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Review

New Approaches to Biological Pacemakers: Links to Sinoatrial Node Development

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Irreversible degeneration of the cardiac conduction system is a common disease that can cause activity intolerance, fainting, and death. While electronic pacemakers provide effective treatment, alternative approaches are needed when long-term indwelling hardware is undesirable. Biological pacemakers comprise electrically active cells that functionally integrate with the heart. Recent findings on cardiac pacemaker cells (PCs) within the sinoatrial node (SAN), along with developments in stem cell technology, have opened a new era in biological pacing. Recent experiments that have derived PC-like cells from non-PCs have brought the field closer than ever before to biological pacemakers that can faithfully recapitulate SAN activity. In this review, I discuss these approaches in the context of SAN biology and address the potential for clinical translation.

Cardiac Conduction System Disease and the Need for Biological Pacemakers

Cardiac electrical impulses originate in the SAN, a 2–3-cm-long comma-shaped structure at the junction of the superior vena cava and right atrium [1]. During each heartbeat, the impulse generated in the SAN is transmitted to the neighboring right atrial myocardium [2]. The ensuing wave of depolarization travels throughout the heart and the rest of the cardiac conduction system in a coordinated fashion, triggering sequential contraction of the atria and ventricles. Over an average human lifespan, this sequence is executed over 2 billion times without a major interruption – an extraordinary output that reflects the robustness of cardiac automaticity and impulse transmission. However, under a variety of common pathological conditions, irreversible degeneration or malformation of the cardiac conduction system results in slow heartbeat, activity intolerance, fainting, or even death. At present, there are no drugs appropriate for long-term use that can safely increase heart rate, so the only available treatment is electronic pacemaker implantation for symptomatic or high-risk patients with conduction system disease. In the USA alone, over 200 000 pacemakers are implanted annually, most commonly for degeneration and malfunction of the SAN [3].

The SAN contains approximately 10 000 specialized PCs (see Glossary) [4]. Several decades of basic research into the electrophysiological mechanisms involved in PCs automaticity resulted in the identification and cloning of the molecular correlates of critical PC ionic currents [5]. With the ability to introduce exogenous genetic material into human cells *in vitro* and *in vivo*, there is an ongoing line of research aiming to transform normally quiescent areas of the heart into **biological pacemakers**. For instance, the introduction of genes encoding ion channels important for PCs, as well as various transplantation approaches of engineered cells, represent important

Trends

Recent progress in the transcriptional regulation of SAN development has guided the derivation of novel biological pacemakers using forward programming of pluripotent cells, or direct somatic reprogramming.

Transduction of embryonic stem (ES) cells with T-box 3 (*Tbx3*) or via selection of leukocyte cell adhesion molecule (ALCAM)+ cells from differentiating ES cells has resulted in cell populations with PC-like gene expression and automaticity.

Tbx18-based somatic reprogramming has altered differentiated ventricular myocytes into a pacemaker-like phenotype by modifying gene expression, cellular morphology, electrical behavior, and improving heart rhythm in a large animal model of heart block.

Multifactor reprogramming methods simultaneously incorporating several sinoatrial node factors have shown promise, but require further development.

Continued progress in understanding early PC differentiation and transcriptional regulation at the genome-wide level is poised to accelerate progress in heart-programming technology.

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advances in the field. These promising studies have been recently reviewed elsewhere and are not described in this article [6–9].

A newer line of investigation is premised on the notion that an ideal biological pacemaker would recapitulate the underlying biological properties of genuine PCs in a more comprehensive fashion. In addition to electrophysiological properties, these would include morphology, autonomic responsiveness, **source–sink matching**, and a dynamic gene expression program unique to PCs. Until fairly recently, the notion of deriving committed PCs from non-PCs would have seemed unfeasible. However, new findings in developmental biology, stem cell biology, and cardiac electrophysiology have now taken PC derivation or reprogramming for cardiac pacing from the purely theoretical realm to active experimental exploration. While recent reviews have explored progress in biological pacing, in this review I contextualize this work by: (i) relating new approaches to biological pacing to recent findings on SAN developmental biology; and (ii) identifying key basic scientific questions that will need to be addressed to move the biological pacing field forward. If existing challenges can be overcome, biological pacemakers created with derived or reprogrammed PCs would constitute a major advance over previous approaches by more faithfully recapitulating the natural pacing mechanisms of the heart.

SAN Structure and Function: Basic Principles

SAN Pacemaker Cells

PCs exhibit **automaticity** and are responsible for generating the initial electrical impulse that drives each heartbeat (Figure 1) [5]. Critical components of the electrical machinery in PCs include the sodium-calcium exchanger [10], voltage-gated calcium channels [11], **hyperpolarization-activated cyclic nucleotide-gated (hcn) ion channels** [12], and spontaneous calcium release from the sarcoplasmic reticulum [13]. Hcn and other channels mediate autonomic responsiveness through their sensitivity to changes in cyclic AMP caused by direct vagal and sympathetic input. Of the Hcn channels, Hcn4 is the most highly expressed in PCs and, when mutated, results in familial sinus **bradycardia** [14,15]. Taken together, the electrophysiological output of PCs is the result of a complex interplay of molecules encoded by numerous genes, including ion channels, receptors, second messengers, and intracellular calcium-handling proteins. Moreover, there is significant debate about the exact mechanisms involved in beat-to-beat regulation of SAN automaticity. Thus, a faithful replication of PC behavior with a biological pacemaker may require reconstitution of the entire PC gene expression program, as opposed to introducing each molecular component individually into quiescent non-PCs.

SAN Architecture

Pacemaker cells within the SAN are surrounded by connective tissue and are heterogeneous in phenotype and morphology. To insure robust source–sink matching, cells at the SAN periphery exhibit tighter electrophysiological coupling to surrounding myocardium than do cells in the interior of the SAN, reflecting heterogeneity in gene expression within the SAN [16]. Loss of PCs through chronic injury, fibrosis, or apoptosis causes **sinus node dysfunction**, as does loss of normal SAN architecture [17]. These findings have important implications for the development of biological pacemakers, since cellular material may have to adopt particular architectural features to achieve robust pacing.

SAN Development and Gene Expression: Recent Discoveries

Developmental Origins of Pacemaker Cells

In an avian model, PC progenitors were shown to arise from right lateral plate mesoderm just posterior to the **heart field** shortly after gastrulation in response to Wnt signaling cues [18]. Based on fate-mapping experiments, these cells migrated to the right inflow region of the heart during mid-development, where they differentiated into PCs (Figure 2A,B).

Glossary

Automaticity: a cellular electrophysiological behavior whereby an electrically active cell fires rhythmic action potentials spontaneously (without depolarization by another cell). This property is critical for PC function and relies on the expression of a particular set of ion channels.

Bradycardia: slow heart beat, defined as less than 60 beats per minute. Symptomatic bradycardia occurs when heart rate is too low to meet physiological demand, causing activity intolerance, lightheadedness, or fainting. If no reversible causes are present, the only safe and effective long-term treatment is permanent pacemaker implantation.

Biological pacemaker: a collection of cells engineered for the purpose of electrically pacing a heart with conduction system disease, thereby preserving heart rhythm. Biological pacemakers can be created by engineering cells *in vitro* for transplantation in the heart, or by delivery of genes into a specific target area of the heart to create a biological pacemaker *in situ*.

Complete heart block: a type of cardiac conduction system disease in which electrical transmission is disrupted at the atrioventricular junction or within the His-Purkinje system, so that SAN impulses activate the atria but do not ultimately trigger ventricular activation. To restore heart rhythm in complete heart block, a biological pacemaker would have to be introduced into the ventricular myocardium.

Embryoid body (EB): an ES-derived structure comprising a heterogeneous group of cells in the process of differentiating from ES cells. Embryoid bodies frequently contain collections of ES-derived cardiomyocytes that can be visualized by spontaneous beating. An EB usually contains cell types from all three germ layers in a disorganized syncytium.

Hyperpolarization-activated cyclic nucleotide-gated (Hcn) ion channels: unlike other voltage-gated ion channels, members of the Hcn family of channels conduct an inward cation current in response to hyperpolarization, creating a diastolic depolarization and rhythmic firing (automaticity). The current carried by Hcn channels is called the 'funny current'.