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Stem cell–based biological pacemakers from proof of principle to therapy: a review

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Abstract

Electronic pacemakers are the standard therapy for bradycardia-related symptoms but have shortcomings. Over the past 15 years, experimental evidence has demonstrated that gene and cell-based therapies can create a biological pacemaker. Recently, physiologically acceptable rates have been reported with an adenovirus-based approach. However, adenovirus-based protein expression does not last more than 4 weeks, which limits its clinical applicability. Cell-based platforms are potential candidates for longer expression. Currently there are two cell-based approaches being tested: (i) mesenchymal stem cells used as a suitcase for delivering pacemaker genes and (ii) pluripotent stem cells differentiated down a cardiac lineage with endogenous pacemaker activity. This review examines the current achievements in engineering a biological pacemaker, defines the patient population for whom this device would be useful and identifies the challenges still ahead before cell therapy can replace current electronic devices.

Key Words: *arrhythmia therapy, cell therapy, gene therapy, pacemaker*

Introduction

Stem cell–based therapy has gained increasing attention over the past 10 years, with most efforts focusing on cardiac repair after myocardial infarction. A 2012 review of 50 studies including 2625 patients with ischemic heart disease has demonstrated a significant long-term improvement in cardiac parameters (left ventricular ejection fraction, infarct size, end diastolic diameter and end systolic diameter) and a possible decrease of the cardiac mortality in patients treated with stem cells derived from bone marrow (1). Alternatively, stem cell–based therapy for arrhythmias or conduction disorders is still in its infancy and has never been tested in humans. Several studies performed *in vitro* and in large animal models have provided proof of concept that both gene-based and cell-based therapies are effective platforms to re-create a biological pacemaker. However, none of these strategies has fulfilled the high safety and quality requirements needed for clinical translation. In this review, we focus on stem cell–based biological pacemaker engineering from proof of principle to therapy. To better address the field, five questions are considered: (i) Why is a bio-engineered pacemaker needed? (ii) What is a biological pacemaker? (iii) What lessons have we learned from gene-based biological pacemaker engineering? (iv) Which stem cells can be used? and (v) What requirements must a biological pacemaker fulfill to compete successfully with its electronic counterpart in human randomized clinical trials?

Why is a biological pacemaker needed?

Electronic pacemakers are currently the standard therapy for symptomatic bradycardia-related symptoms or in the presence of heart failure associated with wide QRS and severe left ventricular dysfunction (2). In the 1950s, electronic pacemakers were cumbersome external devices associated with high complication rates. Device miniaturization, transvenous insertion, demand rather than fixed rate function and battery-life improvement were followed by widespread use of the electronic pacemakers for symptomatic high-degree atrio-ventricular block (AVB) in the late 1960s. Despite the formidable progresses achieved in electronic pacemaker engineering and implantation techniques, there are still shortcomings:

(i) Electronic pacemakers per se have no physiological autonomic responsiveness, leading to

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a lack of rate adaptability during stress, emotion or exercise. Rate-adaptive pacemakers have improved the exercise tolerance of patients but are not a substitute for autonomic responsiveness.

(ii) There is a need for regular pacemaker unit testing and replacement.

(iii) Potentially lethal complications can occur during both the perioperative period (pneumothorax, hemothorax, hemopericardium) and after (pacemaker-related infective endocarditis, lead fracture imposing a lead replacement). Right ventricular apical pacing can lead to pacing induced cardiomyopathy (3). Septal and infundibular approaches are promising, but the long-term effects are still unknown.

(iv) Electromagnetic interferences with pacemakers in medical and nonmedical environment have been reported (4).

(v) There is no ideal pacemaker for children. Thus, the same-size unit designed for adults is implanted. In addition, a surgical procedure for epicardial lead placement can be required for low-weight children. This procedure is painful and associated with a higher risk of lead failure (5).

The primary biological pacemaker: the sino-atrial node

The rhythm of the human heart resides in the cardiac myocytes. This means that all the channels and transporters necessary to initiate and sustain pacemaker activity are resident in the heart. The autonomic nervous system modulates heart rate but does not initiate it. During the 80-year lifetime of a human being, the heart beats roughly 3.5 billion times. In normal conditions, most of these beats are initiated in a highly specialized and heterogeneous structure called the sino-atrial node (SAN) (6). The electrical impulse is then transmitted to the atrias and from them to the ventricles through the atrio-ventricular node (AVN). Conduction through the AVN is slow, which allows atrial contraction to help in filling the ventricles, thus optimizing cardiac output.

The origin of the heartbeat has been one of the most exciting cardiac fields of research for more than a century. The anatomic description of the SAN by Keith and Flack (7) in 1907 and the application of the Hodgkin and Huxley model from the squid axon to the cardiomyocyte roughly 50 years later (8) have been critical steps toward understanding pacemaker activity and conduction of the cardiac action potential. In the early 1980s, the "outward potassium conductance decay" theory was replaced by the view that an inward funny current I_f (also called the pacemaker current) was activated during pacemaker depolarization (phase 4) of the SAN action potential (9,10). Nevertheless, this I_f-focused theory is widely debated (11). Experimental and computational evidence (12) have stressed the importance of other currents such as the late or transient calcium current (ICaT or ICaL, respectively) (13), the persistent tetrodotoxin sensitive sodium current (14), the rapidly delayed potassium current (15), and the Na/K pump (16). The development of submembrane calcium imaging performed simultaneously with the patch clamp technique (17) led some authors to hypothesize that the pacemaking process results from a complex interplay between both the currents generated by the membrane channels ("voltage clock") and the calcium homeostasis ("calcium clock") (18).

Importantly, when the SAN or the AVN fail, the heart does not (always) stop. It is usually driven by a secondary biological pacemaker, the rate of which varies depending of its location: an impulse originating from the ventricles will be slower than one coming from the atria or the AVN. This is primarily determined by the distribution and the biophysical properties of the inward current I_f and the opposing outward background current I_{K1}. The activation curve of I_f is negatively shifted in the ventricles (19), whereas I_{K1} current is larger compared with the AVN, leading to a more negative maximum diastolic potential, less net inward current and a reduced or absent pacemaker depolarization as one proceeds distally in the ventricular conducting pathway (20). One requirement for biological pacing (whether native or induced) is the existence of net inward current. The smaller the inward currents or the larger the outward currents are, the slower the spontaneous rate is.

The gene-based biological pacemaker: a built road

The gene-based biological pacemaker provided proof of concept as well as *in vivo* evidence that a biological pacemaker was feasible. The three initial approaches consisted of (i) overexpression of ß-adrenergic receptors (21), (ii) down-regulation of the outward, hyperpolarizing current I_{K1} (22) and (iii) overexpression of inward depolarizing current I_f (23).

Edelberg *et al.* used a healthy pig model and atrial injection to overexpress the ß2-adrenergic receptor. This increased sinus rate by 50% (21). This strategy enhanced the risk of worsening supra-ventricular arrhythmias (particularly in the setting of sick sinus syndrome in which atrial bradycardia and atrial tachycardia coexist) and was *a priori* not pursued because it required a functional native biological pacemaker as the starting point.

Miake *et al.* were the first to use ion channels as a biopacemaker target. They reduced the outward