al.*, 2003). The isolated beating myocytes were shown to be able to functionally integrate into the neonatal rat ventricular myocytes to induce spontaneous rhythmic activity (Xue *et al.*, 2005). The hESC-derived beating myocytes were implanted into the left ventricle of guinea pig to initiate the spontaneous action potentials in otherwise quiescent ventricular myocytes, recorded by both ECG and optical mapping technique. In the beating hESC-derived myocytes, I_f has been recorded and HCN2 expression was detected (Satin *et al.*, 2004). Consistently, the I_f driven pacemaker activity has also been observed in mouse ESC-derived beating myocytes (Abi-Gerges *et al.*, 2000; Qu *et al.*, 2008; Barbuti *et al.*, 2009). Not surprisingly, in hESC-derived beating cardiomyocytes, the inward rectifier K^+ current, I_{K1}, and Kir2.1 protein expression were barely detected (Satin *et al.*, 2004). In a swine AV block model, injection of these spontaneously beating hESC-derived myocytes into the left ventricle induced a long-lasting ectopic ventricular rhythm (Kehat *et al.*, 2004).

While hESCs-derived beating myocytes hold promises for creating a biological pacemaker, its use is not without risks. Potential tumorigenesis, immuno-reactivity, and pro-arrhythmogenesis are major concerns (Gepstein, 2008). To bypass these concerns, adult

human mesenchymal stem cells have been used as an alternative strategy to deliver HCN genes to the ventricles.
It has been demonstrated that adult human mesenchymal stem cells can form gap junctions with the ventricular myocytes in the co-culture (Valiunas *et al.*, 2004). Using this property, HCN2 gene was introduced into the hMSC and via newly formed gap junctions to the coupled ventricular myocytes for triggering the spontaneous pacemaker activity in the host ventricular myocytes (Rosen *et al.*, 2008; Valiunas *et al.*, 2009).

6. Optimization of biological pacemaker

An effective biological pacemaker must meet the following minimal requirements: 1) a physiological heart rate that can change in response to metabolic needs, usually fulfilled via neurohumoral modulation, 2) the electrical integration of the donor cells with the host cells, 3) the control of ionic currents only during diastole to avoid pro-arrhythmic potential.
Gating of many cardiac ion channels is strongly dependent upon changes in membrane potential. HCN channels are activated at membrane hyperpolarization near diastolic potential, and are closed at membrane depolarization. Kir2.1 channels are not strongly dependent upon membrane potential. However, due to its inward rectifying property, Kir2.1 channels produce little currents in the voltage range from -40mV to 0mV.
Cardiac ion channel properties can also be altered by protein expression levels and post-translational modulation of the channel proteins at the plasma membrane. Signaling molecules such as norepinephrine, acetylcholine, adenosine, adenosine-5′- triphosphate (ATP) and cyclic adenosine monophosphate (cAMP) can all exert dramatic effects on ion channel functions. Sensitivity of the implanted biological pacemaker to cAMP is one of the requirements as the heart rate is modulated by β adrenergic receptor signaling pathway in which cAMP is the key signaling molecule.
Advancement in the cell biology of ion channel has significantly advanced our understanding of how ion channel forward trafficking to the plasma membrane for cell surface expression can critically affect the function of ion channels. Recently, a variety of cells has been differentiated into spontaneously beating myocytes. The potential technical issues as how to use these cells for a working biological pacemaker must be addressed.

6.1 Modulation of Kir2.x, HCN and Cx43 channel levels in the ventricle

Whether gene- or cell-based approach, the essential working principle is the *external* control of the relative levels of outward (I_{K1}) and inward (I_f) currents during diastole. Biophysics and molecular biology studies of I_{K1} and I_f in the past have significantly advanced our understanding how the Kir2.1 and HCN channels are modulated at the plasma membrane. More recently, microRNA studies have provided clues as how these channel transcripts are regulated. Enhanced understanding about regulation of Kir2.1 and HCN channel levels will offer new strategies to create *in vivo* biological pacemaker without the use of the full-length Kir2.1 and HCN channels.

6.2 Gene regulation of channels by microRNA

MicroRNAs (miRs) are small non-coding mRNA ~22nt in length. They bind to the 3′ untranslated region (3′-UTR) of the target genes and inhibit the transcription of the gene expression (Catalucci *et al.*, 2009). Although the working mechanisms of the miRs are still poorly understood, recent works have demonstrated a significant potential of the

endogenous miRs in the treatment of heart diseases such as coronary artery disease and heart failure (Callis & Wang, 2008; van Rooij *et al.*, 2008; Catalucci *et al.*, 2009). The muscle specific forms of miR-1 and miR-133 have been demonstrated to be pivotal in the development of hypertrophy and arrhythmias (Care *et al.*, 2007; Yang *et al.*, 2007).

Overexpression of miR-1 has been detected in individuals with coronary artery disease (Yang *et al.*, 2007). Increased miR-1 has been shown to inhibit the gene expression of KCNJ2 (which encodes Kir2.1 channel proteins) and GJA1 (which encodes connexin 43) (Yang *et al.*, 2007). In the infarct rat hearts, the reduced Kir2.1 and Cx43 channels were linked to the arrhythmogenic potential by slowing conduction and depolarization of the resting membrane potential.

On the other hand, decreased levels of both miR-1 and miR-133 have been shown to enhance the HCN2/HCN4 protein expression and functional channels at the plasma membrane (Xiao *et al.*, 2007). These results suggested that regulation of Kir2.1, Cx3.2, and HCN gene expression by microRNAs may represent an alternative strategy for creation of a biological pacemaker.

6.3 Regulation of channel surface expression

The number of functional channels at the plasma membrane determines the contribution of current to the membrane potential. Decreased I_{K1} (Beuckelmann *et al.*, 1993; Kaab *et al.*, 1996), reduced number of Cx32 channels (Severs, 1994, 2002), and increased I_f as well as the number of HCN channels (Cerbai *et al.*, 1997; Hoppe *et al.*, 1998; Cerbai *et al.*, 2001; Stillitano *et al.*, 2008) have been reported in heart diseases such as ischemia and heart failure. Gene therapy aims primarily to change the number of functional channels. On the other hand, surface expression of ion channels can be regulated by endogenous mechanisms.

In the past ten years, surface expression of the ion channel has been realized as one of the effective ways to alter the function of ion channels. Molecular mechanisms that control the synthesized proteins to exit the endoplasmic reticulum (ER), move forward to the Golgi apparatus, and to the plasma membrane have been intensively investigated, particularly for potassium channel membrane trafficking. Defective membrane trafficking due to mutated Kir2.1/HCN channels has been linked to cardiac arrhythmias (Dhamoon & Jalife, 2005; Herrmann *et al.*, 2007; Baruscotti *et al.*, 2010). Furthermore, endogenous proteins, such as small GTPase Rho1/Rac1, have been found to modulate Kir2.1 surface expression (Boyer *et al.*, 2009).

We have recently discovered that tyrosine phosphorylation of HCN channels mediated by Src kinases and receptor protein tyrosine phosphatase alpha can significantly alter the surface expression of HCN2/HCN4 channels (Huang *et al.*, 2008; Lin *et al.*, 2009). The surface expression and current density of HCN2/HCN4 channels are increased via enhanced tyrosine phosphorylation mediated by Src kinases, and decreased through tyrosine dephosphorylation by RPTPα (Huang *et al.*, 2008; Lin *et al.*, 2009). Using this modulation mechanism, we have shown that Src kinases can restore the normal current and surface expression of a human HCN4 mutant with defective membrane trafficking (Lin *et al.*, 2009).

Future investigation of how the membrane trafficking of Kir2.1, HCN, and Cx43 channels are affected under pathologic conditions may provide novel strategies for efficiently controlling the pacemaker activity in the ventricle when the sinus node is dysfunctional.

6.4 Isolation of homogeneous ESCs-derived beating cardiomyocytes

In theory, ESC derived cardiomyocytes have the potential for arrhythmogenesis (Zhang *et al.*, 2002). At least three different types of action potentials have been recorded in cardiomyocytes derived from ESCs: the sinus node like spontaneously pacing action potential, the atrial like action potential with narrow action potential duration, and the ventricular like action potential.

Although SA node markers such as Cx45/Cx40 and HCN4 have been used to label SA node cells, it will remain a technical challenge to isolate "pure" ESCs derived cardiomyocytes that only exhibit the SA node like action potential. Inclusion of any other types of myocytes in the cell grafts will increase the arrhythmic risk to the host cells. In early-stage of cardiac development, HCN4 is also abundantly expressed in ventricular myocytes (Stieber *et al.*, 2003). More specific markers for SA node will facilitate the isolation of pure ESCs derive cardiomyocytes exhibiting SA node action potentials.

If ESCs-derived cardiomyocytes do express Cx45, an additional challenge would be the properties of possible heteromeric gap junction channels formed by Cx45 and Cx43 in the host ventricular myocytes. A potential solution can be a genetically modified ESC cell body that has suppressed Cx45 and enhanced the expression of Cx43.

6.5 Resources of stem cells for generation of beating cardiomyocytes

The main concerns for the use of hESC are the ethical issues, the anticipated immune rejection and the oncogenic risk. In 2006, mouse fibroblasts were reprogrammed by expression of four transcription factors (oct4, sox2, c-myc, and klf4) to produce cells with characteristics similar to those of the mouse ESCs including the most important pluripotent property (Takahashi & Yamanaka, 2006). These inducible pluripotent stem cells (iPSCs) can also differentiate into cardiomyocytes. In the human iPSCs derived beating myocytes, connexin 43 and HCN2 expression were detected (Zwi *et al.*, 2009). However, no Kir2.x expression was reported. The electrical properties of human iPSCs support the notion that the human iPSCs derived myocytes may represent a better choice than the hESCs derived myocytes for creation of a biological pacemaker.

6.6 Brown adipose tissue

Recently, Takahashi et al has reported the use of brown adipose tissue to create pacemaker like myocytes (Takahashi *et al.*, 2009). Brown adipose tissue (BAT) is one type of mesenchymal stem cells that have the potential to differentiate into several types of cells. Cultured cells isolated from the brown adipose tissue expressed key marker genes for cardiac conduction and pacemaker activity, such as connexins 40 and 45 (Cx40 and Cx45), and HCN1 to HCN4. These BAT-converted pacing cells were injected into the area around AV node of mice. After one week, complete AV block was improved in 50% of mice tested. Although this work is significant in terms of sources for biological pacemaker, the electrophysiology of these BAT-converted pacing cells is unclear. It was not reported whether an I_f-like current is present, I_{K1}-like current is absent or the gap junctions can be formed by the donor and recipients cells.

6.7 Mesoangioblasts

Mesoangioblasts (MABs) have been recently discovered as multipotent and self-renewing cells isolated from the aorta (Minasi *et al.*, 2002). MABs are also found in the small vessels of

the mouse heart ventricle (Galvez *et al.*, 2008). A recent report showed that MABs can be converted into SAN-like pacing myocytes (Barbuti *et al.*, 2010). The MABs-derived myocytes expressed several proteins that characterize the pacemaker cells, such as HCN4 channels and connexin 45. Aggregates of these cells display stable pacemaker activity characterized by SAN-like action potential showing distinct phase-4 depolarization with the most negative potential around -60mV. These cells also exhibited an I_f-like current with threshold activation near -50mV and reversal potential around -20mV. In addition, there is also no I_{K1} in these myocytes.

Further, the pacemaker activity exhibited in these cells is modulated by neurotransmitters. Isoproterenol accelerated, while acetylcholine slowed down the action potentials (Barbuti *et al.*, 2010). These observations recapitulated the autonomous modulation of pacemaker activity in the SA node. These properties make MABs an alternative source for creation of biological pacemaker. It would be interesting to see whether MABs can induce an escape ventricular rhythm after being injected into the left ventricle of the heart in a large animal model such as canine or porcine.

6.8 Global gene transfer and electrical integration

It is known that cardiac ion channels are non-uniformly distributed in different regions of the heart. While the differential expression of ion channels is necessary for physiological heart function, it could also contribute to the pathologic genesis of electrophysiology under pathological conditions. In a neonatal rat ventricular myocyte monolayer model, I_{K1} heterogeneity has been shown to contribute to the generation of ventricular arrhythmias (Sekar *et al.*, 2009). Therefore, local gene transfer by injection not only is inefficient in terms of pacing the whole ventricle, but it could also be pro-arrhythmic. Recently, an improved approach for global gene transfer has been reported (Kikuchi *et al.*, 2005). In an effort to modify atrial electrophysiology without affecting ventricular function in atrial fibrillation, Kikuchi et al applied adenovirus containing HERG-G628S, a long-QT syndrome mutant, directly to the epicardial surface of the porcine atria, by using poloxamer gel to increase virus contact time, followed by using mild trypsin to increase virus penetration. The results showed a prolonged action potential duration and refractory period without changes in ventricular electrophysiology (Kikuchi *et al.*, 2005).

Membrane stability, diastolic depolarizing current and gap junction channels are the minimal requirement for an effective biological pacemaker that can pace the otherwise quiescent ventricles by electrically integrating the donor cells with the host cells. Without electrical integration, the donor cells can provoke the unwanted side effects, even the contractile performance can be improved. Clinical trial with skeletal myoblast has shown a high incidence of ventricular arrhythmias (Smits *et al.*, 2003). The possible reason is due to the lack of gap junction channels formed between the donor skeletal myoblasts and the ventricular myocytes, as skeletal muscle cells are electrically isolated without gap junction channels (Leobon *et al.*, 2003; Abraham *et al.*, 2005).

7. Summary

Cardiac arrhythmias have remained as the leading cause of mortality and morbidity in the developed countries and now also become a major public health problem in the developing countries. Electronic pacemaker has been working well and remains the industry standard. Numerous pharmacological tools have been developed to treat arrhythmias. However, due

to the intrinsic limitations in each strategy, there is still a strong need for novel strategy to normalize these rhythmic disorders with fewer side effects. Advances in gene and cell therapy have made it possible for creation of a biological pacemaker that will be used to overcome the limitations of electronic pacemaker. While there are still many obstacles for an effective and long-lasting biological pacemaker, development of new technologies and more animal experiments for enhanced understanding of mechanisms that control the gene expression and coupling between the donor and host cells will make the use of biological pacemaker a clinical reality.

8. References

Abi-Gerges N, Ji GJ, Lu ZJ, Fischmeister R, Hescheler J & Fleischmann BK. (2000). Functional expression and regulation of the hyperpolarization activated non-selective cation current in embryonic stem cell-derived cardiomyocytes. *J Physiol* 523 Pt 2, 377-389.

Abraham MR, Henrikson CA, Tung L, Chang MG, Aon M, Xue T, Li RA, B OR & Marban E. (2005). Antiarrhythmic engineering of skeletal myoblasts for cardiac transplantation. *Circ Res* 97, 159-167.

Barbuti A, Crespi A, Capilupo D, Mazzocchi N, Baruscotti M & DiFrancesco D. (2009). Molecular composition and functional properties of f-channels in murine embryonic stem cell-derived pacemaker cells. *J Mol Cell Cardiol* 46, 343-351.

Barbuti A, Galvez BG, Crespi A, Scavone A, Baruscotti M, Brioschi C, Cossu G & DiFrancesco D. (2010). Mesoangioblasts from ventricular vessels can differentiate in vitro into cardiac myocytes with sinoatrial-like properties. *J Mol Cell Cardiol* 48, 415-423.

Baruscotti M, Bottelli G, Milanesi R, DiFrancesco J & DiFrancesco D. (2010). HCN-related channelopathies. *Pflügers Archiv European Journal of Physiology* in press, 1-11.

Beeler GW & Reuter H. (1977). Reconstruction of the action potential of ventricular myocardial fibres. *J Physiol* 268, 177-210.

Bers DM. (2006). The beat goes on: diastolic noise that just won't quit. *Circ Res* 99, 921-923.

Beuckelmann DJ, Nabauer M & Erdmann E. (1993). Alterations of K+ currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ Res* 73, 379-385.

Boyer SB, Slesinger PA & Jones SV. (2009). Regulation of Kir2.1 channels by the Rho-GTPase, Rac1. *J Cell Physiol* 218, 385-393.

Brown HF, DiFrancesco D & Noble SJ. (1979). How does adrenaline accelerate the heart? *Nature* 280, 235-236.

Cai J, Yi FF, Li YH, Yang XC, Song J, Jiang XJ, Jiang H, Lin GS & Wang W. (2007). Adenoviral gene transfer of HCN4 creates a genetic pacemaker in pigs with complete atrioventricular block. *Life Sci* 80, 1746-1753.

Callis TE & Wang DZ. (2008). Taking microRNAs to heart. *Trends Mol Med* 14, 254-260.

Care A, Catalucci D, Felicetti F, Bonci D, Addario A, Gallo P, Bang ML, Segnalini P, Gu Y, Dalton ND, Elia L, Latronico MV, Hoydal M, Autore C, Russo MA, Dorn GW, 2nd, Ellingsen O, Ruiz-Lozano P, Peterson KL, Croce CM, Peschle C & Condorelli G. (2007). MicroRNA-133 controls cardiac hypertrophy. *Nat Med* 13, 613-618.

Catalucci D, Gallo P & Condorelli G. (2009). MicroRNAs in cardiovascular biology and heart disease. *Circ Cardiovasc Genet* 2, 402-408.

Cerbai E, Barbieri M & Mugelli A. (1994). Characterization of the hyperpolarization-activated current, I(f), in ventricular myocytes isolated from hypertensive rats. *J Physiol* 481 (Pt 3), 585-591.

Cerbai E, Barbieri M & Mugelli A. (1996). Occurrence and properties of the hyperpolarization-activated current If in ventricular myocytes from normotensive and hypertensive rats during aging. *Circulation* 94, 1674-1681.

Cerbai E & Mugelli A. (2006). I(f) in non-pacemaker cells: role and pharmacological implications. *Pharmacol Res* 53, 416-423.

Cerbai E, Pino R, Porciatti F, Sani G, Toscano M, Maccherini M, Giunti G & Mugelli A. (1997). Characterization of the hyperpolarization-activated current, I(f), in ventricular myocytes from human failing heart. *Circulation* 95, 568-571.

Cerbai E, Sartiani L, DePaoli P, Pino R, Maccherini M, Bizzarri F, DiCiolla F, Davoli G, Sani G & Mugelli A. (2001). The properties of the pacemaker current I(F)in human ventricular myocytes are modulated by cardiac disease. *J Mol Cell Cardiol* 33, 441-448.

Chan YC, Siu CW, Lau YM, Lau CP, Li RA & Tse HF. (2009). Synergistic effects of inward rectifier (I) and pacemaker (I) currents on the induction of bioengineered cardiac automaticity. *J Cardiovasc Electrophysiol* 20, 1048-1054.

Chan YC, Tse HF, Siu CW, Wang K & Li RA. (2010). Automaticity and conduction properties of bio-artificial pacemakers assessed in an in vitro monolayer model of neonatal rat ventricular myocytes. *Europace* 12, 1178-1187.

Dhamoon AS & Jalife J. (2005). The inward rectifier current (IK1) controls cardiac excitability and is involved in arrhythmogenesis. *Heart Rhythm* 2, 316-324.

DiFrancesco D. (1986). Characterization of single pacemaker channels in cardiac sino-atrial node cells. *Nature* 324, 470-473.

DiFrancesco D. (1993). Pacemaker mechanisms in cardiac tissue. *Annu Rev Physiol* 55, 455-472.

DiFrancesco D. (1995). The pacemaker current (I(f)) plays an important role in regulating SA node pacemaker activity. *Cardiovasc Res* 30, 307-308.

DiFrancesco D. (2010). Funny channel-based pacemaking. *Heart Rhythm* 7, 276-279.

Edelberg JM, Aird WC & Rosenberg RD. (1998). Enhancement of murine cardiac chronotropy by the molecular transfer of the human beta2 adrenergic receptor cDNA. *J Clin Invest* 101, 337-343.

Gaborit N, Le Bouter S, Szuts V, Varro A, Escande D, Nattel S & Demolombe S. (2007). Regional and tissue specific transcript signatures of ion channel genes in the non-diseased human heart. *J Physiol* 582, 675-693.

Galvez BG, Sampaolesi M, Barbuti A, Crespi A, Covarello D, Brunelli S, Dellavalle A, Crippa S, Balconi G, Cuccovillo I, Molla F, Staszewsky L, Latini R, Difrancesco D & Cossu G. (2008). Cardiac mesoangioblasts are committed, self-renewable progenitors, associated with small vessels of juvenile mouse ventricle. *Cell Death Differ* 15, 1417-1428.

Gepstein L. (2008). Experimental molecular and stem cell therapies in cardiac electrophysiology. *Ann N Y Acad Sci* 1123, 224-231.

Gonzalez D, Gomez-Hernandez JM & Barrio LC. (2007). Molecular basis of voltage dependence of connexin channels: an integrative appraisal. *Prog Biophys Mol Biol* 94, 66-106.

Han W, Bao W, Wang Z & Nattel S. (2002). Comparison of ion-channel subunit expression in canine cardiac Purkinje fibers and ventricular muscle. *Circ Res* 91, 790-797.

He JQ, Ma Y, Lee Y, Thomson JA & Kamp TJ. (2003). Human embryonic stem cells develop into multiple types of cardiac myocytes: action potential characterization. *Circ Res* 93, 32-39.

Herrmann S, Stieber J & Ludwig A. (2007). Pathophysiology of HCN channels. *Pflugers Arch* 454, 517-522.

Hoppe UC & Beuckelmann DJ. (1998). Characterization of the hyperpolarization-activated inward current in isolated human atrial myocytes. *Cardiovasc Res* 38, 788-801.

Hoppe UC, Jansen E, Sudkamp M & Beuckelmann DJ. (1998). Hyperpolarization-activated inward current in ventricular myocytes from normal and failing human hearts. *Circulation* 97, 55-65.

Huang J, Huang A, Zhang Q, Lin YC & Yu HG. (2008). Novel Mechanism for Suppression of Hyperpolarization-activated Cyclic Nucleotide-gated Pacemaker Channels by Receptor-like Tyrosine Phosphatase-{alpha}. *J Biol Chem* 283, 29912-29919.

Kaab S, Nuss HB, Chiamvimonvat N, O'Rourke B, Pak PH, Kass DA, Marban E & Tomaselli GF. (1996). Ionic mechanism of action potential prolongation in ventricular myocytes from dogs with pacing-induced heart failure. *Circ Res* 78, 262-273.

Kashiwakura Y, Cho HC, Barth AS, Azene E & Marban E. (2006). Gene transfer of a synthetic pacemaker channel into the heart: a novel strategy for biological pacing. *Circulation* 114, 1682-1686.

Kehat I, Khimovich L, Caspi O, Gepstein A, Shofti R, Arbel G, Huber I, Satin J, Itskovitz-Eldor J & Gepstein L. (2004). Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol* 22, 1282-1289.

Kikuchi K, McDonald AD, Sasano T & Donahue JK. (2005). Targeted modification of atrial electrophysiology by homogeneous transmural atrial gene transfer. *Circulation* 111, 264-270.

King WL. (1992). US 5103821.

Lakatta EG. (2010). A paradigm shift for the heart's pacemaker. *Heart Rhythm* 7, 559-564.

Lakatta EG & DiFrancesco D. (2009). What keeps us ticking: a funny current, a calcium clock, or both? *J Mol Cell Cardiol* 47, 157-170.

Lakatta EG, Maltsev VA & Vinogradova TM. (2010). A coupled SYSTEM of intracellular Ca2+ clocks and surface membrane voltage clocks controls the timekeeping mechanism of the heart's pacemaker. *Circ Res* 106, 659-673.

Lakatta EG, Vinogradova TM & Maltsev VA. (2008). The missing link in the mystery of normal automaticity of cardiac pacemaker cells. *Ann N Y Acad Sci* 1123, 41-57.

Leobon B, Garcin I, Menasche P, Vilquin JT, Audinat E & Charpak S. (2003). Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host. *Proc Natl Acad Sci U S A* 100, 7808-7811.

Lin G, Cai J, Jiang H, Shen H, Jiang X, Yu Q & Song J. (2005). Biological pacemaker created by fetal cardiomyocyte transplantation. *J Biomed Sci* 12, 513-519.

Lin Y-C, Huang J, Kan H, Frisbee JC & Yu H-G. (2009). Rescue of a trafficking defective human pacemaker channel via a novel mechanism: roles of Src, Fyn, Yes tyrosine kinases. *J Biol Chem* 284, 30433-30440.

Lipsius SL & Bers DM. (2003). Cardiac pacemaking: I(f) vs. Ca(2+), is it really that simple? *J Mol Cell Cardiol* 35, 891-893.

Liu YM, Yu H, Li CZ, Cohen IS & Vassalle M. (1998). Cesium effects on i(f) and i(K) in rabbit sinoatrial node myocytes: implications for SA node automaticity. *J Cardiovasc Pharmacol* 32, 783-790.

Marvin WJ, Jr., Chittick VL, Rosenthal JK, Sandra A, Atkins DL & Hermsmeyer K. (1984). The isolated sinoatrial node cell in primary culture from the newborn rat. *Circ Res* 55, 253-260.

Miake J, Marban E & Nuss HB. (2002). Biological pacemaker created by gene transfer. *Nature* 419, 132-133.

Miake J, Marban E & Nuss HB. (2003). Functional role of inward rectifier current in heart probed by Kir2.1 overexpression and dominant-negative suppression. *J Clin Invest* 111, 1529-1536.

Minasi MG, Riminucci M, De Angelis L, Borello U, Berarducci B, Innocenzi A, Caprioli A, Sirabella D, Baiocchi M, De Maria R, Boratto R, Jaffredo T, Broccoli V, Bianco P & Cossu G. (2002). The meso-angioblast: a multipotent, self-renewing cell that originates from the dorsal aorta and differentiates into most mesodermal tissues. *Development* 129, 2773-2783.

Much B, Wahl-Schott C, Zong X, Schneider A, Baumann L, Moosmang S, Ludwig A & Biel M. (2003). Role of subunit heteromerization and N-linked glycosylation in the formation of functional hyperpolarization-activated cyclic nucleotide-gated channels. *J Biol Chem* 278, 43781-43786.

Plotnikov AN, Sosunov EA, Qu J, Shlapakova IN, Anyukhovsky EP, Liu L, Janse MJ, Brink PR, Cohen IS, Robinson RB, Danilo P, Jr. & Rosen MR. (2004). Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. *Circulation* 109, 506-512.

Qu J, Plotnikov AN, Danilo P, Jr., Shlapakova I, Cohen IS, Robinson RB & Rosen MR. (2003). Expression and function of a biological pacemaker in canine heart. *Circulation* 107, 1106-1109.

Qu Y, Whitaker GM, Hove-Madsen L, Tibbits GF & Accili EA. (2008). Hyperpolarization-activated cyclic nucleotide-modulated 'HCN' channels confer regular and faster rhythmicity to beating mouse embryonic stem cells. *J Physiol* 586, 701-716.

Ranjan R, Chiamvimonvat N, Thakor NV, Tomaselli GF & Marban E. (1998). Mechanism of anode break stimulation in the heart. *Biophys J* 74, 1850-1863.

Robinson RB, Brink PR, Cohen IS & Rosen MR. (2006). I(f) and the biological pacemaker. *Pharmacol Res* 53, 407-415.

Robinson RB & Siegelbaum SA. (2003). Hyperpolarization-activated cation currents: from molecules to physiological function. *Annu Rev Physiol* 65, 453-480.

Robinson RB, Yu H, Chang F & Cohen IS. (1997). Developmental change in the voltage-dependence of the pacemaker current, if, in rat ventricle cells. *Pflugers Arch* 433, 533-535.

Rosen MR, Brink PR, Cohen IS & Robinson RB. (2004). Genes, stem cells and biological pacemakers. *Cardiovasc Res* 64, 12-23.

Rosen MR, Brink PR, Cohen IS & Robinson RB. (2008). The utility of mesenchymal stem cells as biological pacemakers. *Congest Heart Fail* 14, 153-156.

Ruhparwar A, Kallenbach K, Klein G, Bara C, Ghodsizad A, Sigg DC, Karck M, Haverich A & Niehaus M. (2010). Adenylate-Cyclase VI Transforms Ventricular Cardiomyocytes into Biological Pacemaker Cells. *Tissue Eng Part A* 16, 1867-1872.

Ruhparwar A, Tebbenjohanns J, Niehaus M, Mengel M, Irtel T, Kofidis T, Pichlmaier AM & Haverich A. (2002). Transplanted fetal cardiomyocytes as cardiac pacemaker. *Eur J Cardiothorac Surg* 21, 853-857.

Saez JC, Berthoud VM, Branes MC, Martinez AD & Beyer EC. (2003). Plasma membrane channels formed by connexins: their regulation and functions. *Physiol Rev* 83, 1359-1400.

Sanders L, Rakovic S, Lowe M, Mattick PA & Terrar DA. (2006). Fundamental importance of Na+-Ca2+ exchange for the pacemaking mechanism in guinea-pig sino-atrial node. *J Physiol* 571, 639-649.

Satin J, Kehat I, Caspi O, Huber I, Arbel G, Itzhaki I, Magyar J, Schroder EA, Perlman I & Gepstein L. (2004). Mechanism of spontaneous excitability in human embryonic stem cell derived cardiomyocytes. *J Physiol* 559, 479-496.

Schweizer PA, Yampolsky P, Malik R, Thomas D, Zehelein J, Katus HA & Koenen M. (2009). Transcription profiling of HCN-channel isotypes throughout mouse cardiac development. *Basic Res Cardiol* 104, 621-629.

Sekar RB, Kizana E, Cho HC, Molitoris JM, Hesketh GG, Eaton BP, Marban E & Tung L. (2009). IK1 heterogeneity affects genesis and stability of spiral waves in cardiac myocyte monolayers. *Circ Res* 104, 355-364.

Severs NJ. (1994). Pathophysiology of gap junctions in heart disease. *J Cardiovasc Electrophysiol* 5, 462-475.

Severs NJ. (2002). Gap junction remodeling in heart failure. *J Card Fail* 8, S293-299.

Severs NJ, Bruce AF, Dupont E & Rothery S. (2008). Remodelling of gap junctions and connexin expression in diseased myocardium. *Cardiovasc Res* 80, 9-19.

Shi W, Wymore R, Yu H, Wu J, Wymore RT, Pan Z, Robinson RB, Dixon JE, McKinnon D & Cohen IS. (1999). Distribution and prevalence of hyperpolarization-activated cation channel (HCN) mRNA expression in cardiac tissues. *Circ Res* 85, e1-6.

Shi W, Yu H, Wu J, Zuckerman J, Wymore R, Dixon JE, Robinson RB, McKinnon D & Cohen IS. (2000). The distribution and prevalence of HCN isoforms in the canine heart and their relation to the voltage dependence of if. . *Biophys J* 78, 353A.

Smits PC, van Geuns RJ, Poldermans D, Bountioukos M, Onderwater EE, Lee CH, Maat AP & Serruys PW. (2003). Catheter-based intramyocardial injection of autologous skeletal myoblasts as a primary treatment of ischemic heart failure: clinical experience with six-month follow-up. *J Am Coll Cardiol* 42, 2063-2069.

Stieber J, Herrmann S, Feil S, Loster J, Feil R, Biel M, Hofmann F & Ludwig A. (2003). The hyperpolarization-activated channel HCN4 is required for the generation of pacemaker action potentials in the embryonic heart. *Proc Natl Acad Sci U S A* 100, 15235-15240.

Stillitano F, Lonardo G, Zicha S, Varro A, Cerbai E, Mugelli A & Nattel S. (2008). Molecular basis of funny current (If) in normal and failing human heart. *J Mol Cell Cardiol* 45, 289-299.

Takahashi K & Yamanaka S. (2006). Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* 126, 663-676.

Takahashi T, Nagai T, Naito AT, Kanda M, Tokunaga M, Liu M, Ogura T, Lee J-K, Kodama I, Nakaya H & Komuro I. (2009). Abstract 2966: Brown Adipose Tissue Derived-cells Differentiate Into Cardiac Conduction and Pace-maker Cells in vitro and Improve Complete AV Block in vivo. *Circulation* 120, S721-c-.

Tse HF, Xue T, Lau CP, Siu CW, Wang K, Zhang QY, Tomaselli GF, Akar FG & Li RA. (2006). Bioartificial sinus node constructed via in vivo gene transfer of an engineered pacemaker HCN Channel reduces the dependence on electronic pacemaker in a sick-sinus syndrome model. *Circulation* 114, 1000-1011.

Valiunas V, Doronin S, Valiuniene L, Potapova I, Zuckerman J, Walcott B, Robinson RB, Rosen MR, Brink PR & Cohen IS. (2004). Human mesenchymal stem cells make cardiac connexins and form functional gap junctions. *J Physiol* 555, 617-626.

Valiunas V, Kanaporis G, Valiuniene L, Gordon C, Wang HZ, Li L, Robinson RB, Rosen MR, Cohen IS & Brink PR. (2009). Coupling an HCN2-expressing cell to a myocyte creates a two-cell pacing unit. *J Physiol* 587, 5211-5226.

van Rooij E, Marshall WS & Olson EN. (2008). Toward microRNA-based therapeutics for heart disease: the sense in antisense. *Circ Res* 103, 919-928.

Vassalle M. (1995). The pacemaker current (I(f)) does not play an important role in regulating SA node pacemaker activity. *Cardiovasc Res* 30, 309-310.

Vassalle M, Yu H & Cohen IS. (1999). Pacemaker Channels and Cardiac Automaticity. In *Cardiac Electrophysiology: From Cell to Bedside*, third edn, ed. Zipes DP & Jalife J, pp. 94-103. W.B. Saunders Company.

Wainger BJ, DeGennaro M, Santoro B, Siegelbaum SA & Tibbs GR. (2001). Molecular mechanism of cAMP modulation of HCN pacemaker channels. *Nature* 411, 805-810.

Whitaker GM, Angoli D, Nazzari H, Shigemoto R & Accili EA. (2007). HCN2 and HCN4 isoforms self-assemble and co-assemble with equal preference to form functional pacemaker channels. *J Biol Chem* 282, 22900-22909.

Xiao J, Yang B, Lin H, Lu Y, Luo X & Wang Z. (2007). Novel approaches for gene-specific interference via manipulating actions of microRNAs: examination on the pacemaker channel genes HCN2 and HCN4. *J Cell Physiol* 212, 285-292.

Xue T, Cho HC, Akar FG, Tsang SY, Jones SP, Marban E, Tomaselli GF & Li RA. (2005). 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* 111, 11-20.

Yang B, Lin H, Xiao J, Lu Y, Luo X, Li B, Zhang Y, Xu C, Bai Y, Wang H, Chen G & Wang Z. (2007). The muscle-specific microRNA miR-1 regulates cardiac arrhythmogenic potential by targeting GJA1 and KCNJ2. *Nat Med* 13, 486-491.

Yeager M. (1998). Structure of cardiac gap junction intercellular channels. *J Struct Biol* 121, 231-245.

Yu H, Chang F & Cohen IS. (1993). Pacemaker current exists in ventricular myocytes. *Circ Res* 72, 232-236.

Yu H, Chang F & Cohen IS. (1995). Pacemaker current i(f) in adult canine cardiac ventricular myocytes. *J Physiol* 485 (Pt 2), 469-483.

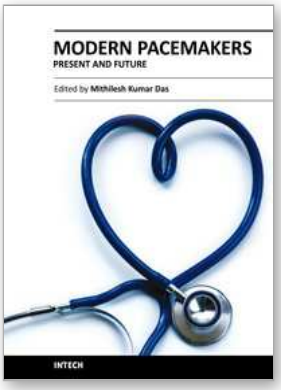
Yu X, Chen XW, Zhou P, Yao L, Liu T, Zhang B, Li Y, Zheng H, Zheng LH, Zhang CX, Bruce I, Ge JB, Wang SQ, Hu ZA, Yu HG & Zhou Z. (2007). Calcium influx through If channels in rat ventricular myocytes. *Am J Physiol Cell Physiol* 292, C1147-1155.

Yu X, Duan KL, Shang CF, Yu HG & Zhou Z. (2004). Calcium influx through hyperpolarization-activated cation channels (I(h) channels) contributes to activity-evoked neuronal secretion. *Proc Natl Acad Sci U S A* 101, 1051-1056.

Zhang Q, Huang A, Lin YC & Yu HG. (2009). Associated changes in HCN2 and HCN4 transcripts and I(f) pacemaker current in myocytes. *Biochim Biophys Acta* 1788, 1138-1147.

Zhang YM, Hartzell C, Narlow M & Dudley SC, Jr. (2002). Stem cell-derived cardiomyocytes demonstrate arrhythmic potential. *Circulation* 106, 1294-1299.

Zwi L, Caspi O, Arbel G, Huber I, Gepstein A, Park I-H & Gepstein L. (2009). Cardiomyocyte Differentiation of Human Induced Pluripotent Stem Cells. *Circulation %R 101161/CIRCULATIONAHA109868885* 120, 1513-1523.



Modern Pacemakers - Present and Future

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The book focuses upon clinical as well as engineering aspects of modern cardiac pacemakers. Modern pacemaker functions, implant techniques, various complications related to implant and complications during follow-up are covered. The issue of interaction between magnetic resonance imaging and pacemakers are well discussed. Chapters are also included discussing the role of pacemakers in congenital and acquired conduction disease. Apart from pacing for bradycardia, the role of pacemakers in cardiac resynchronization therapy has been an important aspect of management of advanced heart failure. The book provides an excellent overview of implantation techniques as well as benefits and limitations of cardiac resynchronization therapy. Pacemaker follow-up with remote monitoring is getting more and more acceptance in clinical practice; therefore, chapters related to various aspects of remote monitoring are also incorporated in the book. The current aspect of cardiac pacemaker physiology and role of cardiac ion channels, as well as the present and future of biopacemakers are included to glimpse into the future management of conductions system diseases. We have also included chapters regarding gut pacemakers as well as pacemaker mechanisms of neural networks. Therefore, the book covers the entire spectrum of modern pacemaker therapy including implant techniques, device related complications, interactions, limitations, and benefits (including the role of pacing role in heart failure), as well as future prospects of cardiac pacing.

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