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Next-generation pacemakers: from electrical devices to biological pacemakers

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Abstract

The invention of an electric pacemaker in the middle of the 20th century led to a revolution in the treatment of cardiac conduction system diseases. The improvement of pacemakers continued. In 1962, the first small series of external pacemakers for percutaneous and direct stimulation was produced in Kaunas. After a while, electric pacemakers became more reliable, smaller and lighter in weight, but the problem of foreign body associated infection and limited service life remained unresolved. Modern high-tech medicine strives to create less invasive electric pacemakers, but nevertheless, biological pacemakers can expand the therapeutic arsenal for the treatment of cardiac patients, being the most physiological for humans. The concept of an artificial biological pacemaker consists of the creation of an organic structure that generates a spontaneous rhythm from the implantation site in the myocardium. Various gene and cellular approaches were used to create biological pacemakers: a functional reorganization approach (use of adenovirus vectors for hyperexpression of genes encoding ion channels in cardiomyocytes); hybrid approach (use of fibroblasts to deliver genes of ion channels that provide heart automation); somatic reprogramming approach (overexpression of the transcription factor TBX18 using adenoviral vectors, which reprograms cardiomyocytes into induced sinoatrial node cells, creating cardiac stimulatory activity); cellular approach (transplantation of stem cells to a specific place in the heart, thereby creating biological stimulation). Modern methods of electrical cardiac stimulation and the developed concepts of the biological pacemaker clearly show the possibility of eliminating current problems associated with the use of an artificial pacemaker by replacing it with a biological one. Each of the approaches (gene, cellular, hybrid-cellular, somatic reprogramming) has its own advantages and disadvantages, which predisposes to further study and improvement in order to introduce a biological pacemaker into clinical practice.

Keywords: biological rhythm driver, gene therapy, cell therapy, electric pacemaker.

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An electrical impulse is generated in the sinoatrial node (SAN), then spreads downwards, and exits in various parts of the heart. With the conduction system pathology, the heart rate slows down, which leads to a discrepancy between the body's need for blood circulation and the actual blood supply [1]. Therefore, medicine resorts to the implantation of electronic pacemakers as a solution.

Over time, electric cardiac pacemakers (ECP) improved, became smaller and lighter in weight, and more advanced two- and three-chamber models appeared. A modern ECP consists of a subcutaneous generator, a lithium-ion battery, and a series of wires with electrodes on tips and can track electrical impulses of the atria and ventricles, respiration rate, and body speed, which adjust the heart rate considering the physiological needs.

Patients with ECPs have several limitations in areas with an electromagnetic field despite the efficiency of ECPs, which affects their quality of life, with a possible risk of a foreign body infection with an infectious agent [2]. Additionally, the use of ECPs also has several problems in children due to the smaller body size than that of an adult and rapid growth, as well as the anatomical changes associated with congenital cardiac defects [3]. Generally, epicardial cardiac pacing is recommended for patients weighing <15 kg and/or with altered anatomy (e.g., with an intracardiac shunt or with a solitary ventricle).

Epicardial pacing leads are often prone to breakage and often need to be replaced with either a new epicardial lead or an endocardial system, if possible. With modern battery technology, the generators must be replaced approximately every 10

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years, which requires multiple replacements of the generators with an appropriate set of risks and complications that are associated with this procedure, such as endocardial lead dislocations, increased stimulation threshold, hematoma in the ECP bed area, pneumothorax, and myocardial perforation. Alternative energy sources, such as piezoelectric energy [4, 5] and solar energy, are currently investigated at the preclinical stages of study by the American scientists, C. Dagdeviren et al. [6].

Biological pacemakers that are derived from gene transfer, cell fusion, or stem cell transplantation provide an alternative to electronic devices. Modern high-tech medicine strives to create less traumatic ECPs; however, biological pacemakers can expand the therapeutic range for the treatment of patients with cardiac disorders, as the most physiological for humans [7]. An increased frequency of pacemaker contractions and activity induction in a new focus is the principal field of biological pacemaker creation [8].

Biological pacemakers. Various biological approaches to improve cardiac autonomy have been analyzed over the years, which aimed to use cells that are functionally similar to SAN cells (heart stimulating natural cells) [9]. Various gene and cellular approaches were described for the development of biological pacemakers.

1. *The functional reorganization approach*. Adenoviral vectors are used to overexpress genes encoding ion channels (one channel or a combination of channels) in cardiomyocytes to increase the number of hyperpolarization-activated cyclic nucleotide-gated channels (HCN) and decrease the number of potassium channels of internal rectification (KIR) by dominant-negative construct overexpression (KIR2.1AAA) [10].

2. *Stem cells*. A stem cell cluster is derived from human embryonic stem cells or induced pluripotent stem cells, which are transplanted to a specific location in the heart to capture the surrounding myocardium, thereby creating biological stimulation.

3. *In the hybrid approach*, cells (human mesenchymal stem cells or fibroblasts) are used to deliver genes of ion channels (e.g., genes encoding components of HCN channels) to ensure cardiac automatism [11]. Delivery using human mesenchymal stem cells requires their connection with cardiomyocytes using gap junction, whereas cell fusion is required for gene delivery using fibroblasts [12].

4. *In somatic reprogramming*, overexpression of the T-box transcription factor TBX18 using adenoviral vectors reprograms cardiomyocytes into induced SAN cells, repeating the properties of SAN and, therefore, creating cardiac stimulatory activity [10, 13].

Gene approaches. The earliest gene approach for increasing cardiac automatism involved the overexpression of genes that encode human β_2-adrenergic receptors in the atria of mice and pigs [14]. This approach did not create a biological pacemaker; however, the rate of endogenous SAN was increased by increasing the number of available β_2-adrenergic receptors for binding to endogenous catecholamines [15].

The first biological pacemaker *de novo* was created using gene therapy, which was reported in 2002. The strategy of the American scientists, J. Miake and E. Marban, consists of the release of an "electric brake" that suppresses automatism in ventricular cardiomyocytes by inhibiting endogenous KIR.

KIR is a specific subset of potassium channels. Currently, seven subfamilies of KIR have been identified in the cells of various tissues of animals of various species. The main role of the KIR channels includes the restoration of the resting membrane potential during hyperpolarization by conducting the weak potassium current into the cell. The overexpression of the KIR2.1 dominant-negative construct (KIR2.1AAA) decreases the amount of functional ionic KIR (encoded by the KIR2 gene family; also known as KCNJ2) in the guinea pig myocardium [15].

Suppression of the incoming rectifying current (IK1) causes spontaneous ventricular cardiomyocyte depolarization, thereby generating the biological activity of the pacemaker. Subsequent studies revealed that KIR2.1AAA overexpression not only influenced the resting potential (causing spontaneous depolarization) but also led to an increased duration of the action potential [10]. Diffuse suppression of IK1 in the ventricular myocardium may predispose to arrhythmias as clinically noted in familial long *QT* syndrome type 7 (REF 82). The potential proarrhythmic effects of focal IK1 suppression, which is necessary to induce the biological activity of the pacemaker, were not described in these small animal validation studies [16]. Therefore, any biological therapy that can increase cardiac automatism should be carefully investigated in several preclinical models, including large animals with low heart rates, to rule out potential proarrhythmic effects.

Moreover, A.N. Plotnikov et al. considered the possibility of sinus node pacemaker f-channels (If-channels) expression in normal working cardiomyocytes by HCN channel overexpression, namely HCN2 [17]. Murine HCN2 adenoviral constructs were delivered by open thoracotomy at the base of the left atrial auricle. The anesthetized dogs, 4 days after injection, had spontaneous rhythms that arose from the left atrium after sinus rhythm suppression by vagal stimulation.

Ion channels, If, are composed of proteins of the HCN family, which exist as four HCN ion channel isoforms. The isoforms 1, 2, and 4 are found only in the myocardium, while isoform 3 in the brain [18]. The HCN2 gene is used for incorporation into the viral vector since the properties of this isoform are closest to the native current [19]. Subsequent studies of the same group demonstrated that HCN2-expressing adenovirus injection into the left branch of the His bundle resulted in spontaneous ventricular rhythms following vagal pacing. These two independent studies have demonstrated the ability to create biological cardiac pacing through functional genetic engineering either by suppressing IK1, thereby causing spontaneous depolarization of ventricular cardiomyocytes, or by expressing If-channels in normal working cardiomyocytes.

Cellular approaches. Human embryonic stem cells can differentiate into spontaneously excited cardiomyocytes [20]. The *in vivo* transplantation of cardiomyocytes that are derived from embryonic stem cells in guinea pigs led to the pacemaker biological activity, which was confirmed by *ex vivo* optical mapping [21]. After the atrioventricular node ablation, previously injected animals with cardiomyocytes that are derived from embryonic stem cells exhibited spontaneous biological cardiac stimulatory activity at the injection site (demonstrated by optical mapping). Given the human origin of these cells, immunosuppression was required to prevent rejection [15].

SAN-like cardiomyocytes that are obtained from human-induced pluripotent stem cells (iPSC) were used to create biological cardiac stimulation *in vitro* and *in vivo* [22]. Another study engrafted iPSC-derived cardiomyocytes into canine hearts through open thoracotomy. The pacemaker biological activity was recorded only in 50% of animals with a heart rate of 40–50 per min [11]. Significant disadvantages of this method include the ability of the transplanted embryonic stem cells to finally differentiate into cardiomyocytes while losing their ECP characteristics, as well as the need for immunosuppression.

Contemporary iPSC technologies create a mixed population of cells with different phenotypes, and it should be considered that immature cells can differentiate into cells of various types (e.g., teratomas) and migrate throughout the body. The rather long term for generation of the iPSCs (up to several months) remains controversial [23].

The Russian scientists, N.Sh. Zagidullin and Sh.Z. Zagidullin, in collaboration with the laboratory of the Cologne University Hospital, electroporated mouse embryonic stem cells with a plasmid that contains the gene for atrial natriuretic hormone, which is significant in atrial development. Spindle-shaped cells with pacemaker activity were found in the culture, which differs in their morphology from triangular and polygonal cells, which are not suitable as biological pacemakers. The experiment revealed that the cultivation of plasmid-loaded embryonic stem cells with endothelin-1 led to a shift in favor of increasing the concentration of spindle-shaped cells with pacemaker-like electrophysiological characteristics for their further use as biopacemakers [24].

Some experimental studies have examined the use of a biological pacemaker in conjunction with an ECP. Potentially, such a combination has additional advantages over their isolated use, as a biological pacemaker will modulate the heart rate based on physical and psycho-emotional stress, while an electrical component will "insure" the heart rate in the event of biological pacemaker "shutdown." Such a tandem will be more physiological for the body and will increase the service life of the ECP batteries.

N.Sh. Zagidullin and Sh.Z. Zagidullin also studied the electrophysiological properties of HCN1, HCN2, and HCN4 isoforms in physiological and hyperpotassium solutions when expressed in the ovarian cells of Chinese hamsters to determine a candidate gene for creating a biological pacemaker. The study revealed that out of the three isoforms, HCN1 showed the fastest kinetics of activation, followed by HCN2, and the "slowest" isoform was HCN4. Additionally, HCN1 was superior to HCN2 and especially HCN4 in current density. In terms of electrophysiological parameters, the HCN2 isoform is closest to the native If-current, which recommends it as a real candidate for biological pacemaker creation [25].

Hybrid gene-cell approach. The hybrid gene-cell approach uses cells that carry genes for cardiac stimulatory activity (e.g., genes encoding HCN channels). Human mesenchymal stem cells that are engineered to express the HCN2 pacemaker channel were injected into dogs with a complete atrioventricular block by open thoracotomy [26]. The biological cardiac stimulatory activity was manifested, and the rate of ventricular contractions was 50–60 per minute, without signs of cellular or humoral rejection.

The potential advantages of this approach include the absence of a viral vector (used in most gene therapy approaches) and the need for immunosuppression (given the low immunogenicity of human mesenchymal stem cells). An obstacle to the viral vector introduction is the activation of the body's immune system, of which the modified cells return to their original state, as well as the presence

of pathogenic potential. Disadvantages of this approach are rather low heart rate (40–45 per minute), the migration possibility, and further human mesenchymal stem cell differentiation [11].

In the next hybrid approach, American scientists and scientists from Taiwan Y.F. Hu et al. used engineered syngeneic fibroblasts expressing HCN1, which was injected into guinea pig hearts to induce cell fusion with surrounding endogenous ventricular cells [27]. The resulting fibroblast-myocyte heterokaryons exhibited biological cardiac stimulatory activity at the injection site. This alternative is a non-stem cell non-viral approach; however, more preclinical studies are required using a minimally invasive delivery system of cells to the myocardium (e.g., using a venous catheter without the need for thoracotomy or arterial access) [7].

Somatic reprogramming approaches. This approach includes overexpression of the gene that encodes the human embryonic transcription factor, TBX18, in ventricular cardiomyocytes, thereby inducing the conversion of cardiomyocytes into SAN cells, which resemble endogenous cells of the SAN11 pacemaker [28]. They possessed all the characteristics that are inherent in natural pacemakers, automatic and cyclic generation of action potentials, which are transmitted to the atrial and ventricular cardiomyocytes and induce their electrical excitation and mechanical contraction [29].

With this approach, the entire gene expression program changes, thereby modifying the physiological and morphological properties of cells. The induced SAN cells had many of the phenotypic and functional characteristics of native SAN11 cells, which is a beneficial promising trait [12]. Moreover, somatic reprogramming *in vivo* with TBX18 created a biological pacemaker rhythm in guinea pig hearts that did not only originate from the injection site but also responded to catecholamines [13]. The reprogrammed pacemakers followed the natural daily cycle of heart rate increase and decrease, and increased heart rate during exercise [10].

Chronology of biological pacemakers. Predicting the duration for biological pacemakers to be introduced and make a significant impact on clinical practice is difficult. The development timeline for the implantable cardioverter-defibrillator provides a useful reference point for this. The original concept, created by the cardiologist Michel Mirowski in the mid-1960s, was first presented in 1970. The idea was not recognized by cardiologists [30].

The first clinical use of an implantable cardioverter-defibrillator was reported 10 years later. Patients with recurrent cardiac arrest episodes despite conventional therapy were selected. The implantation was performed through open thoracotomy in 1980 by the cardiologist, Michel Mirowski, in the operating room of the Johns Hopkins hospital [31]. The device turned out to be quite effective.

Over the next three decades, cardioverter-defibrillator implantation became completely percutaneous. Every year, a cardioverter-defibrillator is prophylactically implanted in hundreds of thousands of patients [32]. Biological pacemakers remain at the preclinical stage at the moment. As with the implantable cardioverter-defibrillator, this technology has not been recognized by the cardiological community [33]. Only time will tell if the biological pacemaker succeeds in clinical practice and, if so, the extent of the effect will be significant in reality.

Conclusion. Currently, one of the methods for treating conduction system disorders is the use of ECPs. If successfully tested, biological pacemakers can provide a therapeutic alternative to modern ECPs [34]. The first approach to a biological pacemaker creation was the expression of β_2-adrenergic receptors in cardiomyocytes to enhance the pacemaker activity or create a *de novo* pacemaker. Therefore, the genes encoding β_2-adrenergic receptors were injected into the right atrial cardiomyocytes in mice and pigs using an adenoviral vector. In both cases, the basal heart rate increased by 40%–50%; however, creating a new focus of pacemaker activity in the myocardial tissue is impossible.

Another option for increasing the heart rate was using a dominant-negative design to reduce the incoming rectifying potassium current, which shifts the resting potential to a more positive side, thereby increasing the heart rate. However, the expansion of the action potential turned out to be a big problem in this case, which could potentially lead to a prolonged *QT* interval and corresponding proarrhythmic effect.

HCN2 gene transfection into the cardiomyocytes using adenovirus turned out to be one of the effective options for creating a biological pacemaker. Recent studies revealed the possibility of transplantation into the myocardium of pacemaker-like cardiomyocytes obtained from embryonic stem cells. These cells express the If-current and rhythmically contract.

Therefore, in the future, real possibilities for creating biological pacemakers by myocardial cell transfection with HCN genes are possible, as well as by genetically modified human mesenchymal stem cells. Each of the approaches (gene, cellular, hybrid cell, and somatic reprogramming) has its advantages and disadvantages, which predisposes to their further study and improvement to introduce a biological pacemaker into clinical practice.

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