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Biological Therapies for Cardiac Arrhythmias:

Can Genes and Cells Replace Drugs and Devices?

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Abstract

Cardiac rhythm disorders reflect failures of impulse generation and/or conduction. With the exception of ablation methods that yield selective endocardial destruction, present therapies are nonspecific and/or palliative. Progress in understanding the underlying biology opens up prospects for new alternatives. This article reviews the present state of the art in gene- and cell-based therapies to correct cardiac rhythm disturbances. We begin with the rationale for such approaches, briefly discuss efforts to address aspects of tachyarrhythmia, and review advances in creating a biological pacemaker to cure bradyarrhythmia. Insights gained bring the field closer to a paradigm shift away from devices and drugs, and toward biologics, in the treatment of rhythm disorders.

Keywords

bradycardia; tachycardia; gene therapy; cell therapy

Present therapy for cardiac arrhythmias relies on pharmacology, ablation, or electronic devices, with varying success. Antiarrhythmic drugs have a propensity to provoke new arrhythmias while suppressing others.¹⁻⁴ Catheter ablation selectively destroys endocardial tissue and thus is curative in destroying inborn wiring errors such as accessory tracts, but falls short in treating more complex cases, such as atrial fibrillation (AF) or ventricular tachycardia (VT).^{5,6} Electronic devices can sustain heart rate, or deliver shocks to terminate tachycardias. However, the high cost of devices, and complications such as pulmonary collapse, hemorrhage, bacterial infection, and lead/generator failure⁷ represent limitations of the technology.

Given these shortfalls, we and others have begun to develop biological alternatives (gene therapy, cell therapy, or combinations of the two) or adjuncts to conventional treatment. Work reviewed here indicates that biological therapy can impact on a diverse variety of arrhythmias, at least at the proof-of-principle level. Figure 1 summarizes schematically the regions of the heart, and the rhythm disorders, which have been targeted by various biological therapies with a view to modifying impulse initiation, conduction or repolarization. Bradyarrhythmia is the clinical target which has received the greatest

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attention. Biological pacemaking can be achieved with astonishing simplicity, as will be discussed below. A major challenge in the field, and one touched on throughout the review, is the gap between conceptual innovation and realistic efforts at translation.

Gene Therapy Approach to Control Ventricular Rate in AF

Accepted therapy for AF includes either antiarrhythmic drugs to maintain sinus rhythm or drugs that slow conduction in the atrioventricular (AV) node to control the ventricular rate. Pharmacological therapies to control heart rate during AF have targeted the conduction properties of the AV node by suppressing the calcium current (calcium channel blockers) or by affecting the balance of adrenergic and cholinergic tone (β -blockers and digitalis compounds, respectively). For patients with normal ventricular function, AV nodal suppressing drugs reduce the heart rate by 15% to 30%.⁸ However, the frequent side effects of these drugs include bronchospasm, hypotension, depression, fatigue, and constipation.⁹ When pharmacological interventions are not tolerated or not effective, and the rhythm itself cannot be controlled, the only remaining option is radiofrequency ablation of the AV node and implantation of an electronic pacemaker.

In the first use of gene therapy to treat an arrhythmia, the α subunit of inhibitory G protein ($G\alpha_{i2}$) packaged into adenovirus was infused into the AV nodal artery of pigs,¹⁰ in an effort to control ventricular rate during AF. The idea was to suppress basal adenylate cyclase activity in the AV node, which in turn would suppress calcium channel activity focally. With the aid of nitroglycerin, vascular endothelial growth factor, and sildenafil to enhance vascular permeability,^{11,12} almost 50% of cells in the AV node showed evidence of gene transfer, whereas transduction outside the AV node was minimal. $G\alpha_{i2}$ overexpression caused clear phenotypic consequences: during sinus rhythm, the transgene decreased conduction velocity and increased refractoriness in the AV node. In acute AF, the heart rate was reduced by 20% when compared to controls, and this relative reduction persisted after β -adrenergic stimulation. In a follow-up study,¹³ a constitutively active mutant of $G\alpha_{i2}$ ($G\alpha_{i2}$ -Q205L) was overexpressed in the AV node of animals with persistent AF and tachycardia-induced cardiomyopathy. Whereas the wild type suppressed heart rate only during sleep, the constitutively active mutant decreased the ventricular rate by 15% to 25% in awake as well as inactive animals, resulting in modest recovery of cardiac function.

Direct suppression of L-type calcium current ($I_{Ca,L}$) in the AV node, independent of adrenergic signal transduction, provides an alternative route for rate control in AF. A GTP-binding protein, Gem, which inhibits the trafficking of calcium channel α subunits to the plasma membrane,¹⁴ was used as a genetic suppressor of $I_{Ca,L}$ in the AV node.¹⁵ Adenoviral gene transfer of Gem in the porcine heart slowed conduction velocity in the AV node, and achieved \approx 20% reduction in the ventricular rate during acute AF. Pharmacological blockade of calcium channels for the treatment of AF is fraught with side effects that are attributable to the drug action outside the AV node. Such localized gene delivery may, in principle, circumvent the problem of nonspecificity inherent to drug therapy.

As a cell therapy approach, Bunch et al injected autologous skin fibroblasts into peri-AV nodal region of dogs.¹⁶ This created a focal scar within the conduction tissue, slowing

conduction velocity. When the fibroblasts were pretreated with transforming growth factor- β , conduction velocity was slowed further compared to the nontreated fibroblasts. However, the clinical relevance of this study is diminished by their protocol of injecting the cells in 80 mL of solution in 320 sites, and the method has no apparent advantage to conventional radiofrequency ablation/modification of the AV node.

Use of clinically available catheters and equipment in these studies suggests that the logistics of translating these concepts to human therapy would be relatively straightforward. Major steps between the present state of development and clinical applicability include the use of longer-lasting, less-inflammatory vectors (such as adeno-associated virus), and tests of safety and efficacy in animal models of chronic AF. The impetus for development of such approaches for rate control has waned, however, with the emergence and increasing popularity of potentially curative catheter ablation methods targeted at isolating irritable foci in the pulmonary veins.¹⁷

Genetic Interventions to Influence Ventricular Repolarization

Repolarization abnormalities increase the likelihood of early afterdepolarizations (EADs) and heighten the susceptibility to arrhythmogenesis.¹⁸ The concept of using somatic gene transfer to alter repolarization was pioneered by Johns et al,¹⁹ who overexpressed K^+ channels in cardiac myocytes and thereby abbreviated action potential (AP) duration (APD). The idea was subsequently applied in vitro to a disease model (cardiomyocytes from pacing tachycardia heart failure).²⁰ In Long QT syndrome, the prolonged ventricular repolarization and its clinically identified, specific ion channel genes provide rational targets for gene therapy. In this regard, overexpression of HERG, the primary gene encoding the rapid component of delayed rectifier K^+ current (I_{Kr}), accelerated repolarization, increased refractoriness, diminished beat-to-beat APD variability, and reduced the occurrence of EADs by more than 4-fold in rabbit ventricular myocytes.²¹ By contrast, when a slow component of delayed rectifier current (I_{Ks}) was augmented by wild-type KCNE1 overexpression (which encodes minK, an ancillary subunit of I_{Ks}) in guinea pig ventricular myocardium, no phenotypic change was observed.²² The mechanism of HERG and the function of KCNE1 channels in repolarization was further examined with their respective dominant-negative mutant channel proteins. HERG-G628S overexpression decreased refractoriness, increased beat-to-beat AP variability, increased EAD occurrence, but without significantly delaying repolarization. In contrast, KCNE1-D76N suppressed I_{Ks} and markedly slowed repolarization, leading to frequent EADs and QT prolongation on the ECG. The drastic undermining of repolarization by KCNE1-D76N is in contrast with the HERG mutant, which facilitated the genesis and propagation of premature beats without prolonging repolarization.^{21,22} These in vitro studies have recently been extended to intact large-animal studies: molecular ablation of ventricular arrhythmia in vivo was achieved by a focal overexpression of HERG-G628S mutant in the infarct scar border area of a porcine model of VT.²³

Calcium-independent transient outward current (I_{to1}) is another important component of membrane repolarizing currents. Using nonviral as well as viral approaches, the significance of I_{to1} during early repolarization was investigated. Guinea pig cardiomyocytes, which

normally lack I_{to1} , were fused with CHO cells overexpressing Kv4.3. This cell fusion instantly reconstituted Kv4.3 channel proteins to the membrane of myocytes. The resultant heterokaryons demonstrated acceleration of early repolarization velocity and abbreviated the APD.²⁴ Cardiomyocytes undergo extensive time-dependent changes of their membrane properties.²⁵ The use of cell fusion avoided culture-related complications by introducing preformed membrane proteins to native cells. In addition, adenoviral delivery of Kv4.3 to guinea pig myocardium complemented the cell fusion approach; ecdysone-mediated, graded expression of Kv4.3 demonstrated that the plateau potential of guinea pig ventricular myocytes is progressively suppressed with increasing density of I_{to1} . When a dominant-negative form of Kv4.3 channels (W362F) was expressed in rat ventricular myocardium, the results mirrored those observed with wild-type Kv4.3; W362F suppressed peak I_{to1} , elevated plateau potential, prolonged APD, and increased the QT interval by 30% on ECG.²⁶ Genetic suppression of I_{to1} may conceivably be useful in the treatment of Brugada syndrome, where haploinsufficiency of Na^+ channels causes a tug-of-war with I_{to1} to render repolarization dangerously unstable.²⁷

A transgenic mouse model of the long QT syndrome has been used to test the feasibility of gene transfer to correct the phenotype. Wild-type Kv1.5 was delivered in either adenoviral²⁸ or adeno-associated viral vector²⁹ into the left ventricular base of transgenic mice bearing a dominant negative of Kv1.1 channel. In both studies, Kv1.5 overexpression and the resultant I_{Ks} led to shortened APD and QT interval. However, the sham-operated animals exhibited a negligible incidence of VT, and the effect of therapy on arrhythmia prevention could not be tested.

Taken together, these studies demonstrate the feasibility of generating a disease-specific animal model by somatic gene transfer. On the flip side, the studies, although mostly limited to proof-of-concept, do highlight the potential utility of gene transfer in blunting ventricular arrhythmogenesis by overexpressing K^+ channels (to accelerate repolarization), or blocking them selectively²³ to achieve focal class III³⁰ antiarrhythmic effects.

Biological Pacemaker: Rationale and Clinical Target

The sinoatrial (SA) node initiates the heartbeat, sustains the circulation and sets the rate and rhythm of cardiac contraction.³¹ Its spontaneous pacemaker activity excites the myocardium, setting the stage for orchestration of cardiac excitation and contraction. Loss of pacemaker activity from the SA node, therefore, results in circulatory collapse, necessitating the implantation of an electronic pacemaker.³² Though effective as a palliative measure, such devices are expensive³³ and associated with risks such as device or lead failure, and chronic infection.

Although the notion of a biological pacemaker replacing an electronic pacemaker is attractive, it must be recognized that, despite their limitations, electronic pacemakers generally work quite well; indeed, they have withstood the test of time for more than 50 years. One approach would target patients with “soft” indications for a pacemaker, such as sick sinus syndrome. If a biological pacemaker fails over time in such patients, life is less likely at risk than it would be in complete heart block, for example. On the other hand,

perhaps it is neither necessary nor wise to aspire to create a permanent, durable alternative to electronic pacemakers; instead, temporary, “niche” applications may turn out to be more clinically compelling. Consider patients that desperately need an electronic pacemaker, but have strong contraindications to indwelling hardware. Biological pacemakers may be ideally suited for the management of pacemaker-dependent bradycardia complicated by bacterial infection of the device, in which the contraindication to re-infectable metal/plastic leads creates a unique advantage for short-term biological pacing.³⁴ When a patient presents with an infected system requiring revision and antibiotics, the biological pacemaker could be used to create a hardware-free interval sufficient to effectively treat the bacterial infection before reimplanting a permanent device. Such “bridge-to-device” applications constitute a logical niche for the initial clinical development of biological pacemakers, given the proven efficacy of electronic devices for bradycardia not complicated by an infectious substrate.

An initial gene therapy approach to enhance chronotropy used overexpression of human β_2 -adrenergic receptor. Edelberg et al reported that heart rate could be increased by 40% in mouse right atria³⁵ and by 50% in porcine atria on exogenous β_2 -adrenergic receptor overexpression.³⁶ These studies demonstrated the ability to upregulate existing sinus rate without introducing rhythm change, but the approach was not designed to convert tissue from quiescent to automatic in terms of electrogenesis.

The first biological approach to create a de novo cardiac pacemaker rendered ventricular myocardium spontaneously active.³⁷ The strategy posits that normally quiescent ventricular myocardium has an innate ability to pace, but is suppressed from pacing by “electric brakes” that stabilize the resting membrane potential. Among the inhibitory currents that are logical candidates to serve as electric brakes, the inward rectifier potassium current (I_{K1}) is notable for its intense expression in nonpacing atria and ventricle and its absence in nodal pacemaker cells.³⁸ I_{K1} , encoded by the Kir2 gene family,³⁹ imparts and stabilizes a strongly negative resting potential and thereby suppresses excitability. We explored the possibility that inhibition of I_{K1} by overexpressing a Kir2.1 dominant-negative channel (Kir2.1AAA)⁴⁰ in the ventricle would cripple I_{K1} and suffice to produce spontaneous, rhythmic electric activity. Kir2.1AAA and GFP were packaged into a bicistronic adenoviral vector and injected into the coronary circulation of guinea pigs during transient cross-clamp of the great vessels.³⁷ Nontransduced left ventricular myocytes isolated from Kir2.1AAA-injected animals, as well as green cells from control hearts, exhibited no spontaneous activity, but fired single APs in response to depolarizing external stimuli (Figure 2A). In contrast, Kir2.1AAA-transduced myocytes exhibited either (1) a stable resting potential from which prolonged APs could be elicited by external stimuli or (2) spontaneous activity (Figure 2B). The spontaneous activity, which was seen in all cells in which I_{K1} was suppressed below 0.4 pA/pF, resembles that of genuine pacemaker cells: the maximum diastolic potential is relatively depolarized, with repetitive, regular and incessant electric activity initiated by gradual “phase 4” depolarization and a slow upstroke.^{31,41} More importantly, premature beats of ventricular origin could be distinguished by their broad amplitude, and “marched through” to a beat independent of, and more rapid than, that of the physiological sinus pacemaker (Figure 2D). In these proof-of-concept experiments, ectopic beats, arising from foci of induced biological pacemakers, caused the entire heart to be paced from the ventricle. The focally intense expression was achieved by chance rather than by design.

A full-fledged biological pacemaker would sustain a wide range of frequencies responding to physiological needs of sleep, exercise, fever, etc. under neurohumoral chronotropic control. β -Adrenergic receptor activation-mediated sympathetic stimulation has a positive chronotropic effect on the native pacemaker. Acetylcholine and vagal stimulation, in contrast, negatively affect chronotropy. I_f and $I_{Ca,L}$ are examples of ionic currents regulated by this neurohumoral influence, accelerating/decelerating the rate of phase 4 depolarization and the AP upstroke.^{42,43} Figure 2E shows that biological pacemakers engineered by suppression of I_{K1} respond to the β -adrenergic agonist isoproterenol with an increase in rate. These effects presumably reflect upregulation of L-type Ca^{2+} channels. Even finer tuning of autonomic responsiveness may be possible when HCN channels are overexpressed (see below), as these channels contain cAMP-response elements.⁴⁴

Thus, specific suppression of Kir2 channels sufficed to unleash pacemaker activity in ventricular myocytes. The conclusions of Miake et al³⁷ have recently been confirmed and extended by Sekar et al,⁴⁵ who mapped the origin of focal pacemaker activity in monolayers of rat ventricular myocytes to islands of suppressed I_{K1} (Figure 3). The crucial factor for pacing was the absence of the strongly polarizing I_{K1} , rather than overexpression of exogenous ionic current(s) that are absent or sparse in ventricular cardiomyocytes (although such currents may play an important modulatory role in genuine pacemaker cells,⁴⁶ and can suffice to induce pacing if overexpressed in atria and ventricles [next section]).

In a follow-up study,⁴⁷ Miake et al found that Kir2.1AAA overexpression not only destabilized the resting membrane potential but also led to prolongation of APD. Conditions that prolong APD could potentiate EADs, thereby predisposing to ventricular arrhythmias.^{48,49} The need for potent suppression of I_{K1} to unleash pacemaker activity without prolonging APs represents a drawback of the Kir2.1AAA strategy. In this regard, the APD prolonging “side effect” of I_{K1} suppression may be obviated by intense focal expression of the Kir2.1AAA transgene, or by coexpression of HERG, in a variation of the previously described dual gene therapy approach.⁵⁰ HERG overexpression would keep the APD short, but not interfere with the resting membrane potential destabilizing effect of Kir2.1AAA at -80 mV.

Gene Therapies to Create Biological Pacemaking in Myocardium

HCN (hyperpolarization-activated, cyclic nucleotide-gated) cation channels have been argued to play an important role in cardiac pace-making.⁵¹ Rather than inhibiting K^+ channels, Rosen and colleagues overexpressed HCN2 channels to elicit ectopic pacemaker activity in a canine model.^{52,53} Although HCN4 is the major isoform in nodal tissues,⁵¹ Qu et al chose to use HCN2,⁵³ which is the dominant isoform in ventricular myocytes, with cAMP responsiveness comparable to that of HCN4. On suppressing sinus rhythm by vagal stimulation, the authors observed spontaneous rhythms from dogs injected with adenovirus encoding HCN2 into left atrium. When the same construct was injected into the left bundle branch, they observed left ventricle-originated ectopic beats at a rate exceeding that of controls.⁵² Similarly, an HCN1 channel with a modified gating property has been used to demonstrate ectopic pacing when the biological was focally injected into left atrium in a

SA node-ablated porcine model.⁵⁴ These works were performed in large-animal models, bringing the biological pacemaker closer to clinical application.

As an alternative to wild-type HCN channels, we have created synthetic pacemaker channels by site-directed mutagenesis on a canonical voltage-dependent K channel backbone.⁵⁵ Such channels activate on hyperpolarization and have nonspecific cation selectivity; moreover, the fact that they do not multimerize with endogenous HCN channels may be advantageous. However, the engineered protein contains extracellularly facing epitopes which may be immunogenic, limiting long-term expression and translational potential.

Gene–Cell Hybrid Approach to Biological Pacemakers

A general hurdle of gene therapies described above lies in the use of viral delivery vehicles. Routine (ie, nongutted) adenoviral vectors elicit acute and prolonged host immune responses, restricting their utility for long-term therapy.⁵⁶ Adeno-associated virus and lentivirus suffer from small packaging capacity and random insertion of transgenes in the host genome, respectively.⁵⁷ Used as an alternative delivery vehicle with genetic transduction by nonviral methods, cells could circumvent at least some of the obstacles faced by somatic gene transfer approaches. Human mesenchymal stem cells (hMSCs) have attracted attention as they can be obtained in a large quantities and are claimed to be immunoprivileged.^{58,59} Mesenchymal stem cells have been used as vehicles to deliver a mouse HCN2 channel. The idea was to impart I_f to myocardium through electrotonic coupling of cardiomyocytes with hMSCs that overexpress HCN2 channels through routine plasmid transfection.⁶⁰ Subepicardial injection of HCN2-overexpressing hMSCs into the canine left ventricular wall induced spontaneous rhythms of left-sided origin on chronic AV block in 5 of 6 animals. The pacemaking activity required 10 to 12 days to become stable, then persisted for at least 6 weeks.⁶¹ A key prerequisite to this approach is a high degree of gap-junctional coupling between the donor hMSCs and the host myocardium. Such gap junctional coupling, however, may not be stable over time, a problem which must be considered in any pacemaker applications. Indeed, major forms of heart disease that carry increased arrhythmic risk often involve gap junction remodeling and decreased cell–cell coupling.⁶² In addition, the donor cells may not stay at the specific site of injection as intended; when quantum dot-labeled hMSCs were injected into a rat left ventricular free wall, only 30% of the total injected hMSCs were found to be in the whole heart at 24 hours.⁶³ Migration of injected cells could affect long-term consistency of the biological pacemaker function or present a substrate for arrhythmia.⁶⁴

A fusion between host myocardial cells and the donor cells, creating heterokaryons, provides a potential solution to uncontrolled migration of injected cells, and obviates the need for gap junctional coupling to deliver the excitatory signal to the host myocardium. We used syngeneic fibroblasts engineered to overexpress a pacemaker ion channel, HCN1, and induced cell–cell fusion using polyethylene glycol.⁶⁵ The polyethylene glycol-induced membrane fusion events have served as a model system to create mouse and human hybridomas,⁶⁶ to study eukaryotic cell–cell fusion events,⁶⁷ and to deliver outward K^+ currents into myocytes.²⁴ Focal injection of HCN1-overexpressing fibroblasts suspended in 50% polyethylene glycol into the apex of guinea pig hearts yielded *in vivo* cell fusion.

Heterokaryons of myocytes and HCN1-fibroblasts exhibited spontaneously oscillating APs with slow phase 4 depolarization (Figure 4B, bottom). In vivo biological pacemaker activities were demonstrated by electrocardiography as early as 1 day after cell injection and were stable for at least 2 weeks (Figure 4C).

In contrast to the direct cell injection approaches, the fusion technology implants the biological pacemakers to the site of injection by somatic cell fusion, creating biological pacing at a specific site by design. Previous studies suggest that the in vivo fusion-induced heterokaryons can maintain the nuclei from each fusion partner separately and stably for at least several months.⁶⁸⁻⁷⁰ To develop this technology for clinical application, future steps would logically include large-animal testing to examine toxicity and efficacy.

Use of Stem Cells as Stand-Alone Biological Pacemakers

Human embryonic stem cells (hESCs) are among the most versatile agents for the development of cell-based therapies; they are pluripotent, clonogenic, and self-propagating.⁷¹ Human ESCs can differentiate in vitro, forming embryoid bodies (EBs) composed of derivatives of all 3 embryonic germ layers. Human EBs (hEBs) are inherently heterogeneous, and some EBs begin to contract spontaneously, containing cardiac myocytes that exhibit phase 4 depolarization.⁷²⁻⁷⁵ We and others reasoned that the hEBs could be used as stand-alone biological pacemakers with no genetic modification.^{76,77} The first step in this strategy is to verify that hEBs can electronically couple with the host myocardium.

An in vitro transplantation model was developed in which a single hESC-derived, spontaneously beating hEB ($\approx 500 \mu\text{m}$ in diameter) was transplanted on top of a quiescent monolayer of neonatal rat ventricular myocytes (NRVMs) serving as the recipient. Coculturing a spontaneously beating hEB on a monolayer of quiescent NRVMs demonstrated expression of gap junction at the hEB-NRVM boundary and synchronous rhythmic contractions of the hEB on the NRVM monolayer. Furthermore, multielectrode and optical mapping recordings revealed that the spontaneous APs were initiated from the hEB and propagated to the NRVMs.

Spontaneously beating hEBs were then injected into the left ventricular anterior wall of a guinea pig in vivo. On cryoablating the sinus rhythm of an animal, ex vivo optical mapping displayed spontaneous APs from the left ventricular epicardial surface, which were not present in control hearts (uninjected or saline-injected). Because the hESC-derived biological pacemakers beat on their own before the tissue transplantation, they could prove to be a quick way to generate a spontaneous rhythm in the bradycardic setting. However, the immune response from the host remains a major hurdle in reducing this technology to clinic, a limitation which may eventually be overcome by induced pluripotent stem cell technologies.⁷⁸ The potential for tumorigenesis, however, plagues both hESC and induced pluripotent stem approaches. Moreover, it is possible, if not likely, that ESC- and induced pluripotent stem-derived cardiac implants would lose their pacing ability as they engraft and mature in vivo.

In recent years, the view that the number of cardiomyocytes we are born with stays unchanged has been challenged and overturned.⁷⁹⁻⁸¹ This shift in paradigm was supported by the discovery of cardiac stem cells. Human adult cardiac stem cells readily form self-aggregating, 3D multicellular structures named cardiospheres.⁸² Methods that allow retrieval of $>10^6$ human adult cells from a single endomyocardial biopsy specimen were found to yield cardiomyocytes with cardiac specific markers, and some of these cells can differentiate into spontaneously contracting cardiac tissue with innate pacemaker function.⁸³ The use of adult stem cells circumvents complications associated with hESCs including ethical concerns,⁸⁴ immunogenic reactions against the donor cells,⁸⁵ and teratoma formation.⁸⁶ Clinical trials of autologous cardiac stem cells for cardiac regeneration have already begun (eg, CADUCEUS; see <http://www.clinicaltrials.gov> for details). Cardiac stem cells may be viable candidates for stand-alone cell therapy, although the stability of resultant pacemaker function has yet to be established.

Conversion of Nonexcitable Cells to Self-Contained Biological Pacemakers

Equipped with what we have learned from the above studies, we asked if expressing a minimal complement of ion channels would convert a nonexcitable cell into a self-contained, entirely engineered pacemaker cell. We reasoned that a minimal pacing apparatus would require a hyperpolarizing current to produce a negative diastolic potential, a depolarizing current to induce excitability, and a repolarizing current to reprime the system. Inexcitable HEK293 cells were engineered to express (1) an inward rectifier current through Kir2.1 to achieve a polarized membrane potential³⁹ (Figure 5A, left); (2) a repolarizing current provided by human ether-a-go-go channel (HERG, Figure 5A, middle)⁸⁷; and (3) an excitatory current to provide the upstroke of an AP. A Na⁺ channel from bacteria⁸⁸ (NaChBac, Figure 5A, right) was chosen for the excitatory current, taking advantage of its slow gating kinetics. In the HEK293 cells expressing all 3 channel proteins, APs could be generated (n=5/31) on stimulation with a brief depolarizing current (0.3 to 0.7 nA) (Figure 5B). The stimulated APs displayed a large variation in their phenotypes, reflecting the randomness of the amount of cDNA taken up by a cell via routine transfection. At room temperature, the maximum diastolic potentials were -78 ± 7 mV with an APD at 90% repolarization (APD₉₀) value of 475 ± 33 ms (n=5). Our own observations and data from others^{89,90} suggested that HEK293 cells express endogenous voltage-gated outward currents. This allowed us to omit HERG, and include HCN1 channel in our assay to test whether additional expression of I_{HCN1} would suffice to generate spontaneously oscillating APs in HEK293 cells. In addition, the compact cDNA of NaChBac allowed us to construct a tricistronic vector containing all 3 transgenes in one plasmid separated by 2 IRES segments under a CMV promoter. Whole-cell recordings from HEK293 cells expressing CMV-HCN1-NaChBac-Kir2.1 revealed spontaneous APs (Figure 5C). The spontaneous APs displayed a maximum diastolic potential of -81.5 ± 11.8 mV, maximum rate of rise (dV/dt_{max}) of 21.6 ± 8.6 V/sec, APD₉₀ of 660 ± 189 ms, and a rather slow frequency of 3 ± 1 bpm, presumably resulting from the slow gating kinetics of NaChBac channels (n=4/85).⁹¹

Recently, a body of evidence has emphasized the role of spontaneous, local calcium release (LCR) events from sarcoplasmic reticulum in the pacing mechanism of SA nodal cells.^{92,93} These studies revisit earlier observations that intracellular Ca²⁺ oscillators produce

spontaneous inward currents,⁹⁴ and implicate rhythmic LCRs as effectors of normal SA nodal automaticity. Although they may be important physiologically, LCRs are not required for pacing: the work in Figure 5 shows that an inexcitable cell, lacking sarcoplasmic reticulum, can be engineered to pace solely via voltage-dependent mechanisms. Moreover, the cellular work of Miake et al³⁷ was performed in myocytes heavily buffered by internal EGTA, such that LCRs would have been suppressed.

Although conceptually interesting, de novo pacemaker cells are not likely to proceed rapidly to translation. Practical development of biological pacemakers would most logically begin with the straightforward single-transgene strategies (Kir2.1AAA or HCN channels) discussed earlier, as these would seem to be more likely to meet regulatory approval.

Genetic Alterations of Electric Conduction

In arrhythmias such as VT, modifying electric conduction would be a useful principle for treatment. Such is the rationale underlying catheter ablation of zones of slow conduction to suppress VT.⁹⁵ Gene transfer, however, offers greater engineering versatility than endocardial destruction, not to mention the conceptual advantage that the targeted heart tissue is modified rather than destroyed. Gap junctional intercellular communication is an intuitive target to alter electric conduction. To this end, an internal loop mutant gene of connexin 43 was delivered in a lentiviral vector to uncouple electric connection in NRVMs.⁹⁶ Transduced cells displayed delayed calcium transients as well as slowed conduction velocity. These data validate a molecular tool targeting gap junctions as a way to modulate electric conduction.

The myocardial infarct border zone is characterized by depolarized membrane potential with low AP upstroke velocity (V_{\max}) and reduced conduction. To correct the low V_{\max} by gene transfer, a skeletal Na^+ channel gene (SkM1) has been ectopically expressed in the epicardial border zones of a canine infarct model. Adenoviral delivery of SkM1 restored V_{\max} and reduced the incidence of inducible sustained ventricular tachycardia/fibrillation.⁹⁷ A skeletal (SkM1) rather than a cardiac (SCN5A) isoform was chosen to take advantage of the positively shifted voltage-dependent activation kinetics of SkM1, which would permit more channel opening than with Nav1.5 channels at depolarized membrane potentials.⁹⁸ Together with gap-junctional modification, these studies begin to develop a novel toolkit for altering electric conduction by focal gene transfer.

Future Directions

Efforts to create biological alternatives to present antiarrhythmic therapies, particularly biological pacemakers, have led to multiple innovations. Figure 6 summarizes the distinct approaches that have been deployed to initiate automaticity in usually quiescent working myocardium. The genes-only strategy (top row) either overexpresses an excitatory gene, or suppresses I_{K1} , to induce automaticity. The hybrid gene–cell approaches (middle rows) rely on gap-junctional coupling or cell fusion to relay the excitatory effects of an HCN gene. Finally, the use of stem cells (bottom row) relies on the endogenous complement of ion channels in hESC-derived cardiomyocytes to drive the enhanced automaticity.

In addition to new conceptual insights that merit consideration in the further development of biological pacing, much more work will be required on focal delivery of constructs/cells to the myocardium to reduce these concepts to practice. For example, injection of biological agents into the endocardium via an intracardiac injection catheter represents a potentially attractive percutaneous delivery route. Likewise, localized coronary circulation may allow isolated delivery to small regions of the heart (as was achieved with the AV node¹⁰). Highly localized biological delivery will be particularly effective for arrhythmias in which focal modifications of electric properties suffice for effective treatment. Because the amount of exogenous biological material delivered can be correspondingly reduced, potential problems attributable to widespread dissemination may be more readily averted. A singular advantage of focal modification is reversibility: if needed, implanted biological pacemakers can be destroyed by conventional electrophysiological ablation, followed by electronic pacemaker implantation.

Different clinical scenarios may call for different gene delivery vectors. Adenoviral vectors, for example, achieve the peak expression earlier than other vectors albeit in a transient manner. This would be ideal for the temporary pacing needs to treat infected pacemaker devices as discussed earlier. In this regard, the high immunogenicity of these vectors could be circumvented by using helper-dependent adenovirus.⁹⁹ On the other hand, adeno-associated virus or lentivirus vectors would be more suitable for long-term applications.¹⁰⁰

Will biological therapies replace drugs and devices? It is too early to tell. Biological pacemakers seem to have a reasonable chance of eventual success. It is our conjecture that the first application will likely be as an alternative to temporary electronic pacemakers in bradycardic patients with pacemaker infections, but more development work in large-animal models will be required before clinical testing can realistically be contemplated. Likewise, biological approaches targeting conduction or repolarization have shown great promise at various levels of preclinical development, but none is ready for prime time. What is certain is that the more we push the envelope, the more likely it is that we will devise creative alternatives to the present palliative approaches.

Further extensive work will be required to optimize the functional effects of biological therapies, to evaluate the toxicology, to exclude potential tumorigenicity in the case of stem cells, and to establish the long-term stability of therapy (in anything other than a purposely temporary indication). Yet, given the promise, the effort to develop biological alternatives to the present therapies appears more justified than ever.

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Non-standard Abbreviations and Acronyms

AF	atrial fibrillation
AP	action potential
APD	action potential duration
AV	atrioventricular
EAD	early afterdepolarization
HCN	hyperpolarization-activated, cyclic nucleotide-gated
hEB	human embryoid body
hESC	human embryonic stem cell
hMSC	human mesenchymal stem cell
LCR	local calcium release
NRVM	neonatal rat ventricular myocyte
SA	sinoatrial
VT	ventricular tachycardia

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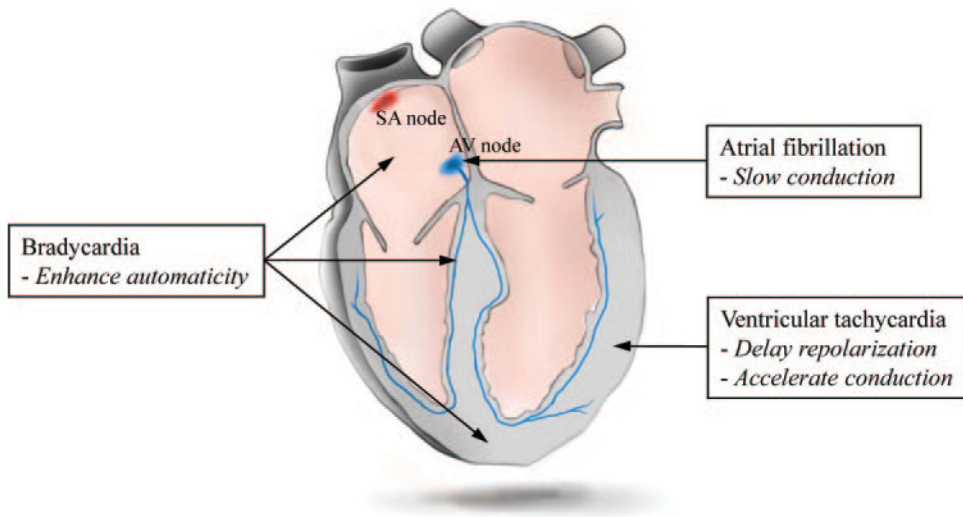


Figure 1. Principles of biological approaches (in italics) recruited to treat major cardiac arrhythmias, and sites within the heart where the various strategies have been tested.

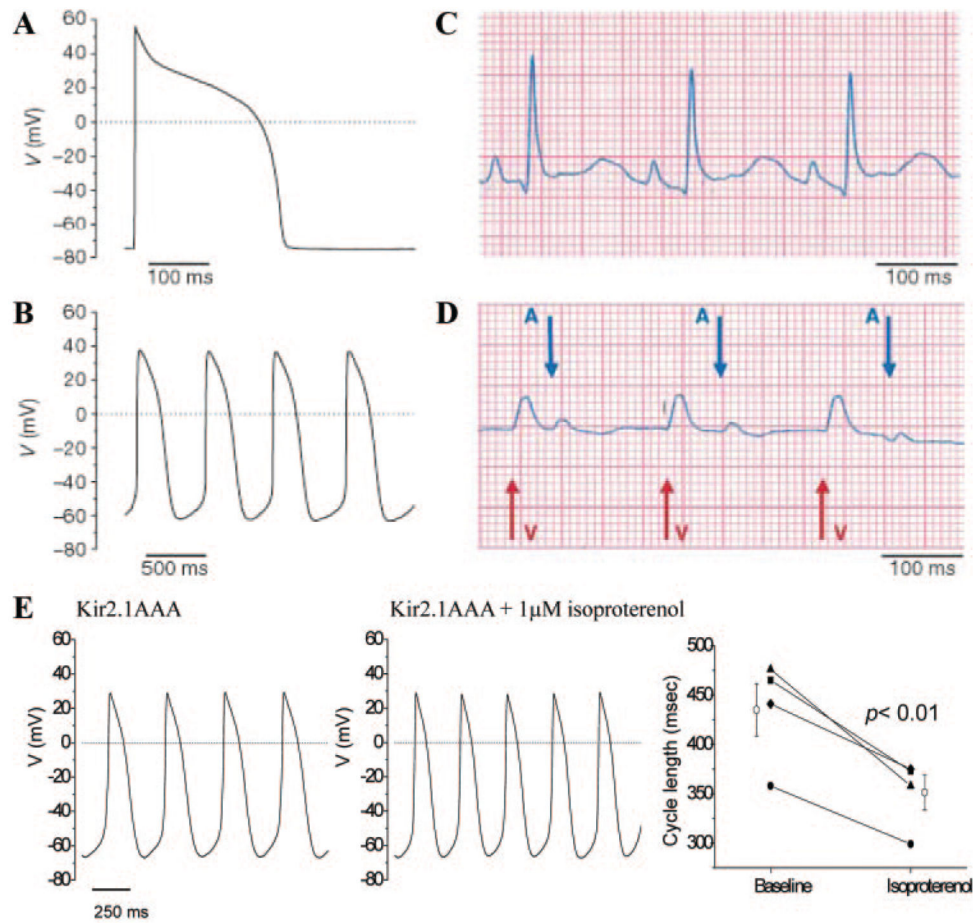


Figure 2. Suppression of Kir2.1 channels unmasks latent pacemaker activity in ventricular cells. A, APs evoked by depolarizing external stimuli in control ventricular myocytes. B, Spontaneous APs in Kir2.1AAA-transduced myocytes with depressed I_{K1} . C, Baseline electrocardiograms in normal sinus rhythm. D, Ventricular rhythms 72 hours after gene transfer of Kir2.1AAA. P waves (A and arrow) and wide QRS complexes (V and arrow) march through to their own rhythm. A through D are reproduced from Miake et al with permission.³⁷ E, Guinea pig ventricular myocytes in which Kir2.1AAA was overexpressed (left) and exposed acutely to 1 μ mol/L isoproterenol (J. Miake, H. B. Nuss, and E.M., unpublished data, 2002).

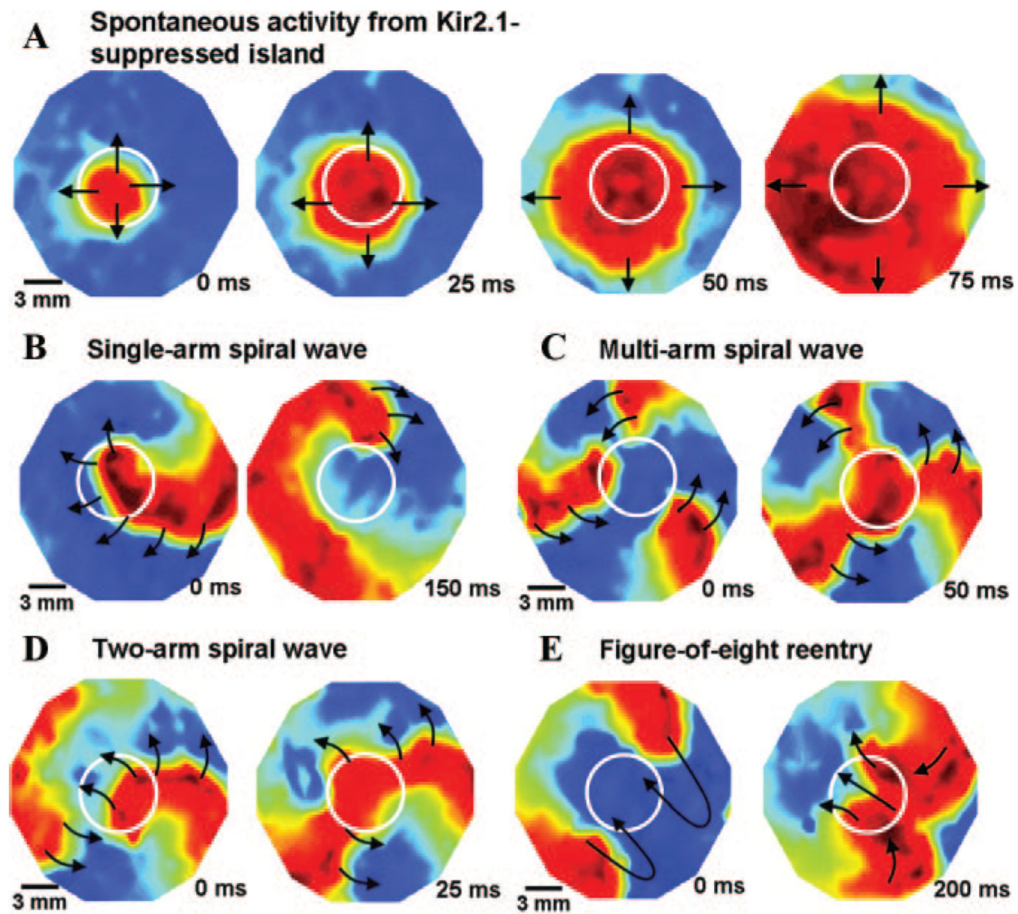


Figure 3. Dynamics of induced reentrant spiral waves in 21-mm-diameter monolayers with a central island of Kir2.1 suppression. The mapping region is 17 mm in diameter. A, Island of Kir2.1-suppressed NRVMs (indicated by white circles) acting as a source of spontaneous activity, as seen by the spread of spontaneous cardiac impulses (shown by black arrows) from an interior region of the island. B, An induced spiral wave anchored momentarily to the region of I_{K1} suppression (shown by white circle). C, Transition of initial spiral wave into a multi-armed spiral wave. D, Transition of initial spiral wave into a 2-armed spiral wave. E, Transition of the 2-armed spiral wave of D into a figure-of-eight reentry that later drifted to the edge of the monolayer and terminated. Reproduced from Sekar et al with permission.⁴⁵

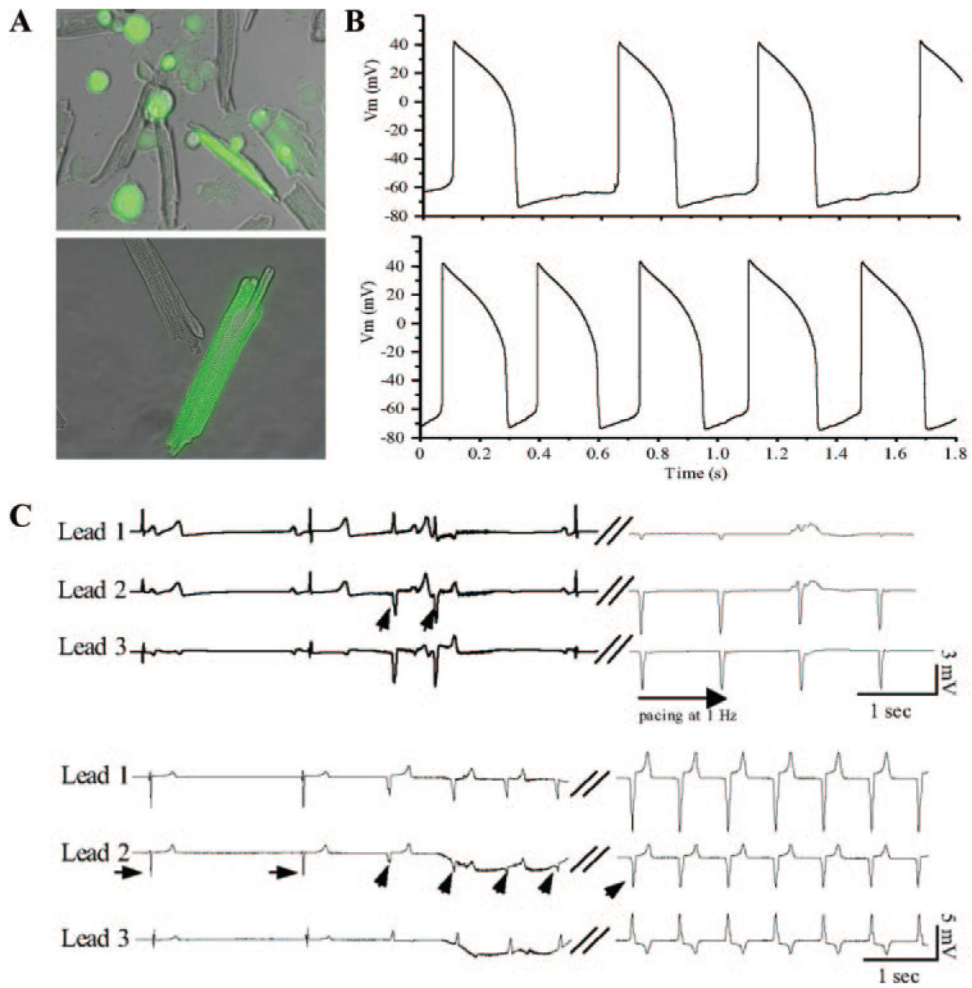


Figure 4. A, In vitro and in vivo fusion of myocytes with HCN1-fibroblasts. GFP-positive heterokaryons after in vitro (top) or in vivo (bottom) fusion of myocytes with HCN1-fibroblasts expressing GFP as a reporter. B, Heterokaryon formed by in vivo fusion of HCN1-fibroblast and a myocyte displays spontaneous AP oscillations in normal Tyrode's (top). Presence of 1 mmol/L isoproterenol in the external solution increased the frequency of spontaneous AP oscillation in the same heterokaryon (bottom). C, ECGs from guinea pig hearts injected with HCN1-fibroblast cells. Top, Ectopic ventricular beats (diagonal arrows) are unleashed on slowing of the heart rate, which share the same polarity and morphology as the electrode-paced ECGs recorded at the site of HCN1-fibroblast injection. Bottom, In another animal, junctional escape rhythms (horizontal arrows) were overtaken by ectopic ventricular beats (diagonal arrows, 16 days after cell injection). Reproduced from Cho et al with permission.⁶⁵

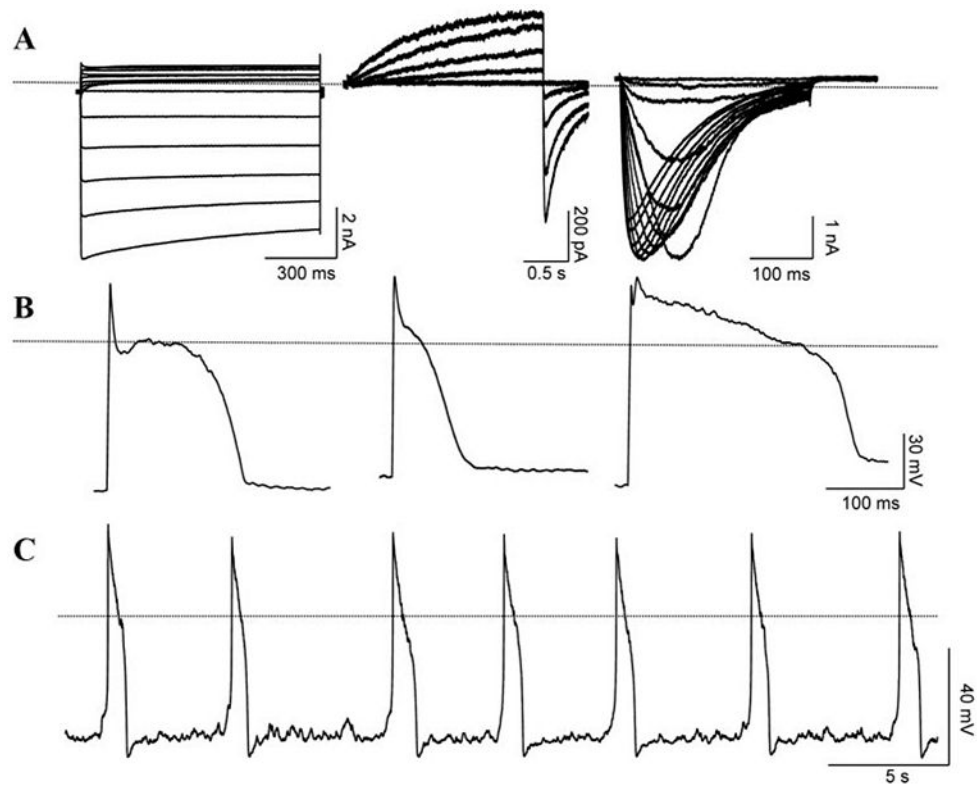


Figure 5. Representative raw recordings from HEK293 cells transfected with the following constructs. A, Voltage-clamp recordings from HEK293 cells transfected with either NaChBac (left), HERG (middle), or Kir2.1 (right). Dotted line indicates zero current level. B, APs from 3 different cells during current-clamp recordings. Each cell expressed all 3 channels, NaChBac, HERG, and Kir2.1. Dotted line indicates zero mV potential. C, Spontaneous APs recorded from a representative cell transfected with single plasmid expressing HCN1, NaChBac, and Kir2.1. Adapted from Cho et al.⁹¹

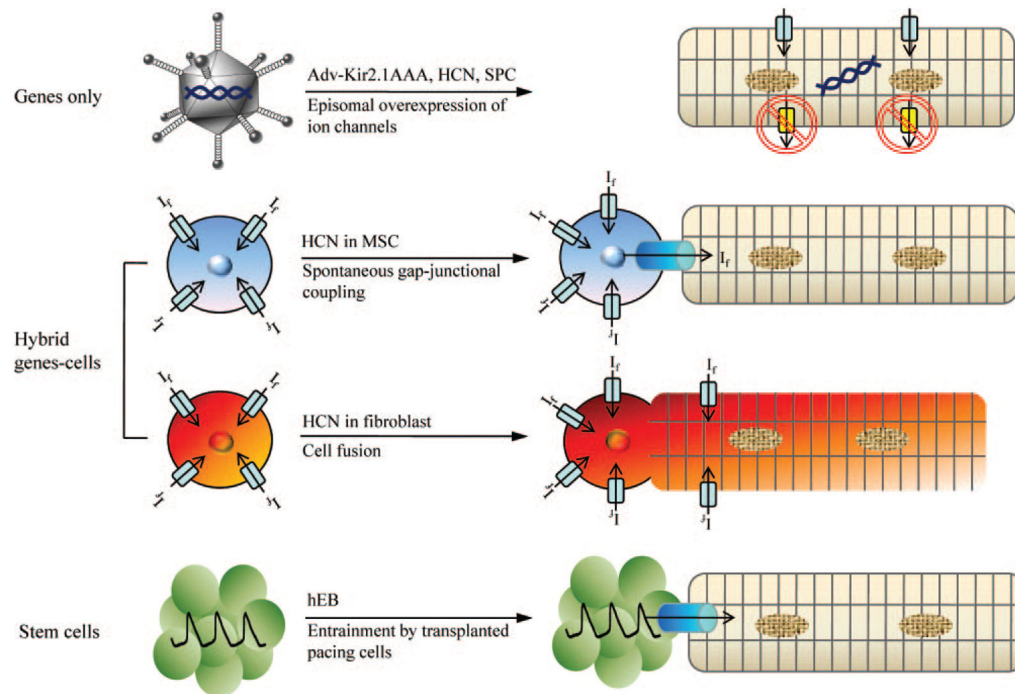


Figure 6.

A summary of different approaches to creating a biological pacemaker. First approach (top row) is a strict gene therapy in which Kir2.1AAA, HCN, or synthetic pacemaker channel genes are overexpressed in myocytes via adenoviral delivery. Kir2.1 dominant negative proteins suppress repolarizing, outward currents whereas pacemaker channels directly contribute to diastolic membrane potential depolarization. Delivering I_f by MSCs requires gap-junctional coupling between myocytes and MSCs (second row). In the cell fusion approach (third row), I_f and the pacemaker activity arise from the HCN channels expressed on the cell membrane of the heterokaryon, without the need for gap-junctional coupling. Spontaneously beating human EBs and cardiospheres transduce their pacemaker activity to cardiomyocytes via electrotonic cell–cell coupling (fourth row).