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Review

Cell-based Biological Pacemakers: Progress and Problems

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The number of permanent pacemaker implantations has been increasing due to the aging of populations worldwide and the increase in the numbers of patients with heart diseases. Commercially available mechanical pacemakers are very useful but still have some problems including short battery life, a risk of infection, the absence of physiological autonomic responsiveness, metal allergy, and electronic interference. A biological pacemaker may resolve these problems and regenerate the cardiac pacemaker. Cell-based therapy and gene therapy have been addressed with the goal of solving the challenges of biological pacemaker. However, the clinical application of a biological pacemaker has not yet been realized. Here we discuss the types of cells that can be used for a biological pacemaker and the problems that remain regarding the clinical applications of cell-based therapy.

Key words: cell therapy, pluripotent stem cells, cardiomyocytes, biological pacemaker, hyperpolarization activated cyclic nucleotide gated potassium channel 4

It is expected that cell-based therapy will eventually be effective for treating various diseases including cardiac arrhythmia. The numbers of patients with heart diseases have been increasing worldwide due to the aging of populations. The number of cardiac pacemaker implantations for bradyarrhythmia has therefore also been increasing [1]. However, pacemaker implantations present various problems including short battery life, lead complications, risk of infection, metal allergy, and electronic interference; alternatives are needed [2-4]. Here we review various types of cells that can be used for a biological pacemaker, and we discuss the problems that must be addressed before the clinical application of cell-based therapy.

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Electronic Prerequisites for the Automaticity of a Pacemaker

The sinoatrial node can generate impulses faster than those generated in other areas. Sinoatrial node cells can spontaneously depolarize during diastole. The I_f current flows through hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, which are cation channels activated by hyperpolarization at voltages more negative than −50 mV. This current is mainly involved in diastolic depolarization [5,6]. HCN4 is one of the isoforms and is highly expressed in the sinoatrial node. *HCN4* mutations have been shown to cause sinus node dysfunction [7-9]. However, transgenic mice overexpressing HCN2 specifically in the heart exhibited no discernible abnormalities under physiological conditions [10].

On the other hand, working cardiomyocytes maintain the resting membrane potentials during diastole.

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The I_{K1} current flowing through K_{ir} channels plays an important role in this phenomenon. Left ventricular cardiomyocytes of guinea pigs transduced with dominant-negative $K_{ir}2.1$ show spontaneous action potentials [11]. Additionally, I_{K1}-enhanced human-induced pluripotent stem cell-derived cardiomyocytes (hiPSC-CMs) lose spontaneous beating and acquire stable resting membrane potentials [12].

Thus, enhancement of the I_f current and attenuation of the I_{K1} current are prerequisites for spontaneous diastolic depolarization of sinoatrial node cells (Fig. 1) [13].

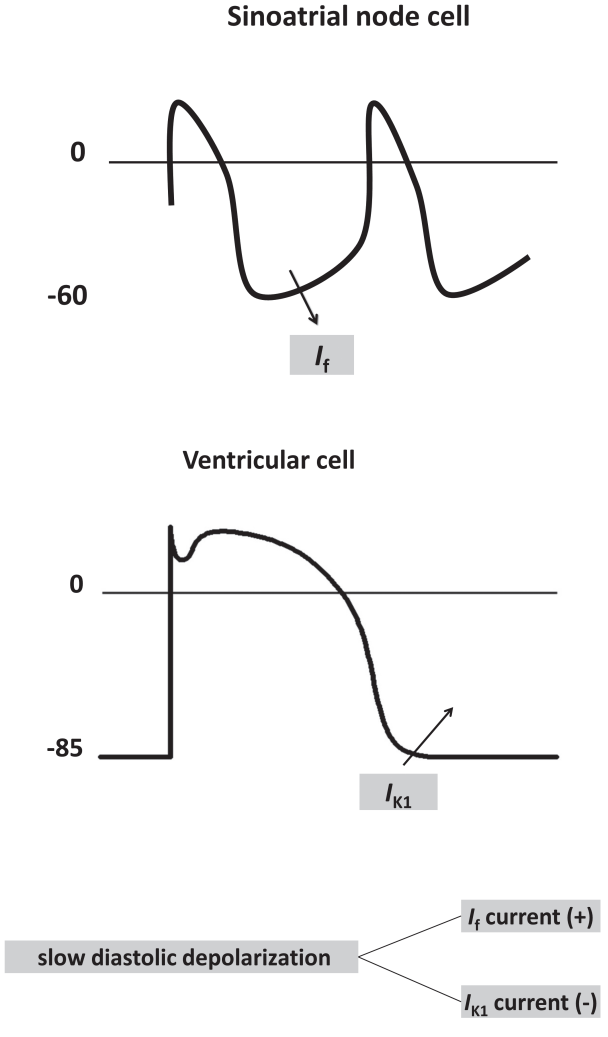

Fig. 1 Differences between the action potential configurations of sinoatrial node cells (*top*) and ventricular cells (*middle*). Sinoatrial node cells have a pacemaking ability via slow diastolic depolarization. The depolarization requires the presence of the I_f current and absence of the I_{K1} current (*bottom*).

Cell-based Therapy

Mainly two types of cells have been used as a platform for a biological pacemaker: mesenchymal stem cells (MSCs) and pluripotent stem cells (PSCs) (Fig. 2).

Mesenchymal stem cell-based therapy. Mesenchymal stem cells (MSCs) can differentiate into several types of mesodermal cells, including osteoblasts, chondrocytes, myocytes and adipocytes [14]. MSCs do not fire spontaneously like cardiomyocytes do (Fig. 2). However, MSCs robustly express connexin 40 and 43, that form the gap junction. MSCs can then electrically couple with cardiomyocytes [15]. MSCs transduced with HCN1, HCN2 or HCN4 can generate I_f current [16-20]. The depolarization of adjacent cardiomyocytes could result in the closing of the HCN channels, and the next repolarization could result in the opening of the HCN channels. That is to say, MSCs can be used as a platform for delivering the I_f current that depolarizes adjacent cardiomyocytes [21]. HCN4-overexpressing porcine MSC transplantation was shown to be able to increase the heart rate in a porcine model of complete atrioventricular block, however, the physiological heart rate could not be recovered [22].

MSCs can possibly migrate and differentiate into other cell types *in vivo* [23] . It has thus been discussed whether the stability of coupling between HCN-transduced MSCs and the myocardium can be maintained for a long time [24 , 25].

Pluripotent stem cell-derived sinoatrial node cell-like cells. Pluripotent stem cells (PSCs) can differentiate into a variety of cells, and they are expected to be a robust source for regenerative medicine [26-30]. Thus, many different methods have been established to induce or sort PSC-derived sinoatrial node cell-like cells. The following methods have been used to isolate sinoatrial node cell-like cells from PSC-derived cells: Sinoatrial and atrioventricular node-like cells derived from mouse embryonic stem cells (mESCs) could be identified and isolated by *Shox2* promoter- and *Cx30.2* promoter-based antibiotic selection, and progenitor cells of sinoatrial node-like cells could be isolated by sorting CD166-positive cells during the cardiac differentiation of mESCs [31 , 32]. *Shox2*-deficient murine embryos exhibited hypoplasia of sinus venosus myocardium (including the sinoatrial nodal region) and a lack of *Tbx3* and *Hcn4* expression, along with an ectopic activation of working myocardium genes, *Nppa*, *Cx40*

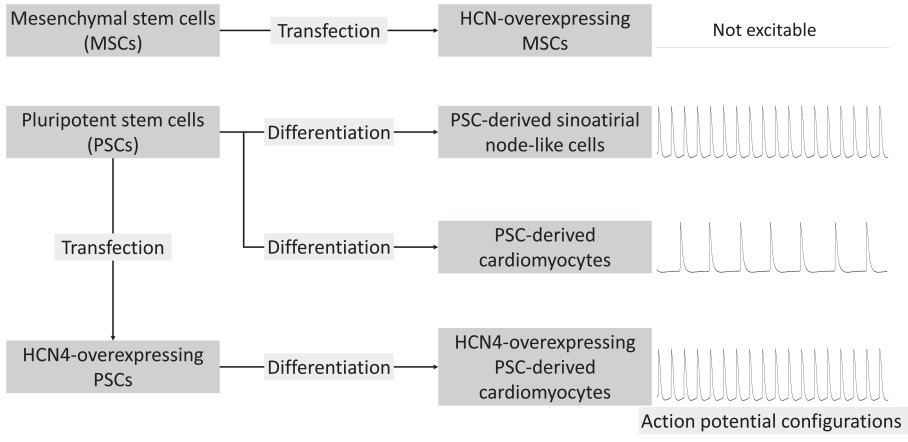

Fig. 2 Various cells that may be used for a biological pacemaker.

and *Nkx2.5* [33,34].

The following methods were shown to induce sinoatrial node-like cells from PSCs. Activation of calcium-activated potassium channels increased the proportion of pacemaker-like cells using mESCs [35]. TBX3 is expressed in the sinoatrial node. *Tbx3*-null murine embryos form sinoatrial nodes with atrial gene expression [36]. TBX3 is required to suppress the atrial and ventricular genes, *Cx40*, *Cx43*, *Scn5a* and *Kcnj2* [37,38]. Mutations in human *TBX3* are also known to be a cause of ulnar mammary syndrome [39,40]. Therefore, TBX3 transduction in the early cardiac differentiation phase and *Myh6*-promoter-based antibiotic selection led to the induction of pacemaker-like cells using mESCs [41].

Additionally, SHOX2 transduction in the early cardiac differentiation phase also increased the proportion of sinoatrial node-like cells using mESCs [42]. These two transcription factors, SHOX2 and TBX3, play an important role in the development of the sinoatrial node [33,34,36,37,43]. Protze *et al.* established a method for inducing sinoatrial node-like pacemaker cells from human PSCs without genetic manipulation [44]. The cells were identified as NKX2-5-negative cardiomyocytes, and their pacemaking ability was demonstrated *ex vivo*.

The sinoatrial node-like cells derived from PSCs have patterns of gene expression and electrical features similar to those of endogenous sinoatrial node cells. However, isolated sinoatrial node cells could not sufficiently perform pacemaking in the right ventricle [45]. Integration between transplanted cells and endogenous cardiac tissue might be important for achieving sufficient pacemaking. Further investigations are needed to determine whether the pacemaking ability of PSC-derived sinoatrial node-like cells is sufficient to treat bradycardia *in vivo*.

HCN4-overexpressing pluripotent stem cell-derived cardiomyocytes. PSC-derived cardiomyocytes have pacemaking ability because they have the ion channels and receptors that are required for impulse generation, propagation and modulation [46-52]. However, the pacemaking ability of PSC-derived cardiomyocytes might be insufficient for treating bradycardia because the new ectopic rhythm rate caused by transplanted PSC-derived cardiomyocytes was shown to be only one-third to one-half of sinus rhythm [53-55].

PSC-derived cardiomyocytes can express $K_{ir}2.1$ with long-term culturing, but they generate a poor I_{K1} current [12,47,56-59]. Additionally, the expression of HCN channels decreases with maturation [47].

We have thus transduced HCN4 into PSC-derived cardiomyocytes and established cardiomyocytes generating an abundant I_f current and a poor I_{K1} current (Fig. 2) [60]. The rabbit HCN4-overexpressing mESC-derived cardiomyocytes (mESC-CMs) showed more frequent spontaneous beating and stronger pacemaking ability compared to those of non-overexpressing mESC-CMs. HCN4-overexpressing mESC-CMs expressed 3- to 5-times higher levels of transcripts of *Hcn4* than did non-overexpressing mESC-CMs. Additionally, the transplantation of HCN4-overexpressing mESC-CMs generated a new rapid rhythm in rats with complete atrioventricular block, and the transplantation partially

recovered the heart rate comparable to the physiological sinus rate [55]. These cells must also be compared with PSC-derived sinoatrial node-like cells.

Advantages and disadvantages of a cell based-biological pacemaker. Some aspects of cell-based therapy are of concern. The duration of cell retention is the most important problem for all cell-based therapies, and it is not yet known how long transplanted cardiomyocytes are retained in the heart, whereas the battery life of a mechanical pacemaker can be examined and determined. Negative chronotropic drugs that are used for treatment of heart failure and tachyarrhythmia can suppress the pacemaking ability of a biological pacemaker, but a mechanical pacemaker can pace regardless of the administration of these drugs [61-64]. Tachyarrhythmia induced by transplanted cells is also a concern, since even PSC-derived cardiomyocytes (PSC-CMs) can induce arrhythmias [65,66].

We and another group have demonstrated that ivabradine, an HCN channel blocker, decreased spontaneous beating, and this drug may be useful for the suppression of excessive pacing by a biological pacemaker with HCN channel overexpression [60,67]. Additionally, PSC-derived cells and genetically edited cells present the possibility of tumorigenesis.

On the other hand, if transplantation can be performed by catheterization, the implantation of a biological pacemaker is minimally invasive and can be performed repeatedly in the same patient [68]. There is also no need to worry about electromagnetic interference. Moreover, the expression of β-adrenoceptor can provide physiologic autonomic responsiveness. The risk of infection in the case of the transplantation of a biological pacemaker might be lower than that with the implantation of a mechanical pacemaker. A biological pacemaker could thus be a complementary treatment for a mechanical pacemaker.

Gene Therapy

Adenoviral gene therapy using different genes has also been studied for the regeneration of pacemakers [67,69-75]. HCN gene overexpression can increase the heart rates of bradycardia model animals. Especially, adenoviral *HCN2/SkM1* transduction into left bundle branches was shown to be able to recover the physiological heart rates in complete atrioventricular dogs [73]. In contrast, the overexpression of chimeric HCN channels, containing N- and C- termini of HCN2 and the transmembrane region of HCN1, caused an excessive tachycardia [67].

TBX18 overexpression makes adult ventricular cardiomyocytes transdifferentiate into sinoatrial node-like cells [74,75]. TBX18 is required for the formation of the sinoatrial node head and the differentiation of sinoatrial node myocardium [36]. *TBX18* gene injection into the left ventricle was shown to recover the physiological heart rate in a complete atrioventricular block porcine model [75]. TBX18 is required for the formation of the sinoatrial node [36]. However, continuous TBX18 expression is necessary to maintain the sinoatrial node-like phenotype even though TBX18 was undetectable in the neonatal and adult sinoatrial nodes [74]. Since adenoviral vector does not integrate into a genome, the expression of a transgene is transient. Thus, a safe and long-lasting vector is required as with other gene therapy.

Challenges Regarding the Clinical Application of Biological Pacemakers

Before clinical application, the efficacy and safety of a biological pacemaker must be validated *in vivo*. Thus far, it has never been demonstrated that transplantation of a biological pacemaker can completely recover the physiological heart rate in large-animal bradycardia models [53,76]. We have reported that HCN4 overexpression enhanced mESC-CMs' pacemaker ability in complete atrioventricular model rats [55]. We are now attempting to create HCN4-overexpressing hiPSC-CMs and transplant them into large-animal bradycardia models.

Moreover, cell delivery systems are required to achieve safe and reliable cell-based therapy. For example, a catheter system and biomaterial carriers have been developed [77,78].

Conclusion

Various methods for the establishment of cell-based therapy for a biological pacemaker have been investigated, but a biological pacemaker has not yet been realized in a clinical situation. The effects of candidate biological pacemakers on bradycardia and arrhythmogenicity must be investigated in large-animal bradycardia models prior to the realization of clinical applications of biological pacemakers.

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