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# Biologic Pacemaker - Role of Gene and Cell Therapy in Cardiac Arrhythmias

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## 1. Introduction

In mammalian heart, the sino-atrial (SA) node is the pacemaker region, which contains a family of ionic currents that contributes to the pacemaker potential. Using SA nodal cells, experiments have shown that dysrhythmias are easily elicited under conditions involving calcium overload that occur during ischemia and cardiac failure. Clinically these SA nodal dysfunctions cause bradyarrhythmias in general and are associated with syncope but rarely with death. To initiate pacemaker function an inward current (*I<sub>f</sub>*) carried by sodium through a family of channels that are hyperpolarization-activated and cyclic nucleotide-gated (HCN channels) (Biel et al 2002).

Recent advances in molecular and cellular biology, specifically in the areas of stem cell biology and tissue engineering have initiated the development of a new field in molecular biology, regenerative medicine, seeks to develop new biological solutions, using the mobilization of endogenous stem cells or delivery of exogenous cells to replace or modify the function of diseased, absent, or malfunctioning tissue. As far as adult cardiomyocytes have limited regenerative capacity it represents an attractive candidate for these emerging technologies. Therefore, dysfunction of the specialized electrical conduction system may result in inefficient rhythm initiation or impulse conduction leading to significant bradycardia that may require the implantation of a permanent electronic pacemaker. Replacement of the dysfunctional myocardium by implantation of external heart muscle cells is emerging as a novel paradigm for restoration of the myocardial electromechanical properties, but has been significantly limited by the paucity of cell sources for human heart cells and by the relatively limited evidence for functional integration between grafted and host cells. Human embryonic stem cell lines may provide a possible solution for the cell sourcing problem.

Although electronic pacing is an excellent therapy, still have disadvantage like the need for monitoring and replacement, indwelling catheter-electrodes in the heart, possibility of infection, and lack of autonomic responsiveness, geometric limitations with respect to pediatric patients make it warrant a search for better alternatives (Rosen et al 2004). The biological pacemaker, a tissue that spontaneously or via engineering confers pacemaker properties to regions of the heart, is an exciting alternative. Several approaches have been

taken in attempting to produce biological pacemakers. These can be considered in 3 headings: 1- The use of viral vectors to deliver genes to regions of the heart such that a pacemaker potential resulting in spontaneous impulse initiation evolves in the region of gene administration. 2- The use of embryonic stem cells grown along a cardiac lineage and manifesting the electrophysiologic properties of sinus node cells; 3- The use of mesenchymal stem cells as platforms to carry pacemaker genes to the heart, relying on gap junctional coupling such that the stem cell and a coupled myocyte form a single functional unit to generate pacemaker function (Edelberg 1998, 2001, Miake et al 2002, Qu et al 2003, Plotnikov et al 2004, Kehat et al 2004, Potapova et al 2004).

## 2. Historical background

Till the mid-20th century, many patients with complete heart block were at risk of death. Therapy in adults was largely limited to positive chronotropic interventions, typically sublingual isoproterenol, the first mass-produced implantable pacemakers were fixed rate units featuring the attractiveness and dimensions of a sterile hockey puck, but they are life saving. Improvements in design and manufacture, insightful adaptation of computer technologies to provide programming and microcircuitry, and the imaginative approaches to a variety of cardiac pathologies have ultimately developed pacing used epicardially or endocardially to treat disorders of heart rate and rhythm and heart failure. The development of cardioverters/defibrillators and their incorporation in the pacemaker industry represent a further major development. The hardware and the methods initially applied to a very limited spectrum of heart rhythm disorders had grown into the medical device industry and into one of the most successful and effective palliative therapies in last 3 decades. (Michaelsson et al 1995, Zivin et al 2001a & b).

## 3. Anatomical and histological bases

The SA node region is located on the endocardial surface at the edge of the right atrium, bounded on two sides by the superior and inferior venae cavae and around the crista terminalis between the venae cavae and the right atrial muscle. Microscopically, the SA node appears as a translucent muscular region near the sino-atrial node artery. With most prominent feature is the ring bundle, which is a thin flap of tissue that extends around most of the periphery of the node and that usually appears to be the most vigorously beating part in an isolated node. On electron microscope, SA nodal cells have a relatively large nucleus and a few myofilaments. There are many caveolar invaginations along the surface membranes of these cells. The intercellular space at 20 nm is wide compared with other tissues. Isolated SA nodal cells are spindle- or spider-shaped and have a maximum length of 25–30  $\mu\text{m}$ , with an irregular profile in cross section and a diameter of less than 8  $\mu\text{m}$ . Isolated spontaneously beating SA nodal myocytes are curved and not flat on their base (Masson-Pevet 1979, Satoh Uchida 1993, Shinagawa et al 2000).

## 4. Physiology of natural pacing

The sinus node depolarizes spontaneously during phase 4 until membrane potential reaches threshold and an action potential is generated. (Phase 4 is initiated at the end of repolarization, when the membrane potential is very negative (about -60 mV), ion channels open that conduct slow, inward (depolarizing)  $\text{Na}^+$  currents. These currents are called

"funny" currents and abbreviated as "If". These depolarizing currents cause the membrane potential to begin spontaneous depolarization). This event occurs rhythmically and regularly for the lifetime. The slope of phase 4 depolarization results from a balance between inward and outward ion currents. The initial inward current, activated on hyperpolarization of the membrane at the end of repolarization, other currents that are inward and contribute to phase 4 depolarization are the T- and L-type Ca currents (upstroke of the sinus node action potential). Providing outward current during the same time frame are the not yet completely decayed potassium currents  $I_{Kr}$  and  $I_{Ks}$  and a weak  $I_{K1}$ . In addition, the Na-Ca exchanger operates during phase 4 to further influence the rate of depolarization of the membrane (DiFrancesco 1981, Biel et al 2002, Bogdanov et al 2006).

The autonomic nervous system modulating the ion channel contribution to pacemaker function. Catecholamine binding to beta adrenergic receptors operates via a Gs protein-linked pathway to increase cyclic adenosine monophosphate (cAMP) synthesis and increase pacemaker rate, whereas acetylcholine binding to M2 muscarinic receptors operates via a Gi protein-linked pathway to reduce cAMP synthesis, thus reduces rate. cAMP is critical to pacemaker rate because of its action on the HCN (hyperpolarization activated cyclic nucleotide gated) channels that determine its function (Biel et al 2002). Taking in consideration, none of the ion currents described is uniquely responsible for pacemaker activity. All contribute, and marked alteration in any one can be balanced by altered function of the others, such that pacemaker activity persists, albeit at different rates. This redundancy in function is important to maintain the initiation and maintenance of the heartbeat under a variety of circumstances. A good example is the effect of ivabradine on sinoatrial rate: The latter may decrease by as much as 30%, accounting for the therapeutic effect of the drug, but effective pacemaker function is preserved (Thollon et al 2007). All currents contribute in such a way to permit the generalization that any event that increases inward current and/or decreases outward current will increase pacemaker rate.

## 5. Transcription factors and conduction system (Table 1)

Cardiac conduction system components work together as a functional unit to provide the rhythmic activity of the heart. Transcription factors, including homeodomain proteins and Tbox proteins, are at the core of pathways specifying the components of the cardiac conduction system. They are essential in activating or repressing a constellation of regulatory genes, most of which still remain unidentified. Together, the transcription factors and regulatory genes specify and maintain the cardiac conduction system in a normally functioning state. Mutations in genes encoding some of these transcription factors produce human disorders defined by the presence of congenital heart defects as well as associated or isolated conduction system abnormalities. In addition to the transcription factors that specify cell lineages destined to become part of the cardiac conduction system, several transcription factors regulate expression of genes encoding the ion channel proteins. Ion channels are essential in contributing to the electrophysiological properties of the conduction system by maintaining the membrane potential of myocardial cells and controlling the release of ions necessary for eliciting a muscle contraction. Dysregulation of these ion channels due to alterations in expression of their modulatory transcription factors can affect proper functioning of the conduction system and lead to the manifestation of arrhythmias. Further characterization of the molecular programs involved in cardiac conduction system specification, maintenance and function, and ion channel expression

should lead to improved diagnosis and therapy of conduction system disease. (Hatcher et al 2009). Recent study report that the *Shox2* homeodomain transcription factor is restrictedly expressed in the sinus venosus region including the SA node and the sinus valves during embryonic heart development. *Shox2* null mutation results in embryonic lethality due to cardiovascular defects, including an abnormal low heart beat rate and severely hypoplastic SA node and sinus valves attributed to a significantly decreased level of cell proliferation. Genetically, the lack of *Tbx3* and *Hcn4* expression, along with ectopic activation of *Nppa*, *Cx40*, and *Nkx2-5* in the *Shox2*<sup>-/-</sup> SAN region, indicates a failure in SA node differentiation. Furthermore, *Shox2* overexpression in *Xenopus* embryos results in extensive repression of *Nkx2-5* in the developing heart, leading to a reduced cardiac field and aberrant heart formation. Reporter gene expression assays provide additional evidence for the repression of *Nkx2-5* promoter activity by *Shox2*. (Ramón et al 2009).

Transcription Factor	Expression in Cardiac Conduction System	Role in Cardiac Conduction System Development
Nkx2.5	AV node, AV bundle, BBs, PF	specification of AV node lineage & peripheral conduction system
Shox2	SA node, BBs	SA node specification and gene expression
Hop	AV node, His bundle, BBs	maintenance of proper CCS gene expression and function
Irx4/Irx5	none (ventricular myocardium)	regulation of ventricular ion channel expression
Tbx2	AV node, AV ring bundle	specification of AV node and AV ring bundle
Tbx3	SA node, AV node, AV bundle, proximal BBs	SA node induction, compartmentalization & maintenance, AV conduction tissue specification and patterning, suppression of myocardial gene expression in atria and ventricles
Tbx18	SA node	SA node compartmentalization
Tbx5	AV node, His bundle, BBs	postnatal maturation of AV node, AV bundle & left BB; right BB patterning
Id2	AV node, AV bundle, BBs	ventricular myocyte conduction system specification and function via cooperative regulation by Nkx2.5 & Tbx5

SA; sinoatrial node, AV; atrioventricular bundle, BB; bundle branch, PF; Purkinje fiber

Table 1. Transcription factors involved in cardiac conduction system specification, patterning, maturation & function. (Hatcher et al 2009).

6. Why biological pacemakers needed

Although electronic pacemakers reduced mortality associated with complete heart block and morbidity of sinoatrial node dysfunction, still they have disadvantages:

1. The imposed limitations on the exercise tolerance and cardiac rate-response to emotion.  
Despite the use of paradigms to improve heart rate response during increased physical

activity, there is no substitute currently available for the autonomic modulation of heart rate.

2. In pediatrics, patient age and size, the mass of the power pack, and the size and length of the electrode catheter are important considerations. The hardware must be tailored to the growth of the patient.
3. The placement site of the stimulating electrode in the ventricle and the resultant activation pathway may have beneficial or deleterious effects on electrophysiologic or contractile function.
4. The long-but-limited life battery expectancy, requiring testing and replacement at periodic intervals.
5. Infection may require removal and/or replacement of the pacemaker.
6. Various devices including neural stimulators metal detectors and magnetic resonance imaging equipment have been reported to interfere at times with electronic pacemaker function. (Furrer et al 2004, Martin et al 2004).

So a biological alternative that might last for the life of the patient, respond to physiologic demands for different heart rates at different times, and activate the heart via a pathway tailored to the anatomy of disease in any individual is an exciting possibility. An ideal biological pacemaker should;

1. Create relatively accepted physiologic rhythm for the life of the individual.
2. Needs no battery or electrode, and no replacement.
3. Effectively compete in direct comparison with electronic pacemakers.
4. Have no inflammatory or infectious potential.
5. Not carcinogenic.
6. Adapt to changes in physical activity and/or emotion with appropriate rapid changes in heart rate.
7. Propagate through an optimal pathway of activation to maximize efficiency of contraction and cardiac output.
8. Not arrhythmogenic.
9. Potentially curative.

## 7. Strategies for building a biological pacemaker

Three strategies reported till now to create biological pacemaker activity:

1. Up-regulation of adrenergic neurohumoral actions on heart rate (Edelberg 1998, 2001).
2. Reduction of repolarizing current (Miake et al 2002).
3. Increasing inward current during diastole (Qu et al 2003).

All three strategies had their foundations in 20th century pharmacology and physiology. In studies of autonomic modulation, increased heart rate via beta-adrenergic catecholamines or sympathetic stimulation through an increase in pacemaker current in the sinus node and in accessory pacemakers, whereas increasing vagal tone or stimulating muscarinic receptors decreased heart rate (DiFrancesco et al 1986, Campbell et al 1989). In studies of ionic determinants of pacemaker activity, augmentation of hyperpolarizing, outward currents decreased pacemaker rate (Di Francesco et al 1995), suggesting that the opposite intervention, i.e. decreasing hyperpolarizing, outward currents, would increase rate (Miake et al 2002). Pharmacological experiments demonstrated that suppressing inward current carried by the T-type or L-type Ca channel slows pacemaker rate. (Lasker et al 1997, Robinson, Di Francesco 2001). What are needed are the tools to apply this knowledge to the molecular and genetic determinants of the pacemaker potential.

The necessary information was provided in part via the identification and cloning of the gene products that determine the beta adrenergic receptors, the inward rectifier current, and the pacemaker current. Also of central importance was the development of tools for; 1- gene therapy, wherein genes encoding the molecular subunits of interest are inserted via plasmids or viral vectors into cells of the myocardium; 2- cell therapy via the use of embryonic stem cells, whose differentiation is directed into myocardial precursors manifesting pacemaker activity, or mesenchymal stem cells used as platforms to implant channels into cardiac myocytes. A critical factor is the development of models in which to test pacemaker constructs. In vitro models of cells in culture are a standard for testing a variety of gene therapies it has been found that infecting neonatal rat ventricular myocytes with replication-deficient adenoviral constructs incorporating the gene of interest (with or without coexpression of GFP) provides a cost-effective and reproducible assay (Qu et al 2001). Using a variation on this model for testing the ability of stem cells to transmit the electrical signal of interest (Potapova et al 2004). It has been considered that a 100 times or more overexpression of current and a statistically significant effect on beating rate as standards that discriminate efficacy, More research is required to establish uniform guidelines permitting reliable correlation of in vitro and in vivo effectiveness. As an intact animal screen, the use of guinea pig (Miake et al 2002), swine (Edelberg et al 2001), and dog (Qu et al 2003, Plotnikov et al 2004, Potapova et al 2004) has been reported. The use of dog is based on its cardiac size, tractability as a chronic model, and similar electrophysiologic properties to those of man.

## 8. Vectors and methods of gene delivery

Gene therapy is defined as the transfer of nucleic acids to somatic cells as therapeutically useful molecules. Human genome has approximately 30,000 genes. The genetic diversity is amplified by alternate splicing of mRNA and post translational modification of proteins. The possible gene targets for arrhythmias are very large. The molecular targets of arrhythmia management are the ion channels and the modulators of ion channels like G proteins (Members of the Sicilian gambit 2001). A vector is the vehicle commonly used to introduce the gene to the target cell. It may be RNA or DNA viruses or non viral in nature. Viruses which have the capacity to incorporate themselves in the host genome are used as vectors for gene therapy. The commonly used viral vectors are genetically modified retroviruses, adenoviruses, adeno associated viruses and lentiviruses. These viral vectors are replication deficient to ensure safety, but require large amounts of vector particles for efficacy. Non viral vectors based on plasmids, DNA- lipid complexes and naked DNA are also used since they lack foreign proteins and avoid immunological problems. The feasibility of gene transfer has been demonstrated in both animals and humans. The extent of gene transfer and expression is low in clinical settings compared to experimental laboratory. The period during which a newly introduced gene is expressed is often short but variable and differs with the tissue. For example, early-generation non-viral vectors express the gene at maximum levels only for a few days (Lee et al 1996). Many adenoviral vectors express the gene for 2-3 week (Armentano et al 1997). Non viral vectors again have short duration of gene expression. This short duration of gene expression may necessitates repeat dosing, although less efficacious. In contrary, expression from adeno-associated viral vectors may not peak for several weeks, but then remain constant in some tissues for several months (Yla-Herttuala & Martin 2000). Retroviruses produce a long lasting effect by integration of

the transfected gene into the host genome (Smith 1999). Various novel methods of transfection have been tried in animal models, including DNA polymer coating on inert materials and subsequent transfer to the atrial myocardium, with sustained gene activity, the classical methods of vector delivery are direct injection into the myocardium, infusion through the coronary arteries or administration to the epicardium. (Labhasetwar et al 1998). Intracoronary perfusion is another modality of gene transduction with near complete expression under optimal conditions (Donahue et al 1997). The gene transfer efficiency depends on the coronary flow rate, virus concentration, exposure time and microvascular permeability. Agents which increase the microvascular permeability have been used to enhance the delivery. Only few generalizations can be made about the vector selection and the method of gene delivery, and each disease has its own target tissue and the amount of gene product required for treatment. None of the currently available vectors satisfy the criteria of an ideal gene therapeutic system.

## 9. Global versus local administration

Permeabilizing agents, vasodilators and vascular endothelial growth factor (VEGF) have been used to facilitate gene delivery to large or localized regions of the heart. Cooling and aortic cross-clamping have been employed to improve gene delivery through the distribution of a coronary artery or the flooding of a chamber or chambers, Not only do these approaches appear excessive for clinical application but the best success to date seen in about 50% of cells in any region transfected, with viral transfer being diffusion-limited and especially problematic in the ventricles. (Lehnart&Donahue 2003, Roth et al 2004). Tempering interest in some viral vectors are concerns about inflammation, chronic illness or neoplasia. These issues led to exploration of hMSCs as platforms for gene delivery. That hMSCs can be loaded with specific gene constructs and delivered to the heart without eliciting inflammation or rejection and not differentiating into other cell types. But long-term stability of hMSC therapies raises concern about migration to other sites, differentiation into other cell types, and duration of expression of genes of interest. The use of various markers to trace cell location should facilitate investigators understanding of the extent of hMSC localization to sites of administration. (Potapova et al 2004, Rosen 2005, Zimmet et al 2005, Plotnikov et al 2007). Cell therapies generally have been intended to regenerate and repair myocardium rather than to be specifically antiarrhythmic. While it has been found that hMSCs to be adequate delivery platforms for ion channel generated currents, it has been followed for 6 weeks only (Plotnikov et al 2007). The question of long-term applicability will await long-term studies of hMSC survival as well as comparison with genomically-incorporated viral constructs.

Somatic gene therapy provides a conceptually attractive strategy for modifying the global cardiac electrophysiological substrate in disease states such as the inherited and acquired long QT syndromes. Another attractive target for local gene therapy may be to selectively modify the conduction properties of the AV node. This may be of value in the treatment of atrial fibrillation. (Nattel 2002). The feasibility of using gene therapy for AV nodal modification in an attempt to control the ventricular rate during atrial fibrillation demonstrated by using adenoviral gene delivery selectively to the AV nodal region via the coronary circulation; the AV nodal conduction properties could be modified by overexpression of an inhibitory G protein (G alpha i2). G alpha i2 overexpression in the AV nodal cells suppressed baseline atrioventricular conduction and slowed the ventricular rate

during atrial fibrillation without producing complete heart block, thus mimicking the effects of beta-adrenergic antagonists (Donahue 2000). More appealing targets in the short term may be arrhythmias in which localized manipulation of the electrophysiological substrate may be sufficient to allow effective treatment.

Recent study, investigated the effect of overexpression of the cardiac potassium channel missense mutation Q9EhMiRP1. This gene mutation is one of the known causes of the long QT syndrome and results in diminished potassium currents following clarithromycin administration. In vitro transfection of the Q9E-hMiRP1 gene resulted in a clarithromycin induced reduction of the potassium outward current in the transfected cells when compared to wild-type hMiRP1 overexpression. With the utilization of a novel gene delivery technique, both plasmids were injected locally into the pig's atrial myocardium with 15% of the atrial cells being transfected. This study conclude that overexpression of this mutated channel gene may have an inducible localized class III-like antiarrhythmic effect on the atrial tissue that may be used in the future for the treatment of reentrant atrial arrhythmias (Burton et al 2003). Viral vector-based therapies are not yet applied clinically to arrhythmia management but have been effective in proof-of-concept experiments suggesting that gene therapy can be of use.

## 10. Cell therapy for the treatment of cardiac arrhythmias (Table 2&3)

An alternative approach to overcome the shortcomings of gene therapy may be the use of genetically modified cell grafts that can be initially transfected ex vivo with excellent long-term efficiency and then transplanted to the in vivo heart. This will require the following:

1. Establish the proper cell sources for transplantation.
2. Assessment of the phenotypic structural and functional properties of the cell grafts, in vitro.
3. Establish transplantation strategies to deliver the cells to the desired locations.
4. Achieve the desired in vivo effect by assuring the survival of the cell grafts, their integration and interactions with host tissue, and their proper function.

Cell therapy can be applied for the treatment of cardiac arrhythmias at three different levels:

1. Replace absent or malfunctioning cells of the conduction system.
2. Modify the myocardial electrophysiological substrate by using cell grafts genetically engineered to express specific ionic channels, which can couple and modify the electrophysiological properties of host tissue through electrotonic interactions.
3. Modify the myocardial environment by local secretion of specific recombinant proteins.

A major limitation for the development of such cell replacement strategies is the paucity of cell sources for human cardiomyocytes. The use of the recently described human embryonic stem cell lines may be solution to this cell-sourcing problem (Gepstein 2002). These unique cell lines have the capability to be propagated in vitro in the undifferentiated state in large quantities and to be coaxed to differentiate to a plurality of cell lineages, including cardiomyocytes (Kehat et al 2001a). This differentiating system is not limited to the generation of isolated cardiac cells, but rather a functional cardiac syncytium is generated with a stable pacemaker activity and electrical propagation (Kehat et al 2002). that can also respond to adrenergic and cholinergic stimuli. The ability to generate, ex vivo, different subtypes of human cardiomyocytes (with pacemaking-, atrial-, ventricular-, or Purkinje-like phenotypes) (Mummery et al 2003) that could lend themselves to genetic manipulation may be of great value for future cell therapy strategies aiming to regenerate or to modify the conduction system.

The ability of the grafted cells (pacemaker cells or conductive tissue) to integrate structurally and functionally with host tissue is a sole requirement. The human ES cell derived cardiomyocytes were able to integrate ex vivo both structurally and functionally with preexisting cardiac tissue and to generate a single functional syncytium (Kehat et al 2001 b). Whereas it is not surprising that cardiomyocyte cell grafts can form intercellular connections with host cells (Isner 2002). Recent studies have demonstrated that other cell types such as fibroblasts (Rook et al 1992, Fast et al 1996, Gaudesius et al 2003) are also capable of forming gap junctions with host cardiomyocytes and that specific electrotonic interactions can be generated between these cells. The feasibility of using genetically engineered fibroblasts, transfected to express the voltage-gated potassium channel Kv1.3, to modify the electrophysiological properties of cardiomyocyte cultures have been examined, in a study, using a high-resolution multi-electrode array mapping technique to assess the electrophysiological and structural properties of primary neonatal rat ventricular cultures. The transfected fibroblasts were demonstrated to significantly alter the electrophysiological properties of the cardiomyocyte cultures. These changes were manifested by a significant reduction in the local extracellular signal amplitude and by the appearance of multiple local conduction blocks (Feld et al 2002). The location of all conduction blocks correlated with the spatial distribution of the transfected fibroblasts as assessed by vital staining and all of the electrophysiological changes were reversed following the application of a specific Kv1.3 blocker.

Genetically engineered cell grafts, transfected to express potassium channels, can couple with host cardiomyocytes and alter the local myocardial electrophysiological properties by reducing cardiac automaticity and prolonging refractoriness. Investigators studied the ex vivo, in vivo, and computer simulation studies to determine the ability of transfected fibroblasts to express the voltage-sensitive potassium channel Kv1.3 to modify the local myocardial excitable properties. Co-culturing of the transfected fibroblasts with neonatal rat ventricular myocyte cultures resulted in a significant reduction (68%) in the spontaneous beating frequency of the cultures compared with baseline values and co-cultures seeded with naive fibroblasts. In vivo grafting of the transfected fibroblasts in the rat ventricular myocardium significantly prolonged the local effective refractory period from an initial value of  $84 \pm 8$  ms (cycle length, 200 ms) to  $154 \pm 13$  ms ( $P < 0.01$ ). Marga toxin partially reversed this effect (effective refractory period,  $117 \pm 8$  ms;  $P < 0.01$ ). In contrast, effective refractory period did not change in nontransplanted sites ( $86 \pm 7$  ms) and was only mildly increased in the animals injected with wild-type fibroblasts ( $73 \pm 5$  to  $88 \pm 4$  ms;  $P < 0.05$ ). Similar effective refractory period prolongation also was found during slower pacing drives (cycle length, 350 to 500 ms) after transplantation of the potassium channels expressing fibroblasts (Kv1.3 and Kir2.1) in pigs. (Yankelson et al 2008).

The possible utilization of cell grafts (fibroblasts, different stem cell derivatives, or other cell sources) that can be genetically manipulated ex vivo to display specific electrophysiological characteristics and then grafted to the in vivo heart may possess a number of theoretical advantages over direct gene therapy. These advantages may be related to a better efficiency and control of the transfection process ex vivo, the ability to screen the phenotypic properties of the cells before transplantation, and the possible achievement of long-term effect because cardiac cell grafts were demonstrated to survive for prolonged periods following transplantation (Muller-Ehmsen et al 2002). Yet, determining the optimal way for the delivery of the cells, controlling their survival following transplantation, assuring appropriate integration of the cells with host tissue, and developing means to control the

required electrophysiological effect are all important obstacles for the future use of this approach as a therapeutic strategy.

Ischemic heart disease represents one of the most important conditions predisposing to arrhythmias. A variety of preclinical and clinical studies have demonstrated the potential utility of gene therapy in the management of chronic ischemic patients through the local secretion of angiogenic growth factors such as vascular endothelium growth factor (VEGF) and fibroblast growth factor (Isner 2002). Cell therapy strategies may similarly play a dual role in promoting angiogenesis. First, cells transfected *ex vivo* may be used for sustained local release of recombinant proteins with angiogenic properties following *in vivo* grafting. Second, transplantation of specific cell types such as endothelial progenitor cells may contribute directly to the neovascularization process. The improved understanding of the molecular pathways involved in the development of heart failure allow definition of several molecular targets for gene therapy to improve systolic and diastolic properties of failing myocytes. To focus on modulating calcium homeostasis, manipulating the beta-adrenergic receptor signaling pathways, and improving cardiomyocyte resistance to apoptosis need to be looked for in future strategies. Similarly, cellular cardiomyoplasty and tissue engineering approaches to regenerate functional myocardium also represent a novel approach for the treatment of heart failure (Reinlib & Field L 2000, Hajjar et al 2000, Kehat et al 2001 b).

## 11. Gene therapy for the treatment of bradyarrhythmias (table 2)

Implanted pacemakers have become the preferred treatment for sinus node dysfunction and high-grade AV block with excellent results with very low morbidity (Kusumoto & Goldschlager 1996). Nonetheless, the ideal therapy for these disorders may be the development of a biological solution allowing reconstitution of the physiological electrical activity of the cardiac conduction system with the same plasticity and adaptability to the human body and to the physiology of the cardiovascular system. Recently; investigators hypothesized that overexpression of an engineered HCN construct via somatic gene transfer offers a flexible approach for fine-tuning cardiac pacing *in vivo*. Using various electrophysiological and mapping techniques, the authors examined the effects of *in situ* focal expression of HCN1- *DeltaDeltaDelta*, the S3-S4 linker of which has been shortened to favor channel opening, on impulse generation and conduction. Porcine models of sick-sinus syndrome by guided radiofrequency ablation of the native SA node were generated followed by implantation of a dual-chamber electronic pacemaker to prevent bradycardia-induced hemodynamic collapse. Interestingly, focal transduction of Ad-CGI-HCN1- *DeltaDeltaDelta* in the left atrium of animals with sick-sinus syndrome reproducibly induced a stable, catecholamine-responsive *in vivo* “bioartificial node” that exhibited a physiological heart rate and was capable of reliably pacing the myocardium, substantially reducing electronic pacing (Tse Hung et al 2006).

Overexpression of the pacemaker-specific current is an interesting strategy for the generation of a biological pacemaker. Investigators assessed the ability of localized overexpression of the hyperpolarization-activated, cyclic nucleotide-gated (HCN-2) isoform pacemaker current to generate stable pacemaking activity *in vivo*. Four days after the injection of adenoviral constructs of the mouse HCN2 into the canine left atrium, the emergence of a new atrial pacemaking activity during vagal stimulation-induced sinus arrest were seen. Electrophysiological mapping localized the source of this activity to the injection site at the left atrium. Whole cell electrophysiological recordings from transfected

myocytes demonstrated the presence of a relatively high-magnitude pacemaker current. (Qu et al 2001).

Enhancement of the chronotropic response of the native pacemaking cells is another strategy proposed to regulate the normal pacemaking activity of the heart by local gene delivery (Edelberg 1998, 2001). Aiming to enhance the responsiveness of the native atrial pace making cells to adrenergic input through up regulation of the Beta 2-adrenergic receptors. Using detailed ex vivo and in vivo studies, the authors demonstrate a significant positive chronotropic effect following overexpression of the human beta 2-adrenergic receptor in atrial tissue.

The above studies demonstrated the ability of local gene delivery to alter the chronotropic properties of the heart; it mainly focused on modifying the function of existing and abnormal pacemaking cells rather than actually creating a new biological pacemaker.

Another strategy for the creation of a biological pacemaker in vivo was described is based on the production of dominant negative inhibition of the Kir2-encoded inward rectifier potassium channels (Ik1) in ventricular myocytes (Kir2.1AAA). The Ik1 current, which is intensely expressed in atrial and ventricular myocytes but not in the pacemaking nodal cells, maintains the negative resting membrane potential of ventricular myocytes and thereby, suppresses any spontaneous diastolic activity. The investigators hypothesized that dominant negative inhibition of this current could restore the latent pacemaking activity in these cells and convert the quiescent ventricular myocytes into pacemaking cells. adenoviral gene delivery of Kir2.1AAA into the left ventricular cavity of guinea pigs was performed. In some of the animals studied, electrocardiogram recordings demonstrated the emergence of a new ventricular source of impulse initiation. In vitro electrophysiological recordings from the transfected myocytes demonstrated, electrophysiological properties and spontaneous activity resembling those of genuine pace making cells. (Kubo et al 1993, Miake et al 2002).



<div> Biological pace maker for Brady arrhythmias</div>	<b>Gene therapy</b>
	-Enhancement of chronotropic response of native pace maker cells by up regulating the B2 adrenergic receptors. (Edelberg1998, 2001). -Shifting the balance between excitatory & inhibitory current using dominant negative inhibition of the Kie2- encoded inward rectifier K channels in cardiac myocytes. (Miake et al 2002). -Over expression of the activated HCN -2 isoform pace maker current in the atria (Qu et al 2003). - Focal expression of HCN1- DeltaDeltaDelta. (Tse Hung et al 2006).
	<b>Cell therapy</b>
<div></div>	Grafting of engineered tranfected fibroblast to express specific K channels to modulate the electrical activity of the cardiac tissue (Yankelson et al 2008).

Table 2. Possible approach for biological pace maker for treatment of Bradyarrhythmias .

12. Gene therapy for the treatment of tachyarrhythmias (Table 3)

Different mechanisms underlying various cardiac tachyarrhythmias (reentry, triggered activity, and abnormal automaticity) result from abnormalities in the myocardial electrophysiological or structural substrate. That may be anatomic or functional and may be localized to a specific area within the myocardium or affect the heart globally. These abnormalities may be inherited (different monogenic ion channel mutations in the congenital long QT syndrome, Brugada syndrome, etc.) or acquired in a variety of clinical

conditions (ischemic heart disease and heart failure leading to ventricular tachyarrhythmias or diseased atria leading to atrial fibrillation) (Keating & Sanguinetti 2001, Marban 2002, Roberts & Brugada 2003).

Understanding of the electrophysiological abnormalities leading to the development of the different rhythm disorders is needed to target specific genes that will either reverse the abnormal phenotype or modify the excitable properties of the myocardial substrate in a favorable way. An attractive target for this type of somatic gene therapy may be to correct the abnormal global electrophysiological substrate in the inherited or acquired long QT syndromes, which can be familial, or inherited (autosomal recessive or dominant trait), or acquired in a variety of clinical conditions, is characterized by the prolongation of the QT interval in the electrocardiogram and by an increased risk for the development of ventricular arrhythmias and sudden cardiac death (Keating & Sanguinetti 2001, Marban 2002).

Heart failure represents a prototype of an acquired long QT condition, which predisposes the patients to the development of ventricular arrhythmias. Experimental evidence have shown that such increased propensity for ventricular arrhythmias may originate partly from the downregulation of  $K^+$  currents (namely  $I_{to}$  and  $I_{K1}$ ) in failing myocytes leading to significant prolongation of the action potential duration (APD) (Beuckelmann et al 1993, Marban 1999). Action potential duration prolongation in failing myocytes may initially be an adaptive response because it increases the time available for excitation-contraction coupling thereby augmenting myocardial contractility. But such process may be maladaptive, predisposing the ventricle to early afterdepolarizations (EADs), inhomogeneous repolarization, and the development of lethal ventricular arrhythmias on the long term bases.

Electrical alternans has been linked to the development of ventricular arrhythmias. Increasing the rapid component of the delayed rectifier current ( $I_{Kr}$ ) may suppress electrical alternans and may be antiarrhythmic.  $I_{Kr}$  in isolated canine ventricular myocytes were increased by infection with an adenovirus containing the gene for the pore-forming domain of  $I_{Kr}$  [human ether-a-go-go gene (HERG)]. The voltage at which peak  $I_{Kr}$  occurred were significantly less negative in HERG-infected myocytes, thereby shifting the steady-state voltage-dependent activation and inactivation curves to less negative potentials (HuaF et al 2004). This has supported the idea that increasing  $I_{Kr}$  may be a viable approach to suppressing electrical alternans and arrhythmias.

Recent study has pursued a novel gene transfer approach to modulate electrical conduction by reducing gap junctional intercellular communication (GJIC) and hence potentially modify the arrhythmia substrate. With ultimate goal of developing a nondestructive approach to uncouple zones of slow conduction by focal gene transfer. Lentiviral vectors encoding connexin43 (Cx43) internal loop mutants were produced and studied in vitro. Transduction of neonatal rat ventricular myocytes (NRVMs) revealed the expected sub-cellular localization of the mutant gene product. Fluorescent dye transfer studies showed a significant reduction of GJIC in NRVMs that had been genetically modified. Additionally, adjacent mutant gene-modified NRVMs displayed delayed calcium transients, indicative of electrical uncoupling. Multi-site optical mapping of action potential (AP) propagation in gene-modified NRVM mono-layers revealed a 3-fold slowing of conduction velocity (CV) relative to non transduced NRVMs. In conclusion; lentiviral vector-mediated gene transfer of Cx43 mutants reduced GJIC in NRVMs. Electrical charge transfer was also reduced as evidenced by delayed calcium transients in adjacent NRVMs and reduced CV in NRVM

monolayers. These data validate a molecular tool that opens the prospect for gene transfer targeting gap junctions as an approach to modulate cardiac conduction (Eddy et al 2007). Because heart failure is characterized by both depressed contractility and delayed repolarization, the unopposed correction of the latter by the strategies described above may further aggravate the already depressed mechanical properties. In vivo, this dual gene therapy approach resulted in abbreviation of the QT interval with preservation of contractility this has been shown by a group of investigators designed a novel dual gene strategy aiming to offset the loss of contractility due to the potassium current-induced action potential duration shortening with the overexpression of the calcium ATPase sarcoplasmic reticulum Calcium ATPase (SERCA). Using a bicistronic adenoviral vector allowing a single promoter to drive the co expression of two genes, the authors co expressed in guinea pig hearts the Kir2.1 cardiac inward rectifier potassium channel together with SERCA1. Myocytes isolated from these hearts demonstrated shortened action potential durations when compared with controls but also displayed larger calcium transients. (Ennis et al 2002). The rational for using SERCA in the dual gene therapy strategy, originates from previous studies showing the ability of SERCA overexpression to augment cardiac contractility by increasing sarcoplasmic reticulum calcium loading (Hajjar et al 2000). Overexpression of SERCA alone also resulted in a favorable electrophysiological effect manifested by shortening of action potential duration and a significant reduction in the incidence of after contractions in the transfected myocytes (Davia et al 2001, Terracciano et al 2002).



<div> <b>Biological pacemaker for Tachyarrhythmia</b> </div>	<b>Gene therapy</b>
	<p><b>*Localized approach ;</b></p> <ul style="list-style-type: none"><li>- Delivering a dominant negative HERG mutant (HERGG628S) via vascular infusion to a peri-infarct zone of pigs (Sasano et al 2006).</li><li>-AV nodal modification by Gai 2 (Donahue et al 2000).</li><li>-Local atrial prolongation of ADP by overexresion of of Q9E –hMi RP1( Burton et al 2003).</li></ul> <p><b>*global approach ;</b> ADP shorting for heart failure &amp;prolonged QT;</p> <ul style="list-style-type: none"><li>-Overexpression of Dorsophilina shakers B K channels in cultured ventricular myocytes.( Nuss et al 1996)</li><li>- Oveexpresion of human K+ channels HERG encoding the Ikr current (Nuss et al 1999).</li><li>-Oveexpresion of the Ca++ ATPase SERCA (Davia et al 2001, Terracciano et al 2002).</li><li>-Oveexpresion of the Ca++ ATPase SERCA with kir 2.1(Ennis et al 2002).</li><li>-Oveexpresion the accessory subunit KCNE 3 to increase the activity of KCNQ1 channel. (Mazhari et al 2002).</li><li>-Lentiviral vectors encoding connexin43 to modulate electrical conduction by reducing gap junctional intercellular communication (GJIC) (Eddy et al 2007).</li></ul>
	<b>Cell therapy</b>
	Grafting of engineered tranfected fibroblast to express specific K channels to modulate the electrical activity of the cardiac tissue (Yankelson et al 2008).

Table 3. Possible approach for biological pace maker for treatment of Tachyarrhythmia.

13. Ventricular tachycardia & fibrillation

Whereas myocardial infarct-induced arrhythmias might respond to local therapy, variations in anatomy from patient to patient require extensive mapping to determine sites at which to localize therapy. Using mapping to identify sites for local radiofrequency ablation reduced

the need for defibrillation in patients who had devices implanted for secondary prevention. Using mapping to identify the border zone of an infarct in a canine model ablation were replaced with intramyocardially-administered gene therapy in preliminary studies and without destroying tissue - achieved a reduction in VT/VF incidence (Reddy et al 2007, Lau et al 2009).

## **14. Specific gene therapies for ischemic arrhythmias**

### **14.1 Speeding conduction via connexins or Na channels**

The importance of connexins and hence gap junctions in arrhythmias has been shown in many studies. the overexpression of Cx45 results in ventricular tachycardia in mice (Betsuyaku et al 2006) while mutations of Cx40 are associated with atrial fibrillation in humans.(Gollob et al 2006) Studies of the epicardial border zone of healing canine myocardial infarcts have demonstrated altered connexin distribution and density in regions of generation of reentrant ventricular tachycardia.(Peters et al 1997) The modulation of gap junctions as an anti-arrhythmic strategy initially attempted to block conduction. However, the gap junctional blockers used to date have not been channel specific neither isoform-specific and in disrupting coupling between cells have been found to cause potentially fatal arrhythmias. On the positive side, antiarrhythmic peptides have been used to increase junctional conductance. One such peptide, rotigaptide, appears to target Cx43 specifically, and may be antiarrhythmic (Dhein et al 2003).

At least 10 different Na channel genes encode alpha subunits in the mammalian genome; these have been cloned from brain, spinal cord, skeletal and cardiac muscle, uterus, and glia (Allessie et al 1977). Since slow conduction is an essential feature of reentrant cardiac arrhythmias, other mammalian Na channels that might have more favorable properties than the cardiac Na channel in circumstances that favor slow conduction were looked for (Lau et al 2009). One such circumstance is membrane depolarization, as in myocardial infarction in such circumstances the voltage dependence of steady state Na channel inactivation is of interest. The midpoint of the cardiac Na channel (SCN5A) is negative to  $-73\text{mV}$ . This is important because in infarcted tissue when myocytes are depolarized to  $-65\text{mV}$  virtually all SCN5A-derived cardiac Na channels are inactivated. In contrast, skeletal muscle (SkM1) Na channels have an inactivation midpoint of  $-68\text{mV}$  and almost half of these channels would be available to open during an action potential in a depolarized cell. This suggests that Na channels such as SkM1 with more favorable biophysical properties than SCN5A might be a useful antiarrhythmic therapy. The effectiveness of this approach has been shown in a canine model in which the incidence of inducible polymorphic VT was 75% of controls and 17% of SkM1-administered dogs 5 days postinfarction. Moreover, SkM1 administration reduced electrogram fragmentation and increased  $V_{\text{max}}$  of phase 0 (consistent with more rapid conduction), as had been predicted for SkM1 (Lau et al 2009).

### **14.2 Targeting diastolic membrane potential**

In ventricular tachycardia in the setting of a partially healed infarct, the viable but depolarized tissue in the border zone provides the substrate for a reentrant arrhythmia (Allessie et al 1977). a logical approach to enhance conduction in these circumstances is to hyperpolarize diastolic membrane potential, thereby making more Na current available. In normal myocytes the diastolic membrane potential is largely set by the inward rectifier IK1 (generated by Kir2.1 with some contribution from Kir2.2) (Zaritsky 2001). Studies overexpressing these channels are needed.

### 14.3 Enhancing rate responsiveness and/or refractoriness

Reentrant arrhythmias require reexcitation of tissue by a propagating waveform. An intervention that facilitates recovery of excitability in the pathway may restore antegrade activation and forestall retrograde invasion of that path by the reentering waveform. Alternatively, it may speed propagation of the reentering waveform such that it encounters tissue that remains refractory. Recent study showed that 6-fold overexpression of native hERG eliminates T wave alternans in isolated canine ventricular myocytes and in computer simulations (Hua et al 2004). Using a different approach, delivering a dominant negative hERG mutant (HERGG628S) via vascular infusion to a peri-infarct zone of pigs. Monomorphic ventricular tachycardia (VT) had been consistently inducible in infarcted animals before gene transfer, but one week later all HERGG628S- transferred pigs showed no such arrhythmia. This result emphasizes the therapeutic potential of yet a different local approach to VT therapy in chronic infarcts (Sasano et al 2006).

## 15. Long QT syndromes (LQTS)

Since 1991, 7 LQTS genes have been discovered and more than 300 mutations have been identified to account for the disease. Gene therapy has been suggested as a possible way to reverse the electrophysiological changes associated with the acquired or congenital long QT syndromes. Studies following short-term in vivo transfection in small animals or in isolated cultured cardiomyocytes demonstrated that overexpression of the KV4.3 gene encoding the Ito can significantly shorten the action potential durations (APD) in myocytes having a normal APD at baseline (Johns et al 1995, Hoppe et al 1999, 2000).

Blockage of the IKr prolongs the QT interval and increases the dispersion of repolarization predisposing to torsades de pointes. Molecular genetic analysis could be useful to solve subclinical mutations or polymorphisms. Individuals with cardiac potassium channel missense mutation, Q9E-hMiRP1 are predisposed to develop QT prolongation after clarithromycin administration. Experimental studies have demonstrated that cells transfected with plasmid DNA containing Q9E-hMiRP1 have reduced potassium currents on exposure to clarithromycin. Site specific gene therapy for arrhythmias by transfecting cell clones with the K<sup>+</sup> channel genes is a feasible approach to the management of LQTS (Burton et al 2003). Mutated K<sup>+</sup> channels resulting in loss of function have been implicated in LQT 1 and 2. The potassium channel alpha subunit genes KCNH2 [HERG] and KCNQ1 [KvLQT1] responsible for IKr and Iks respectively are mutated in LQTS. In normal epithelia, KCNE3 [E3] interacts with the KVQT1 [Q1] thereby augmenting the potassium currents. E3 subunit can be genetically expressed in cardiac tissues, which is normally scarce, to abbreviate the action potential duration and enhance the potassium current. This potentially prevents arrhythmias in LQTS. Adenovirus encoded E3 introduced into guinea pig ventricles shortened QT interval on homogenous transduction, but could be potentially arrhythmogenic if transduction is heterogenous (Mazhari et al 2002). Overexpression of a foreign potassium channel can also effectively abbreviate the prolonged action potential duration (APD) in failing cardiomyocytes. By adenoviral delivery of the inactivated defective Drosophila shaker B potassium channel (ShK) to cultured ventricular myocytes isolated from the rapid-pacing heart failure canine model resulted in significant shortening of the prolonged APDs in these cells. A low level of ShK expression was sufficient to modify the action potential waveform of the failing myocytes to resemble that of normal ventricular myocytes. However, the importance of adequate control of the level of transgene expression

became apparent because higher levels of ShK expression resulted in the generation of bizarre-shaped and overly shortened action potentials leading to significant impairment of the contractile properties of the transfected myocytes. (Nuss et al 1996). An alternative strategy to Ito or Ikr was suggested (Mazhari et al 2002) by over expression of the accessory subunit KCNE3 (E3, encoding MiRP2), a well-known positive regulator of the KCNQ1 (Q1, encoding KvLQT1) channel in different cell types (Schroeder et al 2000) that is not normally expressed in the heart. Ectopic expression of the KCNE3 subunit in ventricular myocytes both *ex vivo* and *in vivo* lead to its co-assembly with Q1 and to a significant increase in the slowly activating delayed rectifier potassium (Iks) current. This in turn resulted in significant shortening of APD at the cellular level and of the QT interval when delivered *in vivo*.

Another candidate current that can be used to shorten the action potential duration is the human ether-a-go-go (HERG) encoding the Ikr rapid component of the delayed rectifier potassium current. Ikr is believed to play an important role in normal repolarization (Trudeau et al 1995). and both naturally occurring mutations as well as pharmacological blockade of this current may result in QT prolongation and induction of ventricular arrhythmias in predisposed individuals (Keating et al 2001). Adenoviral delivery of the HERG gene to cultured rabbit myocytes (which usually develop action potential duration prolongation and increased incidence of early afterdepolarizations after a few days in culture) resulted in significant action potential duration abbreviation, a significant increase in the relative refractory period, and a more than fourfold decrease in the incidence of early afterdepolarizations (Nuss et al 1999).

## 16. Current problems with gene therapy

Gene therapy is in stage of infancy. Majority of trials to date are experimental, Except for a few human trials. The key to success in gene therapy is primarily dependent on the selection of a number of essential elements; an “ideal vector” that can be used to deliver the desired transgene to the relevant tissue with goal of transgene expression in the required quantity, location, and period to exert its beneficial effects. The choice of the specific vector will determine the above properties. It is important to note that only a few vectors, namely recombinant adenoviruses, adeno-associated viruses, and perhaps lentiviral vectors can achieve efficient, high-level transgene expression in post mitotic cells such as cardiomyocytes (Robbins et al 1998). Using the appropriate route of delivery is the next step for success. Intracoronary artery catheter delivery, retroinfusion through the coronary veins, direct injection into the myocardium using an epicardial or endocardial catheter approach, intra-pericardial release, and intra-cavitary catheter delivery during transient cross-clamping of the aorta were applied till today (Hajjar et al 2000).

The expected ideal result from gene therapy is a permanent cure of arrhythmias with a single stage treatment with minimal or no adverse effects. Clearly we are far from the ideal. Problems with vectors include variability in transfection capabilities, inefficient delivery at site, limited period of gene expression, and immunogenicity. The level and efficiency of expression of many trans genes are suboptimal. The tissue expression of many genes is transient. Many viral vectors are potentially immunogenic and carcinogenic.

Successful transfer of the therapeutic gene to all the myocytes at the target site is not fully achieved experimentally. The receptors for many viral vectors are present in many tissues thereby limiting the specificity of gene delivery. The interaction between vector and host

genome can result in the vector being rendered replicant and lose the therapeutic gene. Traditional vectors need to be engineered to increase their affinity for the target tissue or cell and prevent transduction to other cells (Baker 2004). In atrial fibrillation gene needs to be delivered to a wide area, the transfer methods like direct injection into myocardium fails to deliver the gene a short distance from the injection site. Gene therapy for arrhythmia treatment may itself being arrhythmogenic. As well as the incomplete restoration. In a non linear system like biological organisms, making an isolated change in a specific aberration will result in restoration of normal function only if the defect is truly isolated and is the direct cause of the phenotypic response. The long term response of a genetic modification in the myocardium is unknown, continued research and time is needed to solve these problems with certainty. studies described in the previous sections established the feasibility of gene delivery to modify the excitable properties of the myocardial tissue but also raise several limitations, include those that are inherent to other gene therapy strategies such as the possible expression of the transgene in non target organs, the potential to trigger autoimmunity, potential toxic effect of the vector or transgene, and host immune response. In addition the use of gene therapy for the treatment of cardiac arrhythmias may be hampered by a number of specific limitations; 1) limited knowledge of the molecular mechanisms underlying many of the cardiac arrhythmias and complexity of ion channel expression in various regions of the hearts may preclude the utilization of a single ion channel transgene. 2) successful antiarrhythmic gene therapy treatment strategies would require, in most cases, sustained long-term expression of the transgene (months or years). Such option is not feasible with current vector technologies. 3) limitations is related to the inability to adequately control several other key parameters such as the level of transgene expression within the cells, the number of transfected myocytes, their transmural distribution, and their regional distribution within the heart. In vivo myocardial expression using currently available viral vectors is not predictable, is relatively short-lived, is inhomogeneous, may lead to increased dispersion of different electrophysiological properties, and may actually facilitate the generation of arrhythmias.

## 17. Future prospective

Improvement in the understanding of the mechanisms underlying many of cardiac arrhythmias and the development of molecular and cellular tools suggest a future role for gene and cell therapies for treatment of different cardiac arrhythmia. Bridging the gap between the proof-of-concept and the clinical application will require important methodological developments as well as extensive animal experiments. Newer refinements in vector development and design are needed to have better transduction in cardiovascular tissue. Cell specific regulatory elements and promoters to selectively target the cardiac tissue is a potential area of interest (Beck et al 2004). Bacterial gene delivery as an alternative to viral vectors has been proposed (Palffy et al 2006). Hybrid vectors, gutted vectors and new generation non viral vectors may hold the key to future. Evidence from both viral and stem cell approaches state that proof of concept is there. Trials can be designed that permit us to test biological versus electronic pacemakers in relative safety in patients who are protected from failure of the biological unit. Tandem pacing is the proposed way to proceed clinically (patients with chronic atrial fibrillation and complete heart block); i.e. implant both a biological pacemaker and an electronic demand pacemaker in the same individual, this has been tested in dogs in complete heart block an adenoviral HCN2 construct (into the left

bundle-branch system) were delivered and an electronic demand unit, the electrode of which was placed in the right ventricular endocardial apex (Bucchi et al 2006). The biological pacemaker fired 70% of the time and was catecholamine responsive. Moreover, when the biological unit slowed, the electronic unit took over; similarly, the electronic unit sensed the biological unit well and discontinued its function when the biological function emerged, the memory function of the electronic unit can track the function of the biological unit, providing a record for the cardiologist.

Given the imperfections that still reside with electronics, the possibility of a system with no wires, no hardware, and a software that is of the body's own ion channels and autonomic nervous system offers something more appealing, if it can be made to function at the level needed and for the time required. As mentioned above, rate responsiveness is here, and improved and leadless systems have arrived as well. Therefore, there are two competitive approaches evolving. Which will dominate, traditional electronics upgraded to achieve still newer levels of success or biologics, is unknown, and the future will answer.

## 18. References

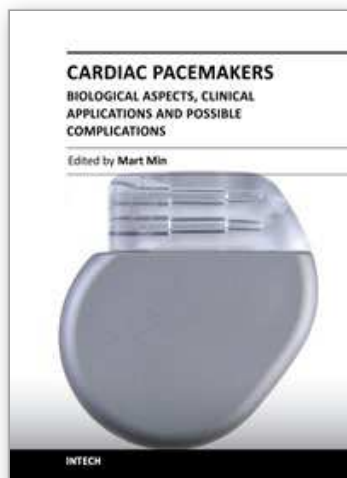
- Allessie MA, Bonke FIM, Schopman FJG. 1977. Circus movement in rabbit atrial muscle as a mechanism of tachycardia. III. The "leading circle" concept: a new model of circus movement in cardiac tissue without the involvement of an anatomical obstacle. *Circ Res*; 41:9-18.
- Armentano D, Zabner J, Sacks C, et al. 1997. Effect of the E4 region on the persistence of transgene expression from adenovirus vectors. *J Virol*; 71:2408-16.
- Baker AH. 2004. Designing gene delivery vectors for cardiovascular gene therapy. *Progs biophys mol* 2004; 84; 279-99.
- Beck C, Uramoto H, Jan Boren, et al. 2004. Tissue specific targeting for cardiovascular gene transfer. Potentials vectors and future challenges. *Curr Gene Therapy*; 4; 457-67.
- Betsuyaku T, Nnebe NS, Sundset R, et al. 2006. Overexpression of cardiac connexin45 increases susceptibility to ventricular tachyarrhythmias in vivo. *Am J Physiol Heart Circ Physiol*; 290(1):H163-H171.
- Beuckelmann DJ, Nabauer M, Erdmann E. 1993. Alterations of K<sup>+</sup> currents in isolated human ventricular myocytes from patients with terminal heart failure. *Circ Res* 73: 379-385, 1993.
- Biel M, Schneider A, Wahl C. 2002. Cardiac HCN channels: Structure, function, and modulation. *Trends Cardiovasc Med*; 12:202-216.
- Bogdanov KY, Maltsev VA, Vinogradova et al. 2006. Membrane potential fluctuations resulting from submembrane Ca<sup>2+</sup> releases in rabbit sinoatrial nodal cells impart an exponential phase to the late diastolic depolarization that controls their chronotropic state. *Circ Res*; 99:979-987.
- Bucchi A, Plotnikov AN, Shlapakova I, et al. 2006. Wild-type and mutant HCN channels in a tandem biological-electronic cardiac pacemaker. *Circulation*. 114:992-999.
- Burton DY, Song C, Fishbein I, et al. 2003. The incorporation of an ion channel gene mutation associated with the long QT syndrome (Q9E-hMiRP1) in a plasmid vector for site-specific arrhythmia gene therapy: in vitro and in vivo feasibility studies. *Human Gene Ther*; 14:907-22.
- Campbell GD, Edwards FR, Hirst GDS, et al 1989. Effects of vagal stimulation and applied acetylcholine on pacemaker potentials in the guinea pig heart. *J Physiol (Lond)*; 415:57-68.

- Davia K, Bernobich E, Ranu HK, et al 2001. SERCA2A overexpression decreases the incidence of after contractions in adult rabbit ventricular myocytes. *J Mol Cell Cardiol* 33: 1005– 1015.
- Dhein S, Larsen BD, Petersen JS, et al. 2003. Effects of the new antiarrhythmic peptide ZP123 on epicardial activation and repolarization pattern. *Cell Commun Adhes*; 10(4–6):371–378.
- DiFrancesco D. 1981. A study of the ionic nature of the pacemaker current in calf Purkinje fibres. *J Physiol.*; 314:377–393.
- Di Francesco D, Mangoni D, Maccaferri M. 1995. The pacemaker current in cardiac cells. In: Zipes DP, Jalife J, editors. *Cardiac electrophysiology: from cell to bedside*. 2nd ed. Philadelphia: WB Saunders; p. 96–103.
- Donahue JK, Heldman AW, Fraser H, et al. 2000. Focal modification of electrical conduction in the heart by viral gene transfer. *Nat Med* 6: 1395– 1398.
- Donahue JK, Kikkawa K, Johns DC, et al 1997. Ultrarapid, highly efficient viral gene transfer to the heart. *Proc Natl Acad Sci U S A.*; 94:4664–8.
- Eddy Kizana, Connie Y. Chang, et al. 2007. Gene Transfer of Connexin43 Mutants Attenuates Coupling in Cardiomyocytes Novel Basis for Modulation of Cardiac Conduction by Gene Therapy. *Circ Res.*; 100:1597–1604.
- Edelberg JM, Aird WC, Rosenberg RD. 1998. Enhancement of murine cardiac chronotropy by the molecular transfer of the human  $\beta$ 2-adrenergic receptor cDNA. *J Clin Invest*; 101:337–343.
- Edelberg JM, Huang DT, Josephson ME, et al. 2001. Molecular enhancement of porcine cardiac chronotropy. *Heart*; 86:559–562.
- Ennis IL, Li RA, Murphy AM, et al .2002. Dual gene therapy with SERCA1 and Kir2.1 abbreviates excitation without suppressing contractility. *J Clin Invest* 109: 393–400.
- Fast VG, Darrow BJ, Saffitz JE, et al. 1996. Anisotropic activation spread in heart cell monolayers assessed by high-resolution optical mapping. Role of tissue discontinuities. *Circ Res* 79: 115–127, 1996.
- Feld Y, Melamed-Frank M, Kehat I, et al. 2002. Electrophysiological modulation of cardiomyocytic tissue by transfected fibroblasts expressing potassium channels: a novel strategy to manipulate excitability. *Circulation* 105: 522–529.
- Furrer M, Naegeli B, Bertel O. 2004. Hazards of an alternative medicine device in a patient with a pacemaker. *N Engl J Med*; 350(16): 1688– 90.
- Gaudesius G, Miragoli M, Thomas SP, et al. 2003. Coupling of cardiac electrical activity over extended distances by fibroblasts of cardiac origin. *Circ Res* 93: 421–428.
- Gepstein L. 2002. Derivation and potential applications of human embryonic stem cells. *Circ Res* 91: 866–876.
- Gollob MH, Jones DL, Krahn AD, et al. 2006. Somatic mutations in the connexin 40 gene (GJA5) in atrial fibrillation. *N England J Med*; 354(25):2677–2688.
- Hajjar RJ, del Monte F, Matsui T et al 2000. Prospects for gene therapy for heart failure. *Circ Res* 86: 616–621.
- Hatcher CJ, Basson CT. 2009. Specification of the Cardiac Conduction System by Transcription Factors *Circ Res.* September 25; 105(7): 620–630.
- Hoppe UC, Johns DC, Marban E, t al. 1999. Manipulation of cellular excitability by cell fusion: effects of rapid introduction of transient outward K<sup>+</sup> current on the guinea pig action potential. *Circ Res* 84: 964–972.
- Hoppe UC, Marban E, Johns DC. 2000. Molecular dissection of cardiac repolarization by in vivo Kv4.3 gene transfer. *J Clin Invest* 105: 1077– 1084.

- Hua F, Johns DC, Gilmore RF Jr. 2004. Suppression of electrical alternans by overexpression of HERG in canine ventricular myocytes. *Am J Physiol Heart Circ Physiol*; 286:H2342–H2352.
- Isner JM.(2002) Myocardial gene therapy. *Nature* 415: 234–239.
- Johns DC, Nuss HB, Chiamvimonvat N, et al .1995. Adenovirus-mediated expression of a voltage-gated potassium channel in vitro (rat cardiac myocytes) and in vivo (rat liver). A novel strategy for modifying excitability. *J Clin Invest* 96: 1152–1158.
- Keating MT and Sanguinetti MC. 2001. Molecular and cellular mechanisms of cardiac arrhythmias. *Cell* 104: 569–580.
- Kehat I, Amit M, Gepstein A, et al 2001b. Functional integration of human embryonic stem cell derived cardiomyocytes with preexisting cardiac tissue: Implication for myocardial repair. *Circulation* 104, Suppl. II: 618.
- Kehat I, Gepstein A, Spira A, et al 2002. High-resolution electrophysiological assessment of human embryonic stem cell-derived cardiomyocytes: a novel in vitro model for the study of conduction. *Circ Res* 91: 659–661.
- Kehat I, Kenyagin-Karsenti D, Snir M, et al. 2001a. Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes. *J Clin Invest* 108: 407–414.
- Kehat I, Khimovich L, Caspi O, et al. 2004. Electromechanical integration of cardiomyocytes derived from human embryonic stem cells. *Nat Biotechnol*; 22:1282–2389.
- Kubo Y, Baldwin TJ, Jan YN, et al 1993. Primary structure and functional expression of a mouse inward rectifier potassium channel. *Nature* 362: 127–133.
- Kusumoto FM and Goldschlager N. 1996 . Cardiac pacing. *N Engl J Med* 334: 89–97.
- Labhasetwar V, Bonadio J, Goldstein S, et al 1998. A DNA controlled-release coating for gene transfer: transfection in skeletal and cardiac muscle. *J Pharm Sci.*; 87:1347-50.
- Lasker SM, Han D, Kline RP. 1997. Zatebradine slows ectopic ventricular rhythms in canine heart 2 hours after coronary artery ligation. *J Cardiovasc Pharmacol*; 29 (5):662– 9.
- Lau DH, Clausen C, Sosunov EA, et al. 2009. Epicardial border zone overexpression of skeletal muscle sodium channel, SkM1, normalizes activation, preserves conduction and suppresses ventricular arrhythmia: an in silico, in vivo, in vitro study. *Circulation*. Jan 6; 119 (1):19-27.
- Lee ER, Marshall J, Siegel CS, et al. 1996. Detailed analysis of structures and formulations of cationic lipids for efficient gene transfer to the lung. *Hum Gene Ther*; 7: 170117.
- Lehnart SE, Donahue JK. 2003. Coronary perfusion cocktails for in vivo gene transfer. *Methods Mol Biol*; 219:213–218.
- Marban E. 2002 . Cardiac channelopathies. *Nature* 415: 213–218.
- Marban E. 1999. Heart failure: the electrophysiologic connection. *J Cardiovasc Electrophysiol* 10: 1425–1428.
- Martin ET, Coman JA, Shellock FG, et al. 2004. Magnetic resonance imaging and cardiac pacemaker safety at 1.5- Tesla. *J Am Coll*; 43 (7):1315– 24.
- Masson-Pevet, M. 1979 . The Fine Structure of Cardiac Pacemaker Cells in the Sinus Node and in Tissue Culture (thesis). Amsterdam, University of Amsterdam.
- Mazhari R, Nuss HB, Armoundas AA, et al 2002 . Ectopic expression of KCNE3 accelerates cardiac repolarization and abbreviates the QT interval. *J Clin Invest.* ; 109:1083-90.
- Members of the Sicilian gambit. 2001. New approaches to antiarrhythmic therapy, partII; *Circulation*; 104: 2990-2994.
- Miake J, Marbán E, Nuss HB. 2002. Gene therapy: biological pacemaker created by gene transfer. *Nature*; 419:132–133.

- Michaelsson M, Jonzon A, Riesenfeld T. 1995. Isolated congenital complete heart block in adult life: a prospective study. *Circulation*; 92: 442-9.
- Muller-Ehmsen J, Peterson KL, Kedes L, et al. 2002. Rebuilding a damaged heart: long-term survival of transplanted neonatal rat cardiomyocytes after myocardial infarction and effect on cardiac function. *Circulation* 105: 1720-1726.
- Mummery C, Ward-van Oostwaard D, Doevendans P, et al. 2003. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation* 107: 2733-2740.
- Nattel S. 2002. New ideas about atrial fibrillation 50 years on. *Nature* 415: 219-226.
- Nuss HB, Johns DC, Kaab S, et al. 1996. Reversal of potassium channel deficiency in cells from failing hearts by adenoviral gene transfer: a prototype for gene therapy for disorders of cardiac excitability and contractility. *Gene Ther* 3: 900-912.
- Nuss HB, Marban E, and Johns DC. Et al. 1999. Overexpression of a human potassium channel suppresses cardiac hyperexcitability in rabbit ventricular myocytes. *J Clin Invest* 103: 889-896.
- Palfy R, Gadlik R, J Hodosy, M Behuliak. 2006. Bacteria in gene therapy; Bactofection versus alternative gene therapy. *Gene Therapy* (13) 101-105.
- Peters NS, Coromilas J, Severs NJ, Wit AL. 1997. Disturbed connexin43 gap junction distribution correlates with the location of reentrant circuits in the epicardial border zone of healing canine infarcts that cause ventricular tachycardia. *Circulation*; 95(4):988-996.
- Plotnikov AP, Shlapakova I, Szabolcs MJ, et al. 2007. Xenografted adult human mesenchymal stem cells provide a platform for sustained biological pacemaker function in canine heart. *Circulation*; 116:706-713.
- Plotnikov AN, Sosunov EA, Qu J, et al. 2004. Biological pacemaker implanted in canine left bundle branch provides ventricular escape rhythms that have physiologically acceptable rates. *Circulation*; 109:506-512.
- Potapova I, Plotnikov A, Lu Z, et al. 2004. Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers. *Circ Res*. Apr 16; 94(7):952-9.
- Qu J, Barbuti A, Protas L, et al 2001. HCN2 overexpression in newborn and adult ventricular myocytes: distinct effects on gating and excitability. *Circ Res*; 89:E8- E14.
- Qu J, Plotnikov AN, Danilo P Jr, et al. 2003. Expression and function of a biological pacemaker in canine heart. *Circulation*; 107:1106-1109.
- Ramón A. Espinoza-Lewis, Ling Yua, et al 2009. Shox2 is essential for the differentiation of cardiac pacemaker cells by repressing Nkx2-5. *Dev Biol*. March 15; 327(2): 376-385.
- Reddy VY, Reynolds MR, Neuzil P, et al. 2007. Prophylactic catheter ablation for the prevention of defibrillator therapy. *N Engl J Med*; 357:2657-65.
- Reinlib L and Field L. 2000. Cell transplantation as future therapy for cardiovascular disease? a workshop of the National Heart, Lung, and Blood Institute. *Circulation* 101: E182-E187.
- Robbins PD, Tahara H, and Ghivizzani SC. 1998. Viral vectors for gene therapy. *Trends Biotechnol* 16: 35-40.
- Roberts R and Brugada R. 2003. Genetics and arrhythmias. *Annu Rev Med* 54: 257-267.
- Robinson RB, DiFrancesco D. 2001. Sinoatrial node and impulse initiation. In: Spooner PM, Rosen MR, editors. *Foundations of cardiac arrhythmias*. New York: Marcel Dekker; p. 151- 70.
- Roth DM, Lai NC, Gao MH, et al. 2004. Indirect intracoronary delivery of adenovirus encoding adenylyl cyclase increases left ventricular contractile function in mice. *Am J Physiol Heart Circ Physiol*; 287:H172-177.

- Rosen M. 2005. Biological pacemaking: In our lifetime? *Heart Rhythm*; 2:418–428.
- Rook MB, van Ginneken AC, de Jonge B, et al. 1992. Differences in gap junction channels between cardiac myocytes, fibroblasts, and heterologous pairs. *Am J Physiol Cell Physiol* 263: C959–C977.
- Rosen MR, Brink PR, Cohen IS, et al. 2004. Genes, stem cells and biological pacemakers. *CardiovasRes*; 64:12–23.
- Sasano T, McDonald AD, Kikuchi K, et al. 2006. Molecular ablation of ventricular tachycardia after myocardial infarction. *Nature Med*; 12:1256–1258.
- Satoh, H. and Uchida, T. 1993. Morphological and electrophysiological changes induced by calciumionophores (A23187 and X-537) in spontaneously beating rabbit sino-atrial node cells. *Gen. Pharmacol.* 24: 49–87.
- Schroeder BC, Waldegger S, Fehr S, et al. 2000. A constitutively open potassium channel formed by KCNQ1 and KCNE3. *Nature* 403: 196–199.
- Shinagawa, Y., Satoh, H. and Noma, A. 2000. The sustained inward current and inward rectifier K<sup>+</sup> current in pacemaker cells dissociated from rat sinoatrial node. *J. Physiol. (Lond.)* 523: 593–605.
- Smith AE. 1999. Gene therapy--where are we? *Lancet*. 354 Suppl 1:SI1-4.
- Terracciano CM, Hajjar RJ, Harding SE. 2002. Overexpression of SERCA2a accelerates repolarisation in rabbit ventricular myocytes. *Cell Calcium* 31: 299–305.
- Thollon C, Bedut S, Villeneuve N, et al. 2007. Use-dependent inhibition of hHCN4 by ivabradine and relationship with reduction in pacemaker activity. *Br J Pharmacol*; 150:37– 46.
- Trudeau MC, Warmke JW, Ganetzky B, et al 1995. HERG, a human inward rectifier in the voltage-gated potassium channel family. *Science* 269: 92–95.
- Tse Hung-Fat ,Tian Xue, PhD; Chu-Pak Lau et al .2006. Bioartificial Sinus Node Constructed via In Vivo Gene Transfer of an Engineered Pacemaker HCN Channel Reduces the Dependence on Electronic Pacemaker in a Sick-Sinus Syndrome Model ,*Circulation* ; 114:1000-1011.
- Yankelson, Yair Feld, MD, Tal Bressler-Stramer et al. 2008. Cell Therapy for Modification of the Myocardial Electrophysiological Substrate *Circulation*;117:720-731.
- Yla-Herttuala S, Martin JF. 2000 .Cardiovascular gene therapy. *Lancet*; 355; 213-222.
- Zimmet JM, Hare JM. 2005. Emerging role for bone marrow derived mesenchymal stem cells in myocardial regenerative therapy. *Basic Res Cardiol*; 100:471–481.
- Zaritsky JJ, Redell JB, Tempel BL, et al. 2001. The consequences of disrupting cardiac inwardly rectifying K<sup>+</sup> current (IK1) as revealed by the targeted deletion of the murine Kir2.1 and Kir2.2 genes. *J Physiol*; 533.3:697–710.
- Zivin A, Bardy GH, Mehra R. 2001, Cardiac pacemakers. In: Spooner PM, Rosen MR, editors. *Foundations of cardiac arrhythmias*. New York: Marcel Dekker; p. 571– 98.a
- Zivin A, Bardy GH, Mehra R. 2001. Implantable cardioverter defibrillators. In: Spooner PM, Rosen MR, editors. *Foundations of cardiac arrhythmias*. New York: Marcel Dekker; p. 599–619.b



## **Cardiac Pacemakers - Biological Aspects, Clinical Applications and Possible Complications**

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Clinical usage of artificial pacing dates back to 1958, when the battery powered cardiac pacemakers became available. Modern implantable pacemakers are the complicated electronic devices operating 10 years continuously without battery exchange. Though the development of devices is not a primary topic of the book, certain efforts towards developing of biologic pacemakers through tissue engineering and studying of cell synchronization are discussed. The main attention is paid to implementations of pacemakers in different medical situations oriented towards widening the clinical indications for implanting the cardiac pacemakers. New methods and devices in cardiac resynchronization therapy (CRT) have received particular attention. Placing of pacing electrodes has been treated soundly. Furthermore, emerging of complexities and complications in new clinical situations and other safety problems have been discussed thoroughly. The authors have derived the used information from their own clinical practice and experiences of their medical colleagues. These and other pragmatic features can be acknowledged as the most valuable asset of the book.

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