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Guest Editorial

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MENDING THE BROKEN HEART

When David Hearse and Roberto Ferrari invited me to edit this issue and to contribute one of the articles I had mixed feelings. While the title proposed suggests a romantic paperback novel, the subject matter it incorporates is potentially groundbreaking: arguably, gene and cell therapies are fields that will apply to much of cardiovascular and other medical therapeutics in the future.

The beginnings have been rocky, especially with regard to gene therapy. This largely reflects the toxicity of viral vectors used in the initial gene therapies that were administered to patients. Cell therapy has had a more successful beginning, first appearing on the scene in 1956. In that year, E. Donnall Thomas obtained long-term survival by transplanting bone marrow into a patient with leukemia. Since that time both the efficacy and safety of bone marrow transplant have been documented and detailed for the treatment of certain cancers and immunodeficiency diseases. Obviously there are toxicities and shortcomings, but the life-saving nature of the therapy is unquestionable. And this history has provided assurance to subsequent investigators studying marrow-derived cells, assurance that the cells they deliver to patients likely will cause no harm. Important with regard to the safety issue is that in most instances the cells administered have been autologous.

Given the limited safety concerns, there has been rapid advancement of cell therapy in humans with myocardial infarction and/or with heart failure. There has been almost marginal benefit, with the occasional report looking hopeful, but the safety of the procedure appears to have been validated.

So if gene therapy has produced death and disease and cell therapy has been shown safe if not necessarily effective (except for bone marrow transplantation as stated above), why devote an issue to “Mending the Broken Heart?” My own prejudice is that

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gene and cell therapies are at a developmental stage not unlike antimicrobials in the 1930s. Sulfa drugs then appeared in a field that previously depended on folk medicine, carbolic acid, arsenicals, and the like. And although sulfa drugs were far from an ideal antimicrobial, they were not only a vast improvement, but they presaged the arrival of antibiotics in the clinic in the 1940s. To succeed, investigators had to accept the germ theory of disease, a nineteenth century concept verified scientifically by Koch in 1875, and then learn a new language: partly that of the germs themselves and partly that of the molds and other sources from which they made their antibiotics. Whereas Tyndall in 1875 had already noted the antimicrobial properties of \textit{Penicillium}, it was not until 1928 that Fleming replicated the observation and did one essential additional experiment: he used the soup in which the mold grew to treat bacterial colonies, saw that it lysed them, called the soup penicillin and gave new impetus to a burgeoning field of therapy. All that remained was to develop a stable, clinically useful system to make penicillin, and this required another decade.

Those individuals now working in the field of cell and gene therapy are also having to learn a language; arguably a far more obscure language than that of the bacilli and molds that dot the antibacterial landscape. What are the signals and factors that determine stem cell growth and fate? How can these be manipulated safely? How reproducibly can cells be made to grow, to mature, but not to evolve into neoplasms? The list is almost endless. These issues and others relating to the use of cells to repair/regenerate myocardium are considered in this issue by Ira Cohen and Glenn Gaudette. The segue from the cells under investigation and problems in understanding their biology to their use in clinical settings of myocardial infarction and heart failure is considered by Kai Wollert and Helmut Drexler. Review of the types of cells available, the means for their administration and the clinical successes and limitations to date is both encouraging and chastening as it indicates how far we have to go to understand what we are doing in the clinic and how to do it better.

Kirk Hammond and Tong Tang look at another side of the coin: their area is the viral approaches to gene delivery in the setting of heart failure. They provide a summary of progress made as well as of the strengths and limitations of different viral approaches. They emphasize preclinical studies showing that gene transfer improves left ventricular contractility and attenuates deleterious remodeling in myocardial infarction–associated congestive failure, and express optimism that these outcomes will be replicated in patients with congestive failure. Finally, my own paper, authored with Peter Danilo and Richard Robinson, considers viral vector–delivered gene therapy as a means to insert novel ion channel constructs into the heart in attempting to prevent induction of lethal ventricular tachycardias. The goal here, in proof-of-concept experiments, is to provide local therapy with these constructs, thereby maintaining an antiarrhythmic action while limiting toxicity.
I encourage the reader to think of the lead article and the three accompanying papers as snapshots of a field that contains a lot of empty space. We are gathering islands of information, and when we have learned a sufficient amount, the continuum of what is needed for us to understand what cell and what therapy to use in any particular situation will have been clarified. But that day is still far off. In the meantime, we learn and we worry: worry that the pressure investigators feel to bring treatments to the clinic and the economic pressures of raising funds to perform research and deliver its benefits to human subjects may poison the field by leading to premature application and unforeseen toxicity or failure. This certainly was the case for gene therapy, even though we are now having another “go” at it; it would be devastating for the same thing to happen with cell therapy. A different concern, but no less dismaying, is the administration of various cell therapies to desperately ill patients in some countries with the same abandon that characterized the selling of snake oil to a gullible public 100 years ago.

Finally, I ask the reader to save this volume for about 20 years, not because of any pretensions about it, but to perform an easy experiment. The experiment is simply to open the volume in 20 years to see, with appropriate hindsight, the extent to which this bold new future we envision for gene and cell therapies has made its way into the mainstream of cardiovascular therapy.
Regenerating the heart: new progress in gene/cell therapy to restore normal mechanical and electrical function

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The last decade has seen the complete sequencing of the human genome and the development of approaches to deliver therapeutic genes to the heart. Equally significant advances have also been made in stem cell biology. The accessibility of these new tools in our therapeutic arsenal raises the exciting question of whether normal mechanical and electrical function can now be regenerated in the diseased heart. In this article, we consider how to choose targets to regenerate mechanical and electrical function. In considering mechanical function, we start by describing its determinants (ie, both active and passive properties), and then review regenerative applications of gene/cell therapy. We also consider arrhythmias, focusing on the potential advantages of gene/cell therapy over pharmacotherapy or devices, and then discuss the development of biological pacemakers as one example. Overall, the future is bright; gene/cell therapy approaches have reached the proof-of-principle stage for both mechanical and electrical regeneration. Some mechanical studies have even reached clinical trials; however, evidence of long-term efficacy is lacking. Ultimately, to achieve therapeutic success with gene and cell therapies, it will be important to gain a better understanding of their mechanisms of action.

In setting the stage for “Mending the Broken Heart,” it is our purpose to consider the potential of gene and/or cell therapy to restore mechanical and electrical function lost to disease. With the development of viral and stem cell technologies, new therapeutic approaches to restore normal function are possible that were not even dreamt of a decade ago. One might think that gene/cell therapy approaches would be brought to the bedside, for electrical and mechanical dysfunction, at the same rate. However, because there is no effective long-term therapy for heart disease, the development of biological pacemakers has had to be accelerated. Indeed, in recent years, several companies have been formed to translate this technology to the bedside. To evaluate the potential of this approach, we focus on islet cells as an example. Islet cells are the beta cells of the pancreas, which secrete the hormone insulin. In diabetes, there is a deficiency of insulin secretion, which leads to hyperglycemia. In 1988, we and others reported that islets could be transplanted and that insulin could be secreted. However, long-term efficacy was limited by the gradual destruction of the transplanted islet cells. To overcome this problem, it was reasoned that islets could be genetically engineered to produce a cytokine that enhances their survival. As a result, several groups have reported that islet cells can be transplanted and can secrete insulin for long periods of time. However, the clinical application of islet cell transplantation is limited by the number of islet cells that can be harvested from a patient and the immune response to the transplanted islets. One alternative is to use stem cells as a source of islet cells. Stem cells are undifferentiated cells that can give rise to all cell types in the body. It is possible that stem cells could be differentiated into islet cells and transplanted to patients with diabetes. However, the use of stem cells as a source of islet cells is limited by the number of islet cells that can be harvested and the immune response to the transplanted cells. Overall, the future is bright; gene/cell therapy approaches have reached the proof-of-principle stage for both mechanical and electrical regeneration. Some mechanical studies have even reached clinical trials; however, evidence of long-term efficacy is lacking. Ultimately, to achieve therapeutic success with gene and cell therapies, it will be important to gain a better understanding of their mechanisms of action.
failure, current approaches employing gene/cell therapy to repair damage induced by myocardial infarction have moved rapidly to clinical trials, resulting in only modest success (see the article by Wollert and Drexler in this issue). By contrast, many existing therapies for electrical dysfunction (electronic pacemakers, implantable cardioverter-defibrillators [ICDs]) are effective and lifesaving, and, thus, the “bar” is higher, and translation to the clinic has lagged.

Our aims in this article are: to briefly discuss current therapies for regenerating myocardial function; consider the targets currently under investigation; discuss the means to evaluate the success of these studies; and in the process, set the stage for new target selection. It is our purpose to emphasize “the dream” of gene/cell therapy; however, only intensive investigation in the laboratory and the clinic over the coming years will generate “the reality.”

**THE PROBLEM**

The World Health Organization predicts 11.1 million deaths from coronary heart disease (CHD) in the year 2020. In the United Kingdom, CHD is the most common cause of death, claiming 101,000 lives in 2005. In the same year in the United States, 16 million Americans were living with CHD, 8.1 million of whom had experienced a myocardial infarction, while 5.3 million suffered heart failure. In 2007 alone, about 1.2 million Americans suffered a myocardial infarction with a mortality of 451,000. The direct and indirect costs of heart failure in the United States are estimated at 34.8 billion dollars. Cardiac arrhythmias are another major affliction. In 2004, they resulted in, or contributed to, 458,800 deaths in the United States. Arrhythmias resulting from sick sinus syndrome or atrioventricular block necessitate the implantation of pacemakers, and in patients at risk of ventricular fibrillation, ICDs are indicated. There were 180,000 pacemakers and 91,000 defibrillators implanted in 2005.

**ACTIVE AND PASSIVE FUNCTION IN THE NORMAL HEART**

The mechanical function of the heart can be separated into active and passive components, both of which are potential targets for regenerative therapy (Figure 1). The active function results from the contraction of the myocytes, and is primarily responsible for systole. In myocardial infarction and heart failure, the myocytes are dysfunctional or destroyed; therefore, active function is decreased. However, the passive function of the ventricle also plays an important role in contraction (Figure 2). In the normal heart, cells are attached to each other and the extracellular matrix, providing a compliant environment. As the heart contracts during systole, this compliant, passive material allows for normal systolic contraction, and does not impede diastolic relaxation. If the environment is stiff, which can occur in infarcted myocardium in which myocytes and extracellular matrix are replaced by a noncompliant scar, systolic contraction is decreased. Diastolic function is also affected, as a stiff environment decreases diastolic relaxation. During contraction, the myocytes shorten, and deform the substrate to which they are anchored. If the myocytes are attached to a stiff material, such as collagenous scar tissue, it is more difficult to deform than normal extracellular matrix. This is likely to occur in nontransmural infarcts, and in the...
border zone between infarcted and spared myocardium. Thus, one means to improve overall function is to restore the passive properties of the myocardium.

**APPROACHES TO IMPROVING PASSIVE MECHANICAL PROPERTIES IN THE INFARCTED HEART**

In most cases, the body’s reaction to a myocardial infarction is scar formation. This fulfills an important need: mechanical stabilization to prevent ventricular wall rupture. However, scar stiffness can impede diastolic filling. Thus, increasing the compliance of the scar may lead to improved ventricular performance. It should be noted that increasing the compliance of the scar excessively has the potential to lead to aneurysm formation (Figure 3, page 10). Scar tissue is largely composed of collagen and fibroblasts. The restoration of blood flow to scar tissue may increase its compliance. For a consideration of angiogenesis employing viral therapies, see the article by Hammond and Tang in this issue.4
Matsubayashi et al seeded vascular smooth muscle cells onto a biopolymer scaffold to replace myocardial scar tissue in a rat model. Eight weeks after scaffold implantation, they noted increased extracellular elastin and increased fractional area shortening. As the patch region was akinetic, these results suggest that the improvement was not due to the restoration of contraction in the region, but probably to scaffold compliance. Implantation of a different biopolymer scaffold on the epicardial surface of a myocardial infarct by Fujimoto et al resulted in an increased presence of smooth muscle cells in the infarct tissue. Improved fractional area shortening was also noted in the patch group, as was compliance of the heart. Confirming the importance of passive properties, Wall et al employed a computational model of the ventricle, and demonstrated that the injection of a compliant material could, in theory, improve mechanical function.

The addition of cells or compliant materials can decrease the stiffness of scarred myocardium. Berry et al recently demonstrated that the injection of mesenchymal stem cells (MSCs) after myocardial infarction leads to increased compliance. These cells also decreased fibrosis in the infarcted region. No evidence of differentiated MSCs was found, suggesting that nondifferentiated MSCs improve compliance.

Interestingly, nearly any type of cell delivered to the heart appears to improve myocardial function, although differentiation of most cells into cardiac myocytes appears to be limited. For example, skeletal myoblasts have been shown to improve myocardial function, yet once differentiated into myotubes, they do not form gap junctions, thereby limiting their ability to contract in synchrony with spared myocardium.

The addition of elastin to collagenous scar tissue may also improve regional mechanical function in the infarcted heart. Recent work from Li’s laboratory has demonstrated that the overexpression of elastin by endothelial cells improved cardiac function compared with nonexpressing cells. The addition of elastin also reduced scar size, suggesting it may have replaced the collagen, or signaled for decreased collagen synthesis. Surgically, the replacement of dysfunctional myocardial tissue can be accomplished through the endoventricular circular patch plasty procedure (also referred to as the Dor Procedure). While most surgeons use Dacron as patch material, the use of a more compliant scaffold may provide the opportunity to improve regional mechanical function. We have recently demonstrated that the implantation of a compliant patch (made from the isolated extracellular matrix of a porcine urinary bladder) improves regional mechanical function in contrast to a Dacron patch. While many other factors probably play a role in the improved function, scaffold compliance may be essential to the improved contraction observed in the patch region.

While increasing the passive function of infarcted myocardium may lead to improved overall heart performance, the regeneration of functional myocardium is necessary for the contraction of the heart. Regenerated myocardium must actively contract, which requires contractile cells.

**APPROACHES TO IMPROVING CONTRACTILE PROPERTIES IN THE INFARCTED HEART**

Contractile cells are needed to fully restore regional mechanical function. In myocardial infarction–associated heart failure, at least 1 billion myocytes are lost and must be replaced.

**Cells used in regenerative therapy: expected properties**

Prior to understanding the different potential mechanisms for regenerating active function in infarcted myocardium, we need to understand the aims of a
successful regenerative strategy. Cells that are successful at regenerating functional myocardium must possess the following (adapted from reference 15):

- **Proteins specific to cardiac myocytes.** Proteins, specific to mechanical function (e.g., sarcomeric α-actinin, ventricular myosin heavy chain), electrical function (gap junctions, cardiac-specific ion channels), or electrical-mechanical coupling of the myocyte, must be present.

- **Sarcomeres.** Efficient contraction of myocytes is dependent on the organization of actin-myosin cross-bridges to produce longitudinal shortening.

- **Ion channels to generate an action potential.** Contraction is preceded by an action potential that facilitates the inflow of calcium from the extracellular space and its triggered release from the sarcoplasmic reticulum, which is essential for the contraction of the myocyte.

- **Functional excitation-contraction mechanisms.** The generation of an action potential needs to be coupled to contraction in the myocyte.

- **Gap junction proteins.** The ability to form gap junctions is essential for the formation of a functional syncytium, necessary for efficient activation to occur, which provides the template that orders the contraction of the heart.

When delivered to the heart, these cells must also:

- **Increase active regional mechanical function.** When the cells are delivered to an infarct, they must increase the contraction of the infarcted regions.

- **Regenerate myocyte mass that correlates with function.** The number of regenerated cells in an infarcted region should correlate with improved mechanical function in the region, if the improved function is directly due to the cardiac differentiation of delivered cells.

- **Increase overall heart function.** The ultimate success of regenerated myocytes is dependent on the restoration of mechanical function to the heart.

While this list does not delineate all the functions of a successful regenerative therapy, it outlines the major accomplishments that need to be fulfilled. With these functions defined, various means can now be considered. All methods to regenerate active mechanical function must include the addition of cell mass to replace the myocytes lost to infarction. These methods can be divided into endogenous and exogenous approaches. Endogenous methods involve recruiting native cells to replenish myocyte mass. Examples would include the induction of native myocytes to proliferate, or the cardiac differentiation of native stem cells that reside in the heart, or that have homed to the infarcted tissue. Exogenous methods rely on the addition of autologous or allogenic cells to the heart. Embryonic stem cells (ESCs), mesenchymal stem cells, cardiac stem cells (CSCs), and induced pluripotent stem cells (iPSCs) can be expanded in culture, and then delivered to the heart. Each serves as a potential means of exogenous regeneration. Both endogenous and exogenous methods are currently under investigation.

### Endogenous regeneration of the heart

**Myocyte proliferation**

Common dogma suggests that cardiac myocytes lack the ability to proliferate. However, in 2001, data from Anversa’s laboratory suggested that myocytes may reenter the cell cycle in regions bordering a myocardial infarction. The data demonstrated that approximately 4% of the myocytes in the border zone between infarcted and viable myocardium were positive for Ki-67, a nuclear molecule involved in cell proliferation. However, after myocardial infarction, the heart does not reconstitute a sufficient number of myocytes, so the damaged tissue is replaced by scar tissue. This does not rule out the ability of myocytes to proliferate; it only suggests that myocyte proliferation is limited, and not favored over scar formation. In fact, myocyte proliferation has also been documented in other models. For example, noninfarcted zebra fish and amphibians regenerate amputated parts of the heart. This occurs as a result of mitotic expansion of cardiomyocytes. Myocyte proliferation has also been noted in a mammalian model, the medical research laboratory (MRL) mouse. These mutant mice regenerate wounds without forming scars, presumably due to an altered mechanism of extracellular matrix remodeling. The mitotic index of myocytes in the MRL mouse was shown to be 10% to 20% during regeneration of cryogenically injured heart.

Other investigators have also documented myocyte proliferation in various environments. Schuster et al induced endogenous myocyte proliferation in a rat infarct model by delivering human endothelial progenitor cells. By using a rat-specific antibody to Ki-67, they assured that native rat myocytes, rather than the exogenously delivered human cells, entered the cell cycle. In vitro experiments have also demonstrated the possibility of inducing myocyte proliferation. Busk et al induced myocyte proliferation through the overexpression of cyclin D2, a cyclin necessary for cells to progress through the G1 phase and into the S phase of the cell cycle. Recently, p38 mitogen-activated protein (MAP) kinase inhibition has been shown to allow adult cardiomyocytes to proliferate in vitro.
In summary, adult mammalian cardiac myocytes can proliferate, and could, in principle, replace the myocytes lost to disease. However, to harness this potential therapy, an improved understanding of cell cycle progression in adult mammalian cardiac myocytes is necessary.

**Mobilizing native stem cells**

The adult human possesses multipotent cells that reside in many different tissues, including bone marrow, fat, and heart. Recent evidence suggests that cells from adipose tissue, bone marrow (described in detail below), and heart may be able to differentiate into myocytes. This property provides an opportunity to develop a therapy that will mobilize these cells, and have them home to the injured myocardium.

While stem cells can home to the infarcted myocardium, increasing homing provides more help. Several homing molecules have been identified, including monocyte chemotactic protein-3, stromal-derived factor-1, and its receptor, chemokine receptor 4 (CXCR4). Increased homing may also be facilitated by the addition of growth factors, which mobilize CXCR4. The initial data are encouraging. However, it remains a challenge to induce a sufficient number of these cells to home to an infarct, and improve mechanical function by differentiating into myocytes, or by releasing paracrine factors that induce native myocytes to proliferate, and provide appreciable recovery of mechanical function.

**Exogenous regeneration of the heart**

The aim of exogenous regeneration is to take a stem cell capable of becoming a myocyte in vivo, and induce it to choose a cardiac lineage. Because of the enormous number of myocytes required, and the paucity of proliferation once fully differentiated (see above section), current strategies have emphasized in vitro expansion and cardiac commitment prior to delivery.

This strategy is illustrated in Figure 4. We now consider the cell types that have been employed. Additional details on the success of cell therapy can be obtained from the article by Wollert and Drexler in this issue.

**Skeletal myoblasts**

Skeletal myoblasts, which are skeletal myocyte progenitors, were the first cellular therapy for myocardial infarction. Like cardiac myocytes, skeletal myoblasts are striated; however, their action potentials are brief in comparison with heart cells. This may account for some of the arrhythmias reported with this therapy. However, delivery of skeletal myoblasts has led to improved mechanical function in animal models, probably due to improved passive function of the heart. These encouraging results have led to clinical trials. Some trials have shown improvements in cardiac function, although ventricular tachyarrhythmia remains a major concern. In February 2006, Genzyme halted its phase 2 clinical trials after enrolling 95 patients, citing that a significant improvement in heart function was unlikely to come from the study. Despite this setback, it appears that clinical trials are continuing.

When skeletal myotubes differentiate from skeletal myoblasts, they do not form gap junctions. Without these intercellular ion channels, electrical activity cannot propagate between the endogenous cardiac myocytes and the exogenous differentiated skeletal myotubes, making it nearly impossible for the transplanted cells to contract synchronously with the native myocytes.

In summary, adult mammalian cardiac myocytes can proliferate, and could, in principle, replace the myocytes lost to disease. However, to harness this potential therapy, an improved understanding of cell cycle progression in adult mammalian cardiac myocytes is necessary.
**Embryonic stem cells**

Many studies have shown the ability of ESCs to differentiate into cardiomyocytes. To differentiate ESCs down a cardiac lineage, embryoid bodies (EBs) are formed. This method can include culturing cells in hanging drops of media to allow them to coalesce and form three-dimensional structures. After formation of the EBs, they are plated for a few weeks. Kehat et al. showed that the resultant cells express cardiac proteins and approximately 8% of the EBs contract 21 days after plating. He et al. demonstrated that some cells grown from the EBs generate action potentials, some of which resemble atrial-, ventricular- and nodal-like action potentials. For more on the cardiogenicity of ESCs, see the review by Laflamme and Murry.

Delivery of cardiomyocytes derived from ESCs has improved mechanical function in the injured heart. Using a myocardial infarction mouse model, Kofidis et al. demonstrated that injected human ESCs improve cardiac function, although they were unable to demonstrate the presence of mature cardiomyocytes. Therefore, improved passive mechanical properties as a reason for improved cardiac performance cannot be ruled out. Kolossov et al. were able to find ESC-derived striated cardiomyocytes after delivering ESCs to mouse heart. Delivery of these cells to the injured heart also significantly improved function, compared with delivery of media alone. However, to improve cell retention, it was necessary to deliver fibroblasts with the ESC-derived cardiomyocytes. When examining the fibroblast-only group with the fibroblast-plus-ESC group, it was difficult to see any difference, bringing into question the added active function benefit resulting from delivery of ESCs. Behfar et al. detected sarcomere formation after delivery of cardiac-committed ESCs to the infarcted mouse heart. They also documented mechanical recovery of function, but did not investigate whether this was due to the contribution of the cells to active contraction or to changes in the passive properties of the tissue.

Aside from the political and moral debate regarding the source of ESCs, a major impediment to the use of these cells is their tumorigenesis. While recent results suggest tumor reduction may be feasible, clinical application will require that there be no tumor formation.

**Bone marrow cells**

In 2001, Orlic et al. documented regeneration of cardiac function and cardiac myocytes through the delivery of bone marrow cells (BMCs). These authors demonstrated the delivery of Lin− c-kit+ BMCs to the infarcted mouse heart resulted in new myocyte formation, confirmed by an enhanced green fluorescent protein tag that marked their BMCs. However, attempts by others to repeat these findings failed. Nonetheless, this initial study has spurred research into specific types of BMCs.

MSCs found in the bone marrow have shown potential for cardiac differentiation. Incubation of MSCs with 5-azacytidine, a DNA-demethylation agent, results in differentiation of some of the MSCs down a cardiac lineage. The differentiated cells express cardiac specific proteins and form sarcomeres. We have recently induced MSCs to express cardiac markers by creating spheroids, a process similar to the formation of embryoid bodies. Approximately 15% of these cells express a calcium current similar in magnitude to that characteristic of adult ventricular myocytes. When implanted into the canine heart, some of the cells develop sarcomeres.

In summary, evidence exists suggesting that MSCs can be induced to choose a cardiac lineage; however, generating a large quantity of a pure population of these committed cells remains a challenge.

**Resident cardiac stem cells**

Early studies from Anversa’s laboratory identified Lin− c-kit+ cells in the adult rat heart that could partially differentiate into cardiac myocytes. These CSCs are mobile, as they are detected in different locations throughout the infarcted heart, and they express cardiac-specific proteins.

Other CSCs were identified, including stem cell antigen-1 (Sca-1) positive cells, side population cells (based on Hoechst dye exclusion), and islet 1 factor (ISL1). These different populations of stem cells appear to have little overlap (although Sca-1 positive and side population cells appear to have some overlap). All have been reported to differentiate into myocytes with contractile properties. Whether these cells exist in humans, and why they are unable to restore
normal function to the infarcted heart, is still unknown. If CSCs could be harvested and expanded, it would enhance their potential as a therapy. However, CSCs appear to be rare. In an attempt to isolate and expand CSCs, Messina et al cultured small pieces of human and mouse myocardium. They collected the round "phase-bright" cells that appeared to migrate from the tissue. When expanding these cells, they formed "cardiospheres," which could be expanded. When delivered to the infarcted mouse heart, cells from cardiospheres improved ejection fraction compared to the delivery of fibroblasts.

### Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are generated by transfecting human dermal fibroblasts with Oct3/4, Klf4, Sox2, and c-Myc, they were able to induce the expression of several ESC-specific markers, suggesting the derivation of iPSCs. Two groups were recently able to derive cardiac cells from iPSCs. Narazaki et al induced vascular cell markers from iPSCs. In addition, by coculturing iPSCs with OP9 stromal cells, they could induce cardiac-specific markers and striated cells. In addition, some of these cells generated a nodal-like action potential. Using an EB-like differentiation process, Mauritz et al generated contracting EBs. Some of these cells also expressed cardiac-specific markers and were striated. These cells also expressed the gap junction protein, connexin 43, and exhibited functional coupling. Major concerns that need to be addressed with iPSCs are the gene infection required to induce pluripotency and the potential for tumorigenesis.

### Delivery of exogenous cells

Another issue complicating exogenous methods of cardiac regeneration is the delivery of these cells to the heart. Current routes for delivering cells used for regenerative therapy are intravascular, intracoronary, and intramyocardial. While intravascular delivery of cells is the least invasive, most of the cells are trapped in the lungs, with less than 1% of the cells residing in the infarcted heart. During angioplasty, cells can be delivered intracoronarily, directly to the region of interest. However, upon restoration of blood flow, the majority of cells are washed away from the region of interest, and only 3% of those delivered are engrafted into the heart. The intramyocardial route for injection of cells resulted in 11% engrafting in the heart.

While many researchers have developed tissue constructs that incorporate fetal or neonatal rat cardiac myocytes into engineered cardiac tissue (see Radisic et al, 2007 for review), only a few of them have investigated scaffold-based strategies for delivering stem cells to the heart. Materials used include alginate, collagen, collagen/glycosaminoglycan (GAG), and Matrigel. However, stem cells delivered via scaffolds appear to have a difficult time traversing the myocardial wall to reach the endocardium, where many clinical myocardial infarctions reside. Recently, Simpson et al, using a scaffold-based delivery vehicle, demonstrated that only 1% of engrafted human mesenchymal stem cells (hMSCs) reside in the endocardial space.

### Summary of exogenous regeneration

The search for a pure population of cells that can differentiate into myocytes continues. While embryonic stem cells appear to differentiate reliably into myocytes, issues with tumorigenesis continue to be a major concern. Other cell types have been shown to differentiate into myocytes, but none on a consistent basis in large enough quantities to effectively restore myocardial function.

Indeed, there is no reported correlation between the number of new myocytes and the change in regional mechanical function, a necessary criterion to evaluate success. In addition, efficient delivery of any cell to the heart remains elusive.
RESTORING MECHANICAL FUNCTION TO THE INFARCTED HEART

Restoring mechanical function to the heart appears to be achievable, at least through passive mechanisms. However, to fully regenerate myocardium, a significant quantity of contractile cells must be produced. Neither endogenous nor exogenous methods have been able to fully restore mechanical function to the infarcted heart. Although hopeful signs for regeneration exist, it remains to be seen whether myocyte proliferation or stem cell differentiation will make functional myocardial regeneration a clinical reality.

ELECTRICAL REGENERATION WITH GENE/CELL THERAPY

The heart is a rhythmic pump, whose mechanical function requires orderly electrical activation. In this section, we lay out the reasons why gene/cell therapy has been considered as an alternative, and describe the means by which targets are selected. It should be noted at the outset that there are fundamental differences in approaches to gene/cell therapies for mechanical and for electrical regeneration. When the heart fails as a rhythmic pump, it is a global problem, and the optimal solution requires the replacement of a billion or more myocytes. Arrhythmias, in contrast, can often be treated focally by influencing a million or fewer cells.

NORMAL ELECTRICAL ACTIVITY: THE ACTION POTENTIAL AND PACEMAKER ACTIVITY

The electrical activity of the heart, delivered via orderly propagation of the cardiac action potential, precedes and initiates the mechanical event, the contraction. All the ion channels and transporters necessary to initiate the heartbeat reside in the membranes of the sinoatrial (SA) node myocytes. The nervous system modulates this activity, but does not initiate it.62

The action potential is generated by a population of ion channels and transporters, each of which is comprised of one or more proteins generating either inward or outward current across the myocyte membrane. The voltage waveform of the action potential is the result of the summed ion movements through all the channels and transporters of a cell. A net inward current (positive ions moving into the cell) generates a membrane depolarization, while a net outward current (positive ions moving out of the cell) generates a membrane repolarization.

The pacemaker potential is a slow depolarization generated by a net inward current flow. In the normal heart, the action potential propagates from the primary pacemaker cells in the SA node, to the most distal cells in the ventricular epicardium by current flow through intercellular channels, called gap junctions.62 While gene therapy can directly deliver genetic material to myocytes, cell therapy requires electrical integration of the delivered cells. This is most often accomplished by gap junction formation between delivery and target cells.

COMPARISON OF PHARMACOTHERAPY AND DEVICE THERAPY WITH GENE/CELL THERAPY

The last half-century of pharmacological discovery has focused on development of small molecules capable of influencing excitability. Device therapy has focused on the development and implantation of electronic pacemakers and cardioverter-defibrillators, as well as on surgical or catheter ablation of arrhythmogenic foci, or interruption of reentrant pathways. A comparison of gene/cell therapy with either pharmacotherapy or device therapy suggests some potential advantages of the former:

Pharmacotherapy

• Specificity. Most pharmacologic agents are nonspecific. In addition to their actions on the channel/transporter of interest, they frequently have actions on others. For example, Class I drugs that slow or terminate conduction via Na channel blockade can also block K channels. Class III drugs that prolong refractory period do so by prolonging repolarization as well. Excess prolongation of repolarization can be proarrhythmic.
• Types of actions. There are far more channel or transporter blockers than there are openers, however, more often than not, the electrical abnormalities that generate arrhythmias are due to reduced ion channel/transporter activity. For example, in the border zone of an infarct the myocytes are depolarized, inactivating Na channels and reducing Na conductance on depolarization, resulting in slow conduction that predisposes to reentry.
• Targets. Drugs are limited to actions on those channels or transporters already present in the myocytes. That is, one cannot “import” a target into the cell that then is subject to therapeutic drug action.

The sum total of these limitations means that pharmacologic therapy for electrical dysfunction is not usually aimed at restoring normal function, but at limiting the
consequences of abnormal electrical activity instead, by making offending regions electrically unexcitable.

**Device therapy**

- **Maintenance.** Pacemakers require regular maintenance due to battery rundown and occasional lead fracture.
- **Autonomic responsiveness.** Pacemakers are not autonomic. However, rate-responsive units do increase rate in response to the demands of exercise.
- **Placement.** It would be ideal to position electrodes at sites that maximize mechanical performance. Yet, this cannot be routinely done. Even using biventricular pacing, electrode positioning is limited by the anatomy of the coronary venous system.
- **Inappropriate responses.** Inappropriately delivered shocks are a complication of ICDs that can cause physical and psychological trauma.
- **Venous stricture and recurrence of arrhythmia.** Atrial fibrillation originating from the pulmonary veins is often treated with ablation. A complication of this therapy is venous stricture. Furthermore, antiarrythmic success with ablation can be complicated by later recurrence.

To summarize, pacemakers were the panacea of 20th-century therapy for some disorders of rate and rhythm. However, maintenance is required, and rate responsiveness is an imperfect solution to the absence of autonomic control. ICD implantation has saved tens of thousands of lives, but patients are exposed to both physical and psychological trauma from inappropriate firing. Ablation therapy has worked for arrhythmias where other alternatives have failed; however, it carries risks of anatomical damage, and is not always effective in the long-term.

**Gene/cell therapy**

We will now consider whether gene/cell therapy offers the possibility of achieving a more successful therapy.

When compared with pharmacotherapy:
- **Specificity.** A gene encodes for a single protein and, as such, is specific. There can be nonspecific effects associated with overexpression or silencing of a specific protein, but, ideally, the only action of the delivered genetic material is to alter expression of a single protein.
- **Types of actions.** Channel/transporter activity can be enhanced by successful delivery of its cDNA, or reduced by a dominant negative construct, antisense, or small interfering RNA.

- **Origin of targets.** The entire human genome has been sequenced. Therefore, channels/transporters expressed in the heart can be used to correct abnormalities, as can those normally located in other tissues. Furthermore, if the properties of native channels are less than optimal, then a more optimal gene can be created by modifying existing genes or by creating new ones by genetic engineering.

When compared with device therapy:
- **Maintenance.** No maintenance is required.
- **Autonomic responsiveness.** Normal sympathetic and parasympathetic responsiveness should occur as long as innervation is present at the site of implantation.
- **Placement.** With regard to biological pacemakers, positioning of the implant by catheter delivery can be used to optimize mechanical performance of the ventricles.
- **Inappropriate responses.** These can be terminated either by regulated expression of the exogenous gene (the promoter-inducing expression can be turned off), by pharmacotherapy that blocks the overexpressed channel/transporter protein, or by ablation of the site. There is minimal tissue damage with delivery of either gene or cell therapy.
- **Venous stricture and recurrence of arrhythmia.** There is minimal tissue damage with delivery of either gene or cell therapy.

In summary, gene/cell therapy has the capacity to be more specific than pharmacotherapy, to enhance as well as reduce channel function, and to employ a larger array of targets optimized by genetic engineering. Compared with devices, gene/cell therapy is maintenance-free, autonomically responsive, and optimized for mechanical performance. Inappropriate responses can be prevented or reversed by regulating exogenous gene expression, or with appropriate pharmacotherapy, and no anatomical damage should occur with deployment. In short, it has the capacity to restore normal electrical function.

**CHOOSING A DELIVERY SYSTEM**

There are three approaches to delivering a therapeutic electrical change to the heart:
- The first is conceptually the simplest. The cDNA for the channel/transporter is delivered in a naked plasmid. However, the transfection efficiency is low and the action very transient.
• Second, the gene of interest can be packaged in a virus. The delivery can be either localized\textsuperscript{63} or to the chamber as a whole,\textsuperscript{64} a decision dictated by both the safety and the efficacy of each approach. A second decision is which virus to choose. Adenoviruses are largely safe and induce high levels of expression of the transgene, but are not persistent because they are episomal (not incorporated into the genome). Lentiviruses are more persistent because of genome incorporation, but have higher risks of neoplasia. The choice of which particular virus, as well as the properties of adeno-associated viruses, are discussed in detail in the article by Hammond and Tang\textsuperscript{4} in this issue.

• The third is cellular delivery. This approach is, by definition, focal, and requires that the chosen delivery cell be able to electrically influence the target cell. In most cases, this is achieved through the formation of gap junctions. These are formed from subunit proteins, called connexins. HMSCs and ESC-derived cardiac myocytes make connexins, and can form functional gap junctions with cardiac myocytes as well as with each other, in vitro and in vivo.\textsuperscript{65-68}

Finally, fusing delivery and target cells is possible. Cho et al\textsuperscript{69} transfected lung fibroblasts and induced fusion of these fibroblasts to myocytes in the guinea pig heart. This approach avoids the potential loss of function during ischemia where gap junctions might tend to uncouple. However, the long-term effects of cell fusion as well as the agent that induces it (polyethylene glycol) remain unknown.

In their article in this issue, Rosen et al\textsuperscript{70} discuss approaches of gene/cell therapy to life-threatening tachyarrhythmias. It is clear that native cardiac, mutated cardiac, and even noncardiac genes are showing proof of principle as therapies in animal studies in that arena. Below, we discuss an alternative to devices: biological pacemakers.

### Strategies for the Development of Biological Pacemakers

The area showing the widest array of conceptual approaches to gene/cell therapy has been the development of biological pacemakers. Beginning in 1998,\textsuperscript{71} a number of investigators have used a variety of approaches to create biological pacemakers as an alternative to these electronic devices. These approaches display not only some of the principles of gene/cell therapy design, but also point out some of the limitations of this approach.

#### General Principles of Biological Pacemaker Design

Figure 5 (page 18) shows the action potentials of a pacemaker cell from the SA node, and that of a ventricular myocyte. In the sinus node, a small net inward current during diastole generates the spontaneous pacemaker depolarization, while in the ventricle and much of the atrium, there is no net current flow (and thus no change in membrane potential) between action potentials. Figure 5 also indicates the major membrane conductances responsible for the pacemaker activity in the SA node myocyte, and for quiescence in the ventricular cell. \(I_f\) is an inward current (carried largely by Na\textsuperscript{+}), activated by hyperpolarization in the sinus node. This “pacemaker” current is absent in the physiologic voltage range in ventricular myocytes. \(I_{K1}\) is generated by a large outward conductance present in ventricular myocytes, but is largely absent in the pacing cells of the SA node.\textsuperscript{72,73}

Whether placed in the atrium or in the ventricle, the challenge in creating a biological pacemaker is to convert a diastolic interval with zero net current flow into a period of small net inward current.

Current gene and cell therapy approaches to this problem are illustrated schematically in Figure 6 (page 19) and discussed in detail below.

**Reducing background outward**

\(I_{K1}\) current leads to pacemaker activity in normally quiescent myocytes

Since \(I_{K1}\) is the dominant conductance during diastole in ventricular myocytes, reducing this outward current in normally quiescent tissue should result in a net inward current generating a pacemaker-like membrane depolarization toward threshold. Miao et al\textsuperscript{64} used a dominant negative form of Kir2.1 (a molecular correlate of the \(I_{K1}\) channel), and an adenoviral delivery system in the guinea pig ventricle, to reduce the number of functioning \(I_{K1}\) channels.

This approach works in the manner illustrated in Figure 7 (page 19). The \(I_{K1}\) channels, like most K channels, are composed of 4 identical subunits. A dominant negative construct is a genetically modified form of the channel subunit, which combines with the native functional channel subunits to form nonfunctional channels that do not permit current flow. The disadvantage of such an approach is illustrated in Figure 5.
I\textsubscript{K1} contributes to final repolarization of the ventricular action potential. Thus, in addition to inducing pacemaker activity, delivery of this dominant negative form of I\textsubscript{K1} also prolongs the action potential and the QT interval.\textsuperscript{74} Adding inward pacemaker current also creates pacemaker activity 

Native HCN genes

If net inward current is required for pacemaker activity, the obvious alternative approach to reducing an outward current is to add an inward current (Figure 5, lower right panel). As stated above, the pacemaker channel that carries the major inward current during the pacemaker potential in the sinus node is called I\textsubscript{f},\textsuperscript{73} and is not activated at diastolic potentials in quiescent atrium or ventricle. The \(\alpha\)-subunit of this channel is encoded by the hyperpolarization-activated–cyclic nucleotide-gated (HCN) gene family.\textsuperscript{75,76} As its name indicates, this channel opens when the action potential repolarizes as well as during diastole, so decreasing its magnitude lengthens the action potential (top right panel: control action potential in orange, minus I\textsubscript{K1} in green). Since I\textsubscript{f} is activated only during diastole, adding it has little or no influence on the reaction potential duration (lower right panel: control action potential in orange, plus I\textsubscript{f} in green). Both approaches create a biological pacemaker, but removing I\textsubscript{K1} results in a longer QT interval.\textsuperscript{74}

An important lesson learned from this approach is that individual channel types can have effects on different phases of the action potential. In this case, the reduction of I\textsubscript{K1} had the desired effect of producing net inward current during diastole. However, it also had the undesired effect of increasing action potential duration.

Adding inward pacemaker current also creates pacemaker activity

Figure 5. Pacing requires net inward current. Schematic action potentials from a sinus node (top left panel) and a ventricular myocyte (bottom left panel). The sinus node myocyte paces, and, thus, has a net inward current flowing during diastole, while the ventricular myocyte reaches a constant “resting potential” because the sum of all currents flowing across the membrane is zero. The major “pacemaker” current in the sinus node is I\textsubscript{f}, while the dominant conductance setting the resting potential in the ventricular myocyte generates a background outward current, called I\textsubscript{K1}. Panels on the right show approaches to creating a biological pacemaker by removing I\textsubscript{K1} from, or adding I\textsubscript{f} to, the ventricular myocyte. I\textsubscript{K1} flows during the final repolarization as well as during diastole, so decreasing its magnitude lengthens the action potential (top right panel: control action potential in orange, minus I\textsubscript{K1} in green). Since I\textsubscript{f} is activated only during diastole, adding it has little or no influence on the action potential duration (lower right panel: control action potential in orange, plus I\textsubscript{f} in green). Both approaches create a biological pacemaker, but removing I\textsubscript{K1} results in a longer QT interval.
700,000 hMSCs (roughly half carrying the HCN2 gene) were delivered to the canine left ventricular free wall. The hMSCs carrying the gene showed no evidence of humoral or cellular rejection, and the rhythm was catecholamine sensitive. The absence of hMSC rejection in this xenograft was not a complete surprise, as these cells are known to possess some immune privilege. Although native genes demonstrated both acceptable basal rates (roughly 60 beats per minute) and some increase in rate, in response to catecholamines, neither was optimal.

**Modified HCN genes**

If the native pacemaker genes are not optimal, it is possible an improved channel form could be created by genetic engineering. When a new ion channel is cloned, investigators study the relationship of its structure to its function. Such was the case for the HCN gene family, in which the portion of the channel responsible for its voltage dependence, its kinetics, and its cAMP binding were defined. To increase the rate of pacemaker activity, more \( I_f \) channels must open during diastole. This can be achieved by shifting the voltage dependence of opening to more positive poten-

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**Figure 6. Gene and gene/cell therapy approaches to creating a biological pacemaker.**

**A.** Gene therapy approaches. **B.** Approaches that use either cell therapy or a combination of gene and cell therapy. Details provided in text.

**Figure 7. Dominant negative suppression of ion channel function.**

Upper panel: A representation of a longitudinal section through a functional channel (top right) in a membrane (green), composed of 4 endogenous subunits (orange) that allow ions (violet) to pass through the pore. A nonfunctional channel (top left) comprised of 3 endogenous subunits (orange) and 1 exogenous (dominant negative) subunit (grey). The grey barrier is there to illustrate that the nonfunctional channel does not allow ions (violet) to permeate. Lower panel: Cross-section through a functional (4 orange subunits) and a nonfunctional (3 orange subunits and 1 grey subunit) channel. These illustrate that 4 subunits come together to form the channel and create a pore.
tentials, or by speeding the kinetics of channel opening. We chose a mutant HCN channel with a more positive voltage dependence, but it expressed more poorly in myocytes than the native channel. There was no increase in basal pacemaker rate, although there was enhanced catecholamine sensitivity. Tse et al. used a mutated form of the most rapidly activating HCN isoform, HCN1, whose voltage dependence favored channel opening. They delivered it by adenovirus to the pig atrium (where the opposing I_K current is smaller than in ventricle). They were able to achieve physiologic rates in that location, with some autonomic responsiveness. If adding more I_f speeds pacemaker rate, is there any limit to this approach? The theoretical answer is yes. Since I_f is an inward current, it could cause a steady depolarization making cells unexcitable. The same group produced larger magnitudes of I_f with the same HCN1 mutant, and demonstrated that too much pacemaker current can lead to termination of pacemaker function.

As stated above, the other approach to increase I_f is to accelerate the kinetics of channel opening. We employed a chimeric channel formed from HCN1 and HCN2. The piece from HCN1 guaranteed faster kinetics than HCN2, while the portion from HCN2 guaranteed better cAMP-sensitivity than HCN1. This chimeric HCN1/HCN2 channel was delivered by adenovirus to the canine left bundle branch where it generated a ventricular tachycardia. Fortunately, this arrhythmia was eliminated by the I_f-blocker ivabradine, demonstrating the feasibility of terminating runaway biological pacemaker activity.

Thus, the lesson learned from these approaches is that more inward current is not always better. Even if the action of a gene/cell therapy is limited to the diastolic range of potentials, the amount of inward current can overshoot the optimum, leading to either arrhythmias or quiescence. The inward current added must be titrated against the outward current present in the delivery location to achieve the optimal pacemaker rate.

Synthetic genes carrying inward current

When using HCN genes in a cardiac region that natively expresses them, channels formed of native and overexpressed subunits may have unpredictable properties. To avoid this potential difficulty, Kashiwakura et al. used a voltage-dependent K channel (Kv1.4) that would generate net outward current at diastolic potentials, as a template. They mutated the channel to change its selectivity, converting it from K-selective to nonselective. This resulted in a change in direction of the current flowing through the channel, from net outward to net inward. The native channel opened on depolarization. They engineered the channel to open on hyperpolarization. This synthetic “HCN-mimic” was delivered by adenovirus to the guinea pig ventricle where it generated a biological pacemaker. This tour de force of genetic engineering demonstrated that existing channels could be radically reengineered to produce pacemaker function. Unfortunately, unlike the native HCN channels, this synthetic channel does not possess a cAMP binding site, and so is unlikely to be directly regulated by the autonomic nervous system. However, autonomic regulation might be achieved through effects on other ion channels/transporters present in the infected myocytes.

Alternative gene/cell therapy approaches

Overexpression of the β2-adrenergic receptor

The first biological pacemaker was created by delivery of the cDNA for the β2-adrenergic receptor to the murine right atrium. Expression of this receptor increased pacemaker rate by 40%. The disadvantage of this approach was that the overexpression of a single protein had a nonspecific action. This was because a myriad of ion channels/transporters, as well as other cellular functions, are regulated by cAMP. This approach demonstrated proof of principle for biological pacing, but was potentially arrhythmogenic, so unlikely to progress to a therapeutic modality.

Embryonic stem cells

As discussed in the section on mechanical regeneration, ESCs can be forced into a cardiac lineage by forming three-dimensional structures, called embryoid bodies (EBs). Cells within these cardiogenic EBs are spontaneously active. Kehat et al. transplanted 40 to 150 spontaneously beating EBs into the posterolateral wall of the pig left ventricle to create a biological pacemaker. In those animals in which persistent pacemaker function was demonstrated, the rate was similar to the idioventricular rate of native secondary pacemakers (roughly 60 bpm). It was also responsive to adrenergic stimulation.

The major advantage of this approach is that all the elements necessary for generating the pacemaker potential are included in the delivered cell.
However, the disadvantages include the risk of neoplasia from undifferentiated embryonic stem cells, and the need for immunosuppression to prevent the rejection of those embryonic stem cells that had differentiated.

*Autologous SA node myocytes*

If the aim of biological pacemakers is to simulate sinus node function, it is natural to ask whether autologous transplantation of sinus node myocytes into a ventricular location could generate a functional biological pacemaker. Zhang et al\(^9\) successfully removed and dissociated myocytes from the canine SA node, and then delivered roughly 500,000 of these cells to the right ventricular subepicardial free wall of the same animals. Three weeks after implantation, an idioventricular rhythm of approximately 60 beats per minute was observed. The rate increased on catecholamine infusion. However, neither the basal rate nor the catecholamine responsiveness was better than that observed with HCN2-transfected hMSCs delivered to the canine left ventricular free wall, or with adenoviral delivery of HCN2 to the canine left bundle branch.\(^6\),\(^8\)

**CONCLUSIONS FOR ELECTRICAL REGENERATION**

The 20th century brought us lifesaving pharmacologic and device therapies for disorders of rate and rhythm. However, these therapies are not perfect. Drugs are nonselective and, because of this, can be proarrhythmic. Devices also come with a price: regular maintenance, inappropriate responses, and anatomic damage. Gene/cell therapy has the potential to be more selective, and has a wider array of targets, than pharmacologic agents. It also has the potential to be maintenance-free and more biologically responsive than devices, yet without their associated anatomical damage. As stated in the introduction, our aim was to show you the “dream” of gene/cell therapy. Our current “reality” is nowhere near this ideal. Although “proof of principle” has been demonstrated for some gene/cell therapies for both tachyarrhythmias and bradyarrhythmias, these therapies are far from perfect. The electrical activity of the heart is dependent on the complex interactions of many ion currents. Unfortunately, because of these interactions, influencing the expression of even a single channel type can often result in outcomes that influence the activity of others, resulting in proarrhythmia. Although the successes of gene/cell therapy for electrical dysfunction are modest to date, they hold the hope of a return to normal function that must be pursued.

**LOOKING TO THE FUTURE**

The title of our article raises the question, “Can gene/cell therapy restore normal mechanical and electrical function?” The answer is not a simple yes or no. Proof of principle has been demonstrated for a wide variety of approaches, in both the mechanical and electrical arena, and this has raised hopes. However, definitive therapies remain an elusive target. Part of the problem is theoretical, as clear objective criteria for success have not been defined. The other part is technical, and relates to the scale of the mechanical problem requiring billions of myocytes, and the complex nature of the electrical problem due to the interactions of multiple time- and voltage-dependent ion currents. Progress can be accelerated if studies reporting measures of success also pursue an understanding of the mechanism of action. However, given the new tools in our therapeutic arsenal, the future looks bright. We have come a long way in a short time. Myriad targets show promise, and many therapies may be just beyond the horizon. How long we take to reach that point will be determined by the rigor of our investigations and the support provided by our funding institutions.

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Mending the Broken Heart

Expert Answers to Three Key Questions

1

Gene therapy for myocardial infarction–associated congestive heart failure: how far have we got?

H. K. Hammond, T. Tang

2

Does cell therapy for myocardial infarction and heart failure work?

K. C. Wollert, H. Drexler

3

Gene and cell therapy for life-threatening cardiac arrhythmias: will they replace drugs, surgery, and devices?

M. R. Rosen, P. Danilo Jr, R. B. Robinson
Gene therapy for myocardial infarction–associated congestive heart failure: how far have we got?

H. Kirk Hammond, MD; Tong Tang, PhD

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Department of Medicine - University of California San Diego - La Jolla - Calif - USA

With the advancement of vectors, delivery methods, and newly identified molecular targets, preclinical studies have shown that gene transfer is effective in improving left ventricular contractility and attenuating deleterious left ventricular remodeling in myocardial infarction–associated congestive heart failure (CHF). We are optimistic that these favorable effects will also be seen when tested in patients with CHF associated with myocardial infarction, as well as in patients with CHF from other etiologies. Gene therapy has the potential to be tailored to meet the needs of individual patients. Moreover, when used in conjunction with pharmacological and device management of the patient with CHF, it provides hope for a brighter future for the 23 million patients worldwide with this devastating disease.

Congestive heart failure (CHF) is an inexorable disease associated with high morbidity and mortality. CHF has several etiologies, including hypertension, diabetes mellitus, excessive alcohol consumption, valvular heart disease, myocarditis, and familial cardiomyopathy. The majority of cases in developed nations result from coronary artery disease, and subsequent myocardial infarction (MI). Many of these etiologic factors can be altered to avoid the occurrence of CHF altogether—proper treatment of hypertension, surgical intervention for valvular heart disease, risk factor reduction to prevent coronary artery disease, and so forth.

Once CHF is associated with symptoms at rest or with mild exertion (New York Heart Association [NYHA] class III and IV), 50% of such patients are expected to die within 4 to 5 years—even on optimal medical and device therapy—a prognosis worse than many cancers. Worldwide, it is estimated that 23 million patients have CHF. 1 million new cases are diagnosed yearly, and that every 3 minutes there is another death due to CHF. In the US, CHF is the most common admitting diagnosis in patients >65 years old, in whom there is a 1% incidence. Incidence in this age group is expected to double by 2037. Therefore, despite recent advances, there is an unmet medical need for the millions of patients with CHF. These statistics provide the rationale to develop new, unconventional therapies, such as cell- and gene-based therapies.

**CARDIAC GENE TRANSFER**

In general, any gene therapy has three essential components: (i) a vector to carry and express the gene; (ii) a delivery method that safely and efficiently delivers the vector to the organ, tissue, or cell of interest; and (iii) a therapeutic gene tailored to an aspect of the disease being treated. For cardiac gene therapy, there are few suitable vectors, but many candidate genes. The most troublesome component has been the method of delivery. Let us briefly review the most suitable vectors and

**SELECTED ABBREVIATIONS AND ACRONYMS**

<table>
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<th>Abbreviation</th>
<th>Description</th>
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<tr>
<td>AAV</td>
<td>adeno-associated virus</td>
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<tr>
<td>BAR</td>
<td>β-adrenergic receptor</td>
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<tr>
<td>CHF</td>
<td>congestive heart failure</td>
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<tr>
<td>FGF</td>
<td>fibroblast growth factor</td>
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<td>GH</td>
<td>growth hormone</td>
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<td>IGF-1</td>
<td>insulin-like growth factor–I</td>
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<td>MI</td>
<td>myocardial infarction</td>
</tr>
<tr>
<td>SERCA2a</td>
<td>sarcoplasmic reticulum Ca2+-ATPase 2a</td>
</tr>
<tr>
<td>VEGF</td>
<td>vascular endothelial growth factor</td>
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possible delivery methods, before proceeding to a discussion of potential therapeutic genes in MI-associated CHF.

Vectors

The ideal features of a vector for cardiac gene transfer include: (i) high efficiency, (ii) high specificity (heart and not elsewhere, or cardiac myocyte and not elsewhere, for example); (iii) minimal toxicity or immunogenicity; and (iv) regulated expression. Nonvirus vectors (eg, naked DNA, protein-DNA complexes, and liposomes), despite their easy production, minimal toxicity, and low immunogenicity, have poor transduction efficiency, and their use for CHF gene therapy is limited. Virus vectors have been the main focus for cardiac gene therapy because of their higher myocardial transduction efficiency, and their utility for vascular delivery.

The most used virus vectors are adenovirus, adeno-associated virus (AAV), and lentivirus (Figure 1). These three vectors, unlike most retrovirus vectors, can be used to obtain gene transfer in cardiac myocytes and other nondividing cells (Table I). Both AAV and lentivirus provide persistent transgene expression, as the transgene becomes integrated in the host chromosome. Adenovirus, in contrast, provides extrachromosomal expression.

Delivery method

Several delivery methods for virus vectors have been used for cardiac gene transfer (Table II). Lentivirus vectors are generally thought to be unsuitable for intracoronary delivery because they do not readily cross the endothelial cell border, and, therefore, direct intramyocardial in-
Injection is a preferred method. Intracoronary delivery of adenovirus appears to cause less inflammation in heart compared with other organs, and also circumvents the inflammatory response induced by intramyocardial injection. However, the intramyocardial injection method is associated with substantial gene transfer efficiency, albeit confined to the areas directly adjacent to the needle tract. Recently, it has been reported that intravenous delivery of AAV vectors—AAV6, AAV8, and AAV9 in particular—may provide substantial gene transfer to heart, lung, and other organs, although very high amounts of the virus are required, using doses that may not be feasible in human subjects. Routine intracoronary delivery of AAV is much less efficient than adenovirus.

Pharmacological agents and mechanical procedures have been used to increase gene transfer efficiency with AAV and adenovirus. Histamine, nitroprusside, serotonin, acetylcholine, sildenafil, vascular endothelial growth factor, and substance P, which increase transcytosis via increases in nitric oxide and cGMP, promote transvascular movement of adenovirus (and perhaps AAV) when delivered by the vascular route—either intracoronary or intravenous. AAV and adenovirus vectors can be efficiently delivered to the myocardium using indirect intracoronary delivery, in which the virus vector is injected into the left ventricular (LV) chamber during sustained occlusion of the ascending aorta and proximal pulmonary artery, thus forcing egress into the coronary arteries. Hypothermia, which enables prolonged dwell time without ischemic injury to the heart and brain and simultaneous use of serotonin or acetylcholine, facilitates gene transfer using this method. Recently, a percutaneous closed-loop recirculation system—essentially a cardiopulmonary bypass without thoracotomy—was shown to provide cardiac gene transfer with AAV—presumably, at least in part, because of the substantially increased dwell time enabled by continuous coronary recirculation of the vector. In clinical settings, where safety is the first priority, the gene delivery method that is effective, safe, and easy to apply will prevail as the best choice. Safe methods of delivery will be particularly germane to patients with CHF—invasive methods requiring thoracotomy, cross-clamping of the aorta, or cardiopulmonary bypass will likely be poorly tolerated.

### Table I. Virus vectors for cardiac gene therapy.

<table>
<thead>
<tr>
<th>Virus vector classification</th>
<th>Chromosomal integration</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Adenovirus</td>
<td>No</td>
<td>Efficient direct intracoronary delivery</td>
<td>Immunogenicity</td>
</tr>
<tr>
<td></td>
<td>No risk of insertional mutagenesis</td>
<td>Expression not permanent</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Easy to produce</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adeno-associated virus</td>
<td>Yes</td>
<td>Low immunogenicity</td>
<td>Risk of insertional mutagenesis</td>
</tr>
<tr>
<td></td>
<td>Vascular delivery may be feasible</td>
<td>Difficult to produce</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Permanent expression</td>
<td>Limited insert size</td>
<td></td>
</tr>
<tr>
<td>Lentivirus</td>
<td>Yes</td>
<td>Very large insert size</td>
<td>Risk of insertional mutagenesis</td>
</tr>
<tr>
<td></td>
<td>Permanent expression</td>
<td>Vascular delivery difficult</td>
<td>Difficult to produce</td>
</tr>
</tbody>
</table>

### Table II. Delivery methods for cardiac gene therapy.

<table>
<thead>
<tr>
<th>Method</th>
<th>Advantage</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous</td>
<td>Simple; relatively noninvasive</td>
<td>Low efficiency gene transfer, generalized distribution of vector</td>
</tr>
<tr>
<td>Myocardial injection</td>
<td>Can select region of interest</td>
<td>Requires thoracotomy or sophisticated catheter methods; inefficient gene transfer</td>
</tr>
<tr>
<td>Direct intracoronary</td>
<td>Simple; reduced morbidity vs other methods</td>
<td>Less efficient than indirect intracoronary</td>
</tr>
<tr>
<td>Indirect intracoronary</td>
<td>Efficient gene transfer</td>
<td>Requires pulmonary artery and aortic cross-clamping and thoracotomy or cardio-pulmonary bypass</td>
</tr>
</tbody>
</table>

There are three phases in the development of CHF associated with MI, in which a gene therapy may be envisioned: (i) reduction of MI size; (ii) attenuation of LV dilation; and (iii) treatment of fully devel-
oped CHF. We will consider the application of gene therapy to these three phases by examining peer-reviewed publications that have used preclinical models of CHF—at this writing (2008), there are no placebo-controlled clinical trials of gene therapy for CHF to review.

**Reduction of MI size**

Since acute MI, and consequent loss of LV mass, is the major cause of CHF in developed nations, it is reasonable to ask whether or not gene therapy could reduce infarct size in the setting of acute myocardial infarction, which has been, over the past 25 years, the goal of both thrombolyis and percutaneous coronary interventions. There is overall agreement, however, that reduction of infarct size requires that an occluded vessel be made patent—the earlier, the better—and that a delay of 6 or more hours is generally not associated with substantial reduction in infarct size. With this backdrop, it is difficult to provide a rationale for gene transfer to reduce infarct size in acute MI, given the required delay in vector delivery, transgene expression, and myocardial preserving element (eg, angiogenesis) to result. Indeed, taken together, these requirements of gene therapy, would, in the best circumstances, take many hours to occur.

Surprisingly, there have been a few published examples in which gene therapy, administered at the time of MI, or very shortly thereafter, has been associated with a reduction in MI size. Although it seems unlikely that a gene therapy-associated angiogenesis could have occurred rapidly enough to limit infarct size, it is possible that effects related to the inhibition of apoptosis may have contributed. Even so, these possible reductions in MI size are rare, and require experimental coronary occlusion with antecedent gene transfer, or vector injection into the myocardium at the time of coronary occlusion—situations that are unlikely to be relevant in clinical acute MI.

**Attenuation of LV dilation**

There are several examples in which delivery of genes to the heart, at the time of MI or shortly thereafter, has been associated with a reduction of post-MI LV dilation (adverse remodeling). This is important because, unlike the challenge to reduce infarct size in acute MI—a challenge better achieved with percutaneous coronary interventions or thrombolysis—it is clinically feasible that a gene therapy could be administered after the acute phase of MI, if it were to influence LV remodeling in a favorable manner. Transgenes that have been shown to have this favorable effect on LV remodeling after completed MI include angiogenic genes, antiapoptosis genes, and growth hormone (GH)/insulin-like growth factor-I (IGF-I).

**Treatment of fully developed CHF**

Given the abundance of patients with fully developed CHF (23 million worldwide), this phase of treatment may be the most suited to gene therapy, particularly since pharmacological agents have been used so successfully to reduce LV remodeling after MI. On the other hand, one wonders how successful a gene therapy can be when directed to an LV that may have 30% to 40% of its mass already scarred from infarction. Even so, 50% survival has increased from 18 months to 4 to 5 years over the last 25 years with the use of pharmacological agents. It is the hope of those in the field that gene therapy, working on such fundamentally different mechanisms than current medical therapy, may provide better outcomes—giving a patient with severe symptoms the possibility of surviving even longer and feeling better. There are a large number of transgenes that have been used in animal models of CHF with some success, in terms of improved LV function and geometry, exercise capacity, and survival (which will be reviewed in the next section).

**GENES OF INTEREST WITH SUPPORTIVE PRECLINICAL DATA**

We have selected promising genes that appear to have favorable influences on the failing heart. Stringent criteria were imposed: (i) convincing demonstration of the presence of CHF, and (ii) gene transfer was performed when CHF was actually present, and not merely given at the time of MI, for example (which may suggest a therapeutic effect but falls short of providing support for a clinically applicable gene therapy per se).

**Angiogenic and vasculogenic factors**

The most common cause of CHF in the US and the European Union is MI, and such patients have persistent myocardial ischemia, both in the border zone of the healed infarction, as well as elsewhere in the heart. Even patients with dilated failing hearts unassociated with coronary artery disease have measurable myocardial ischemia. Improving myocardial blood flow by gene transfer of angiogenic/vasculogenic factors is an attractive approach, not only for MI-associated CHF, but also for CHF in general.

In pacing-induced CHF in pigs (associated with myocardial ischemia), intracoronary delivery of an adenovirus encoding fibroblast growth factor (FGF)–4, in the setting of ad-
vanced CHF, was associated with improvements in global LV function and geometry. In the same animal model of CHF, direct intramyocar- dial injection of plasmid encoding vascular endothelial growth factor (VEGF), showed favorable effects on regional function. Finally, a recent paper showed improved regional and global heart function in a pig model of ischemic cardiomyopathy, using intracoronary delivery of an adenovirus encoding FGF5. Interestingly, improved LV function was associated not only with the anticipated increase in blood flow in Ad FGF5-treated animals, but also with increased cardiac myocyte mitosis and reduced apoptosis in the viable ischemic zone.

Intramyocardial gene transfer of either hepatocyte growth factor or angiopoietin has been reported to increase regional and global LV function—when delivered at the time of the experimentally induced MI. However, in none of these cases was CHF present at the time of gene transfer, so it remains to be proven that these genes can improve function of the failing heart.

**β-Adrenergic receptor signaling pathway**

A hallmark of CHF is impaired β-adrenergic receptor (BAR) signaling, associated with BAR downregulation, Gs/BAR uncoupling (Gs for stimulatory G protein), and abnormalities of adenyl cyclase (AC) function. These alterations impair cAMP generation, and thus depress myocardial contractility. In the 1980s, treatments for clinical CHF focused on increasing myocardial cAMP levels using pharmacological agents that stimulated the BAR (dobutamine), or decreased the breakdown of cAMP (milrinone). These strategies failed, perhaps owing to sustained high levels of cAMP produc-
tion. Current therapy for clinical CHF embraces inhibition, not stimulation of the BAR. Mirroring the poor outcomes seen with BAR-agonist treatments in clinical CHF, are two classic examples from animal studies: (i) chronic infusion of isoproterenol leads to CHF in mice and rats, and (ii) cardiac-directed expression of BAR leads to CHF in transgenic mice.

With this backdrop, it is quite surprising that AC, the effector mole- cule in the BAR signaling pathway, appears to have beneficial effects on the failing heart. AC6 gene expression not only prevents LV hypertrophy and reduces mortality in a genetic model of CHF, but also increases LV function, and survival after acute myocardial infarction. In addition, AC6 expression prevents further deleterious LV remodeling, and improves cardiac function in MI-associated CHF. Why should this element of the BAR signaling pathway—a pathway that governs cAMP production—be so different in its effects compared with strategies designed to stimulate the BAR? Several recent publications have documented that some of the favorable effects of AC6 may not be directly related to increased cAMP generation per se. For example, increased expression of cardiac AC6 corrected calcium-handling defects in cardiomyopathy, and, after MI, was associated with normalization of cardiac troponin I phosphorylation, and reduced apoptosis in mice with severe CHF. AC6 gene transfer influences the phosphorylation and activation of important signaling proteins—individually, and in concert—with increased AC6 expression, in contrast to BAR stimula-
tion, benefits the failing heart. Gene transfer of AC6 has shown promising results in treating CHF in pigs, and a randomized, double-blind, placebo-controlled Phase 1/Phase 2 clinical trial using AC6 gene transfer in patients with stable, but severe CHF has been approved by the Food and Drug Administration, and will be initiated in early 2009.

Finally, gene transfer of BARct, an inhibitor of G-protein–coupled receptor kinases, increases contractility in MI-associated CHF in rabbits. These results suggest that controlled increases in BAR signaling, unlike β-agonists and phosphodiesterase inhibitors that lead to unrelenting stimulation of the BAR pathway, may be beneficial for the failing heart.

**Calcium handling**

Calcium plays a crucial role in controlling the cardiac contractile process. In every heart beat, calcium is taken up by sarcoplasmic reticulum, through the sarcoendoplasmic reticulum Ca++-ATPase 2a (SERCA2a) calcium pump, and then released through calcium-release channel ryanodine receptor. Failing hearts exhibit defective calcium uptake and calcium release. Because of the pivotal role of calcium in LV contrac-
tion and relaxation, it is a logical focus for gene therapy.

Phospholamban is an endoge-
nous muscle-specific inhibitor of SERCA2a, and its phosphorylation at Ser16 releases the inhibition on SERCA2a function. CHF is associated with decreased phospholamban phosphorylation at Ser16. Reduction of phospholamban inhibition of SERCA2a, which promotes the favorable effects of SERCA2a on calcium handling, appears to have favorable effects in genetic cardiomyopathy in mice. To test this ap-
Gene therapy for myocardial infarction-associated CHF - Hammond and Tang

approach in fully developed CHF, rats with large infarcts received indirect intracoronary delivery of AAV encoding a pseudophosphorylated mutant phospholaminban—a dominant negative mutant that does not inhibit SERCA2a. This resulted in increased cardiac systolic and diastolic function, and reductions in heart weight, cardiac myocyte size, and fibrosis, even 6 months after gene transfer.19

There are controversies over whether increased cardiac expression of SERCA2a can improve LV performance in human hearts as it appears to do in rodent hearts, and over how long this effect lasts. However, long-term increases in cardiac SERCA2a content, achieved by AAV-mediated gene transfer, preserved LV systolic function and prevented deleterious LV remodeling in a volume overload–induced CHF model in pigs.20 Two phase 1 clinical studies have been approved that propose to use intracoronary delivery of an AAV encoding SERCA2a to treat patients with CHF.

S100A1 belongs to a family of proteins containing 2 EF-hand calcium-binding motifs. S100A1 interacts with SERCA2a, and increases SERCA2a activity. There is also evidence that S100A1 blocks sarcoplasmic reticulum calcium leak during diastole and increases systolic sarcoplasmic reticulum calcium release. Intracoronary delivery of an adenovirus encoding S100A1 restored impaired calcium handling, and improved LV function in myocardial infarction–associated CHF in rats.21 Calcium handling and LV function were restored after intracoronary delivery of an AAV vector with cardiac-specific S100A1 expression into failing rat hearts 10 weeks after MI, where significant calcium-handling impairment, LV dysfunction, and deleterious LV remodeling were evident.22 Gene transfer of other calcu- mulation-handling regulators, for example, protein phosphatase inhibitor 1 and EF-hand calcium-binding proteins sorcin and parvalbumin, has also shown favorable effects on LV function in cardiomyopathic animal models. These methods may be transferrable for CHF treatment, and warrant further studies in MI-associated CHF models.

**Other candidates**

**Anti-apoptosis**

During and after acute MI, there is increased apoptosis in myocardium, especially in the border zone of the infarct. Thus, inhibiting apoptosis may attenuate cell loss, thereby preserving myocardial mass. Hepatocyte growth factor and JAG6 (discussed previously) appear to reduce apoptosis. Indirect coronary delivery of an adenovirus encoding Bcl-2, an inhibitor of apoptosis, was performed in a rabbit model of regional ischemia-reperfusion injury.23 Bcl-2 expression increased LV ejection fraction and reduced deleterious LV remodeling, which were associated with reduced apoptosis, but the demonstration that this works to treat CHF is lacking.

**Reduction of reactive oxygen species**

Myocardial infarction increases reactive oxygen species generation, which has deleterious effects on LV function and geometry. While no studies have conclusively shown that gene therapy with reactive oxygen species scavengers to be effective in fully developed CHF, data in models of myocardial ischemia using delivery of inducible nitric oxide synthase, cyclooxygenase-2, heme oxygenase-1, and extracellular superoxide dismutase are promising.24

**Cell cycle reentry**

Reduced cardiac function after MI is largely caused by cardiac myocyte loss. Although spontaneous cardiac myocyte regeneration may occur after MI, the magnitude of this intrinsic process is insufficient to restore substantial cardiac mass. An intriguing idea is to induce adult cardiac myocytes into the cell cycle, and to promote cardiac myocyte regeneration with the hope that this would promote functional recovery after MI. Cardiac-directed expression of cyclin D2 promotes cardiac myocyte proliferation, and new myocardial tissue formation in the infarcted area after MI.25 Associated with these changes were synchronized calcium transients, within the newly regenerated cardiac myocytes and within cardiac myocytes in uninfarcted areas, and increased LV function. Cyclin A2, which is similar to cyclin D2, induces cardiac myocyte proliferation and cardiac regeneration after MI. Presently, these approaches have not yet been used to treat fully developed CHF.

**GH and IGF-I**

Although GH (peptide) therapy has been used in clinical CHF, no placebo-controlled, blinded trials have shown efficacy. It is possible that if sustained delivery of GH (or IGF-I) could be achieved—with gene transfer—the approach might be more effective. Although many papers have shown that GH or IGF-I gene therapy—administered at the time of MI—result in improved regional LV function,26 no study has determined whether GH or IGF-I gene therapy is effective in the treatment of fully developed CHF. Given the generally cardiac-friendly effects of GH and IGF-I—both angiogenic and antiapoptotic—they are promising gene therapy candidates.

**FUTURE DIRECTIONS**

The pathophysiology of MI-associated CHF is complex. Future studies on molecular signaling pathways will
continue to identify new and more effective targets for gene therapy. For example, recent advancement in RNA interference research has identified endogenous small RNAs that specifically silence the expression of target genes. Some of these small RNAs indeed are downregulated after MI. Specific delivery of exogenous small interfering RNA and microRNA into infarcted myocardium presents a new venue for gene therapy.

Although there have been several clinical trials of cell-based CHF treatment, cardiac myocyte regeneration has been difficult to document, and the modest clinical benefits reported in these trials may be the result of angiogenesis, which, it can be argued, could be more easily achieved using virus vectors. However, the next phase of gene therapy for CHF will be advanced only when more effective gene transfer vectors and (especially) methods of delivery become available.

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Viral gene transfer of the antiapoptotic factor Bcl-2 protects against chronic postischemic heart failure.

The late phase of preconditioning and its natural clinical application—gene therapy.

Cardiomyocyte cell cycle activation improves cardiac function after myocardial infarction.

Strategic advantages of insulin-like growth factor-I expression for cardioprotection.
Does cell therapy for myocardial infarction and heart failure work?

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Department of Cardiology and Angiology - Hannover Medical School - Hannover - GERMANY

Modern reperfusion strategies and advances in pharmacological management have resulted in an increasing proportion of patients surviving after an acute myocardial infarction (AMI). The resulting loss of viable myocardium sets the stage for progressive left ventricular remodeling in many patients. The extent of cardiac remodeling after an AMI is closely related to the size of the infarct: larger infarcts result in a greater extent of left ventricular remodeling and a worse prognosis. As a consequence, AMI has become the most common cause of heart failure in many countries. However, none of our current therapies addresses the underlying cause of the remodeling process, ie, the critical loss of myocardium in the infarcted area.

THE CONCEPT OF CELL THERAPY

It has recently been observed that cardiomyocytes in the infarct border zone may reenter the cell cycle and divide after an AMI. In addition, genetic fate-mapping studies support the notion that endogenous stem cells may be a source of new cardiomyocytes after ischemic injury. Moreover, there are data to suggest that the injured myocardium attracts circulating stem cells and progenitor cells that may positively affect the healing response and functional recovery after an AMI via the release of paracrine factors. The overall capacity of the adult heart for regeneration is limited, however, and the vast majority of necrotic cardiomyocytes are replaced by scar tissue after an AMI. Still, the existence of endogenous regenerative mechanisms may open the way to mimicking and amplifying these processes therapeutically.

Two main approaches to achieve myocardial tissue replacement and functional enhancement have been envisioned: the use of isolated cells delivered directly to the diseased myocardium (cell therapy—the focus of the present paper), or the use of a combination of cells and biomaterials to generate functional three-dimensional tissues in vitro before implanting them into the body (tissue engineering). As the heart is composed of 30% cardiomyocytes and 70% nonmyocytes, such as endothelial cells, smooth muscle cells, and fibroblasts, cardiac regeneration is not only a matter of cardiac myocyte addition, but also of nonmyocyte supplementation.

POTENTIAL DONOR CELLS

There are two different cell sources that might be used for cell transplantation: autologous and allogeneic cells. Autologous cells are obtained from the patients themselves, and pose no risk of immune rejection. However, the functional activities of autologous cells may be negatively affected by underlying cardiovascular risk factors and dis-
ease. Transplantation of most allogeneic cells will require immunosuppressive therapy to avoid immunological reactions. Experimentally, differentiated cells as well as stem and progenitor cells have been employed for cell therapy. Each cell type has its own profile of advantages, limitations, and practicability issues, and may have an impact on cardiac structure and/or function through distinct mechanisms (Table I). In general, differentiated cells show a lower proliferation rate and a diminished survival rate after transplantation when compared with stem and progenitor cells. Stem cells are capable of self-renewal, transformation into dedicated progenitor cells, and differentiation into specialized progeny. Depending on their differentiation potential, stem cells are classified as being pluripotent (capable of differenti-
ating into any of the three germ layers) or multipotent (giving rise to a limited number of other cell types).

**CELL TRANSPLANTATION STRATEGIES**

The goals of any cell delivery strategy are to transplant sufficient numbers of cells into the myocardial region of interest and to achieve maximum retention of cells within that area (Table II). Cell retention may be defined as the fraction of transplanted cells retained in the myocardium for a short period of time. The local milieu is an important determinant of cell retention, as it will influence short-term cell survival and, if a transvascular approach is used, cell adhesion, transmigration through the vascular wall, and tissue invasion. Transvascular strategies are especially suited to the treatment of recently infarcted and reperfused myocardium, when chemoattractants are highly expressed. Selective intracoronary application delivers a maximum concentration of cells homogeneously to the site of injury. Unselected bone marrow cells have been delivered via the intracoronary route in patients after AMI. In these studies, cells were delivered through the central lumen of an over-the-wire balloon catheter during transient balloon inflations to maximize the contact time of the cells with the microcirculation of the infarct-related artery. In experimental models, intravenous delivery of endothelial progenitor cells and mesenchymal stem cells has been shown to improve cardiac function after AMI. However, cell homing to noncardiac organs limits the applicability of this approach. Indeed, in a recent clinical study, homing of unselected bone marrow cells to the infarct region was observed only after intracoronary stop-flow delivery, but not after intravenous infusion. Direct injection techniques may be more appropriate for patients presenting late in the disease process, when an occluded coronary artery precludes transvascular cell delivery or when homing signals are expressed at low levels in the heart (scar tissue).

<table>
<thead>
<tr>
<th>Cell delivery strategies</th>
<th>Pros</th>
<th>Cons</th>
</tr>
</thead>
<tbody>
<tr>
<td>Intravenous infusion</td>
<td>- Least invasive</td>
<td>- Cell trapping in the lungs and other tissues</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Limited cell delivery to the heart</td>
</tr>
<tr>
<td>Intracoronary infusion</td>
<td>- Homogeneous cell delivery to the site of injury</td>
<td>- Open infarct-related artery required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Limited cell retention in infarcted area</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Not suitable for delivery of large cells which may cause microembolization (eg, skeletal myoblasts, mesenchymal stem cells)</td>
</tr>
<tr>
<td>Transendocardial injection</td>
<td>- Electromechanical mapping of the endocardial surface can be used to delineate viable, ischemic, and scarred myocardium before cell injections</td>
<td>- Creates islands of cells with limited blood supply and poor cell survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- May not be safe in patients with acute myocardial infarction (AMI), when cells are injected in friable necrotic tissue</td>
</tr>
<tr>
<td>Transepicardial injection</td>
<td>- Allows for direct visualization of the myocardium, and a targeted application of cells to scarred areas and/or the border zone of an infarct</td>
<td>- Creates islands of cells with limited blood supply and poor survival</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Open heart surgery required</td>
</tr>
<tr>
<td></td>
<td></td>
<td>- Invasiveness hampers its use as a stand-alone therapy, or its use in the setting of AMI</td>
</tr>
</tbody>
</table>

Table II. Cell delivery strategies.

**CURRENT STATUS OF CELL THERAPY IN PATIENTS WITH ACUTE MYOCARDIAL INFARCTION AND HEART FAILURE**

Preclinical studies have shown that transplantation of bone marrow–derived hematopoietic stem cells, endothelial progenitor cells, or mesenchymal stem cells can promote functional improvements in animal models of AMI. Transdifferentiation of the transplanted cells into cardiomyocytes and endothelial cells has been offered as an explanation, but the quantitative importance of stable cell engraftment and transdifferentiation for the functional effects has been challenged. It is now believed that the reported improvements in these models are mediated predominantly by paracrine effects (Figure 1, page 40). Clinicians welcomed these animal studies with great enthusiasm, and, fairly rapidly, clinical trials were designed to translate these findings into the clinical scenarios of post-AMI or heart failure patients.
Randomized trials using unselected bone marrow cells

Most clinical investigators have chosen a pragmatic approach by using unfractionated bone marrow cells, which contain different stem and progenitor cell populations, as well as more differentiated hematopoietic cell types. In all of these studies, the cells were delivered into the reperfused and stented infarct-related artery by using a stop-flow balloon catheter approach. In four trials, cells were delivered a few days after coronary reperfusion to enhance systolic function and prevent adverse remodeling; in one trial, bone marrow cells were transplanted in patients with ischemic heart failure, months or years after an AMI (Table III).14-18 The combined experiences from these studies indicate that intracoronary delivery of unselected bone marrow cells is feasible and probably safe. The outcome of these randomized trials has been mixed, however, with some studies showing significant improvements in global and regional left ventricular systolic function,14,15,18 one trial showing improvements in regional function only,16 and one trial reporting no significant improvements at all.18 While the reasons for these heterogeneous results are difficult to resolve, it has been argued that differences in cell preparation methods and in timing of cell transfer may have been critical.15-17,19

Recent meta-analyses of published randomized and nonrandomized studies, involving a total of approximately 1000 patients, support the notion that bone marrow cell transfer contributes to modest improvements in cardiac function after AMI, above and beyond current interventional and medical therapy.20-22

Skeletal myoblast transplantation

Recently, the first randomized, placebo-controlled study of skeletal myoblast transplantation after myocardial infarction has been published.23 Patients were treated with culture-expanded, autologous skeletal myoblasts or placebo, at least 4 weeks after AMI. Cells were injected into the infarct border zone during bypass surgery. Myoblast transplantation did not improve regional or global left ventricular function, the primary end points of the trial. Notably, however, a significant decrease in left ventricular volumes was noted after cell therapy. A greater incidence of arrhythmias was noted in the myoblast-treated patients, but this did not translate into differences in major adverse cardiac events after 6 months.

ISSUES TO ADDRESS AT BENCH AND BEDSIDE

The mixed results from the randomized bone marrow cell trials remind us that procedural issues, such as the cell preparation method, cell dosage, and timing of cell transfer need to be further refined in upcoming studies. Different cell populations and cell delivery methods may be required depending on
whether the AMI occurred recently or not to achieve optimum therapeutic effects early or late after AMI. Patient subgroups that derive the greatest benefit from cell transfer need to be identified prospectively, eg, patients presenting late after symptom onset in whom little myocardial salvage can be expected from reperfusion therapy. The impact of bone marrow cell transfer on clinical end points is currently unknown. Although a significant reduction in the combined end point of death, myocardial infarction, or revascularization was observed in one trial,24 no such effects were observed in other (smaller) trials.25 Ultimately, large outcome trials using optimized cell preparation and delivery strategies need to be performed. Cell-labeling studies indicate that less than 5% of unselected, nucleated bone marrow cells are retained in the infarcted area after intracoronary delivery in patients.7 It is conceivable that pharmacologic strategies might be used to enhance the homing capacity or other functional parameters of the cells; experimental studies are pointing in this direction.26,27

Table III. Randomized cell therapy trials in patients with acute myocardial infarction or ischemic heart failure. In BOOST, cells were prepared by gelatine polysuccinate density gradient sedimentation, which retrieves all nucleated cell types from the bone marrow. REPAIR-AMI, TOPCARE-CHD, and Leuven-AMI employed a Ficoll gradient, which recovers the mononuclear cell fraction. Although a similar cell isolation protocol was used in ASTAMI, the cell yield was lower as compared with REPAIR-AMI. Remodeling was assessed by MRI in Leuven-AMI, BOOST, and in ASTAMI, by left ventricular angiography in MAGIC. Abbreviations: AMI, acute myocardial infarction; BMC, bone marrow cell; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; n, number of patients; LVESV, left ventricular end-systolic volume.

Abbreviations: AMI, acute myocardial infarction; BMC, bone marrow cell; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; n, number of patients; LVESV, left ventricular end-systolic volume.

Study acronyms: ASTAMI, Autologous Stem-cell Transplantation in Acute Myocardial Infarction; BOOST, BOne marrOw transfer to enhance ST-elevation infarct regeneration; Leuven-AMI, Leuven-Acute Myocardial Infarction; MAGIC, Myoblast Autologous Grafting in Ischemic Cardiomyopathy; REPAIR-AMI, Reinforcement of Enriched Progenitor cells And Infarct Remodeling in Acute Myocardial Infarction; TOPCARE-CHD, Transplantation Of Progenitor Cells And REcovery of LV [left ventricular] function in patients with Chronic ischemic Heart Disease.

The ultimate goal of stem cell therapy is to replace the infarcted area with new contractile and fully integrated cardiomyocytes. While unselected bone marrow cells may have a favorable impact on systolic function, they probably do not make new myocardium. The lack of true cardiac regeneration should stimulate further basic research into the therapeutic prospects of cardiomyocyte progenitor cells. Recent data support the idea that paracrine effects play an important role in patients undergoing bone marrow cell transfer. In fact, bone marrow cells have been shown to secrete a number of proangiogenic factors,28 consistent with the improvements in microvascular function observed after bone marrow cell transfer in patients.29

Experimental studies suggest that enhanced angiogenesis after AMI may improve infarct healing and energy metabolism in the infarct border zone. Further investigation of candidate factors in animal models may ultimately enable more specific and powerful therapeutic strategies after AMI.
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   Bone marrow cells are a rich source of growth factors and cytokines: implications for cell therapy trials after myocardial infarction.

Gene and cell therapy for life-threatening cardiac arrhythmias: will they replace drugs, surgery, and devices?

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Ventricular tachycardia and/or fibrillation result in 200 000 - 400 000 sudden cardiac deaths each year in the US alone and atrial fibrillation currently afflicting up to 2.3 million Americans each year. Gene and cell therapies for cardiac arrhythmias are nascent fields whose raisons d’être derive from: (i) the problematic state of arrhythmia treatment today (especially atrial and ventricular tachyarrhythmias for which drugs, devices, and ablation remain more stopgap measures than optimal interventions); and, (ii) the opportunity to learn, and potentially treat and cure these arrhythmias, by exploring new technologies. Our review examines the state of antiarrhythmic therapy today and the new directions being taken.

Ventricular tachycardia (VT) and/or ventricular fibrillation (VF) lead to 200 000 to 400 000 sudden cardiac deaths each year in the US.1 Atrial fibrillation (AF) today afflicts about 2.3 million Americans, and may reach 12 to 16 million by 2050.2 Reentry accounts for nearly all AF and approximately 85% of arrhythmias in ischemic heart disease (IHD).3 In one conceptualization, the reentrant waveform follows a well-defined anatomic pathway, reaching its point of origin after the standing wave that is the action potential has ended, and the tissue at that site is no longer refractory. For this to happen, a number of changes in conduction must occur, including unidirectional block and slow retrograde propagation.4,5 Another concept is functional reentry,6 in which the length and/or position of the reentrant path changes over time.

Therapeutic approaches to reentry use drugs to prolong the effective refractory period (ERP), and/or to slow/block conduction, and/or surgery or catheter ablation to interrupt the pathway. For example, in AF, flecainide, dofetilide, sotalol, and amiodarone are representative drugs having therapeutic roles.7 Regrettably, clinical trials have demonstrated virtually all antiarrhythmics tested to be proarrhythmic.8 A different pharmacological approach uses angiotensin-converting enzyme inhibitors or angiotensin II type 1 (AT1) receptor blockers to reduce paroxysmal AF recurrences.9 Radiofrequency ablation terminates AF caused by triggered foci in pulmonary veins prevents recurrences, and appears more effective than antiarrhythmic drugs. However, ablation manifests variable success rates: recurrence is more frequent than anticipated.

No antiarrhythmic drug is acceptable primary or sole therapy for VT/VF in the setting of IHD.8 In contrast, several clinical device trials8 have shown survival benefit in treating potentially lethal arrhythmias.

SELECTED ABBREVIATIONS AND ACRONYMS

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>AF</td>
<td>atrial fibrillation</td>
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<td>AV</td>
<td>atrioventricular</td>
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<tr>
<td>ERP</td>
<td>effective refractory period</td>
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<td>hERG</td>
<td>human ether-a-go-go gene</td>
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<td>hMSCs</td>
<td>human mesenchymal stem cells</td>
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<td>IHD</td>
<td>ischemic heart disease</td>
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<tr>
<td>SkM1</td>
<td>skeletal muscle Na channel</td>
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<td>VF</td>
<td>ventricular fibrillation</td>
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<td>VT</td>
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in patients with IHD. But device and combined drug/device approaches to VT/VF carry a high economic price. Emotional and physical tolls comprise a different and important cost. All told, new approaches to prevention/termination would be welcome.

**NOVEL APPROACHES TO PREVENTING/TERMINATING REENTRY**

Nearly 100 years ago, Mines\(^4,5\) conceptualized and experimentally demonstrated reentry and communicated its potential role in clinical arrhythmias. He postulated that the relationship between path length, propagation velocity, and refractoriness determines whether reentry will evolve and persist. Much subsequent discovery derives from Mines’ observations: in the process we have learned of the multiple wavelet and leading circle concepts of reentry, of anisotropy, and of rotors.

The actions of clinically-used \(I_{Kr}\) and \(I_{Na}\)-blocking antiarrhythmic drugs dovetail with Mines’ concepts: they prolong ERP and slow conduction to interrupt reentry.\(^10\) Yet Mines’ concepts avail us of much more information that has not been applied to antiarrhythmic advantage. Figure 1 (from Schmitt and Erlanger’s 1928 adaptation of Mines\(^5\)) explores this: a waveform activates the peripheral conducting system, but if there is a region of depressed conduction (grey), antegrade propagation may fail (Panels A-C) and reentry may occur (Panels D-E). We can terminate the reentrant loop by further depressing conduction until it is blocked bidirectionally and/or by prolonging ERP. Both are outcomes of antiarrhythmic drug therapy: the conundrum in prolonging ERP is that we usually prolong repolarization as well and encounter proarrhythmia.\(^10\) We can cut the loop surgically or using catheter ablation, in the process perhaps creating a scar that may then serve as a nidus for further reentry.

Mines recognized therapeutic options other than prolonging ERP or blocking conduction.\(^4,5\) We should be able to speed conduction such that a waveform “catches its tail,” thereby encountering refractory tissue and failing to propagate further. We also should be able to prolong ERP relative to repolarization, but without prolonging repolarization itself.\(^10\) To date, neither approach has achieved consistent success. Moreover, a clear derivative of Mines’ model is the concept of regional therapies that might modify a portion of a pathway. For example if the Figure 1 pathway were made to propagate normally (Panel F), then reentry would not commence.
CURRENT STATUS OF ANTIARRHYTHMIC GENE AND CELL THERAPIES

Antiarrhythmic gene and cell therapies have: (i) resulted in biological pacemakers for treating heart block; (ii) utilized regional delivery of viral constructs for treating VT or AF; and (iii) developed alternatives to surgery for ablating the atrioventricular (AV) junction in AF.

General approaches to gene and cell therapy are summarized in Figure 2.

To modify cardiac rhythm directly, we can use gene therapy carried via viral vectors. These include: (i) adenovirus, which expresses briefly and episomally, and is used largely for proof-of-concept experiments; (ii) adeno-associated virus, which shows long persistence in expression, but whose genomic incorporation is uncertain; and (iii) lentivirus, which results in genomic incorporation. Despite persistent questions regarding long-term impact on human subjects, these viruses are being used in clinical trials. Cells loaded via electroporation or viral vectors have also been used to carry gene constructs. These include fibroblasts and the human mesenchymal stem cells (hMSCs) in Figure 2.

The leading edge of gene/cell arrhythmia research has focused on bradyarrhythmias and creation of biological pacemakers or AV bridges. Biological pacing strategies have included overexpressing β2-adrenergic receptors, transfecting a dominant negative construct to reduce currents, overexpressing the hyperpolarization-activated cyclic nucleotide-gated (HCN) gene family to increase pacemaker current and using mutagenesis to create designer pacemakers based on HCN or K channel genes. Cell therapy has seen human embryonic stem cells coaxed into a pacemaker line, adult hMSCs used as platforms to carry pacemaker genes, and fibroblasts used to carry pacemaker genes to myocytes with which they have been fused. Finally, in AV block cell-engineered bypass tracts have carried sinus impulses to the ventricles.

The treatment of tachyarrhythmias has been more challenging than that of bradyarrhythmias. Issues include design of therapeutic constructs and whether to administer treatment globally or locally, as discussed below.

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, either through the coronary artery or by the flooding of a chamber or chambers. Not only do these approaches appear excessive for clinical application, but the best success to date has seen about 50% of cells in any region transfected, with viral transfer being diffusion-limited and especially problematic in the ventricles.

Tempering interest in some viral vectors are concerns about inflammation, chronic illness, or neoplasia. These issues led us to explore hMSCs as platforms for gene delivery. It is exciting that hMSCs can be loaded with specific gene constructs and delivered to the heart without eliciting inflammation or rejection, and without differentiating into other cell types. However, the long-term stability of hMSC therapies raises concern (eg, 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 our understanding of the extent of hMSC localization to sites of administration.

Hence, viral vector–based therapies have not yet been applied clinically to arrhythmia management, but have been effective in proof-of-concept experiments (see below), suggesting that gene therapy can be of use. Cell therapies, generally, have been intended to regenerate and repair myocardium rather than to be specifically antiarrhythmic. While we have found hMSCs to be adequate delivery platforms for ion channel generated currents, we have only followed them for 6 weeks. The question of long-term applicability will have to await long-term studies of hMSC survival as well as comparison with genomically-incorporated viral constructs.

**Novel ion channel constructs as antiarrhythmic interventions**

Given the feasibility of gene/cell therapy approaches, a potential advantage is that, unlike drugs, they do not limit us to the channels and transporters expressed by native cardiac myocytes. Instead, channels resident in other tissues, or man-made mutant or chimeric channels with more favorable biophysical properties can be employed. Such a unique arsenal of antiarrhythmic tools allows a “rational” approach to antiarrhythmic therapy, in which the biophysical properties of an ideal therapeutic agent are defined, synthesized, and delivered. A general approach to administering gene therapy constructs is suggested in Figure 3. Theoretically, we might employ novel Na channels or connexins to speed conduction, and/or alter the properties of inward or repolarizing currents to produce postpolarization refractoriness. Alternatively, we might deliver small interfering RNA to target specific channels for inhibition and to block conduction at localized sites.

**Atrial fibrillation**

A major goal of gene therapy experiments on atrial fibrillation has been to induce atrioventricular block. To this end, G-αi2 overexpression via AV nodal artery injection in pig was used to suppress basal adenyl cyclase activity and, via amplified vagal tone, to indirectly reduce the Ca current. During sinus rhythm, AV conduction slowed and ERP was prolonged, and, during AF, there was a 20% reduction in ventricular rate. Other strategies reported are the creation of an AV nodal site of Ca channel blockade, or the implantation of fibroblasts to induce AV nodal scarring and block. All these approaches exemplify local gene delivery whose therapeutic intent is to produce rate control. Whether they will become a practical alternative to radiofrequency ablation is uncertain.

Another experimental AF therapy aimed at rhythm control uses an ion channel mutation Q9E-hMiRP1 (a contributor to long QT syndrome induced by I<sub>Kr</sub>-blocking drugs). Levy et al administered the construct into the atrial epicardium of pigs: about 15% of cells manifested up-
take. Clarithromycin was then infused and profoundly blocked $I_{Kr}$. This led the investigators to hypothesize that, in AF, they might achieve regional atrial $I_{Kr}$ blockade without prolonging the QT interval.\(^{24}\)

**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. For example, Reddy et al demonstrated that mapping to identify sites for local radiofrequency ablation reduced the need for defibrillation in patients who had devices implanted for secondary prevention.\(^{25}\) Using mapping to identify the border zone of an infarct in a canine model, we have replaced ablation with intramyocardially administered gene therapy in preliminary studies and—without destroying tissue—achieved a reduction in VT/VF incidence.\(^{26}\)

**Specific gene therapies for ischemic arrhythmias**

- **Speeding conduction via Na channels or connexins**

  At least 10 different Na channel genes encode α-subunits in the mammalian genome, and these have been cloned from brain, spinal cord, skeletal and cardiac muscle, uterus, and glia.\(^{6}\) Since slow conduction is an essential feature of reentrant cardiac arrhythmias, we sought other mammalian Na channels that might have more favorable properties than the cardiac Na channel in circumstances that favor slow conduction (Figure 4).\(^{26}\) One such circumstance is membrane depolarization, as in myocardial infarction (Figure 3). Here, the voltage dependence of steady state Na channel inactivation is of interest. The midpoint of the cardiac Na channel (SCN5A) is negative to -73 mV. This is important because, in infarcted tissue when myocytes are depolarized to -65 mV, virtually all SCN5A-derived cardiac Na channels are inactivated. In contrast, skeletal muscle (SkM1) Na channels have an inactivation midpoint of -68 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 with more favorable biophysical properties than SCN5A, such as SkM1, might be a useful antiarrhythmic therapy (Figure 4). Data from our laboratory have demonstrated the effectiveness of this approach 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.\(^{26}\) Moreover, as shown in Figure 5, SkM1 administration reduced electrogram fragmentation and increased $V_{\text{max}}$ of phase 0 (consistent with more rapid conduction), as had been predicted for SkM1. Several studies have lent credence to the importance of connexins and, hence, gap junctions in arrhythmias. For example, overexpression of Cx45 results in ventricular tachycardia in mice,\(^{27}\) while mutations of Cx40 are associated with atrial fibrillation in humans.\(^{28}\) Studies of the epicardial border zone of heal-
ing canine myocardial infarcts have demonstrated altered connexin distribution and density in regions important to the generation of reentrant ventricular tachycardia. The modulation of gap junctions as an antiarrhythmic strategy initially attempted to block conduction. However, the gap junctional blockers used to date have not been channel-specific or 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 is purportedly antiarrhythmic.

- **Targeting diastolic membrane potential**
  In VT in the setting of a partially healed infarct, the viable, but depolarized tissue in the border zone provides the substrate for a reentrant arrhythmia (Figure 3). 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 Kir2.1 (generated by Kir2.1 with some contribution from Kir2.2). Studies on the overexpression of these channels are in progress.

- **Enhancing rate responsiveness and/or refractoriness**
  Reentrant arrhythmias require reexcitation of tissue by a propagating waveform. Here, 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 spread propagation of the reentering waveform so that it encounters tissue that remains refractory. Hua et al. showed that 6-fold overexpression of native human ether-a-go-go gene (hERG) eliminates T-wave alternans in isolated canine ventricular myocytes and in computer simulations. Using a different approach, Sasano et al delivered a dominant negative hERG mutant (HERG-G628S) via vascular infusion to a peri-infarct zone of pigs. Monomorphic VT had been consistently inducible in infarcted animals before gene transfer, but, 1 week later, all HERG-G628S-transferred pigs showed no such arrhythmia. This result emphasizes the therapeutic potential of a different local approach to VT therapy in chronic infarcts.

### CONCLUSIONS

How best to prevent/treat arrhythmias that confer significant morbidity and/or are life-threatening is a question that 40 years of targeted pharmacologic therapy and many more years of empirical drug therapy have not answered. While surgery, ablation, and cardioverter-defibrillators are newer, robust alternatives, all appear draconian when compared with a targeted therapy that can be administered via catheter
and doesn’t destroy tissue. Gene and cell therapies meld principles for rational drug design with a new approach to treatment. The approach starts by simulating an optimal change in an ion current present in the heart, and then selects constructs independent of their tissue origins based on their close mimicry of the proposed optimal change. Additionally, use of novel genes not normally found in heart expands our therapeutic universe.

Also innovative are the focus on regional delivery of these novel ion channel constructs, the use of hMSCs as platforms for therapeutic intervention, and the harnessing of these new therapies to mechanistically test old, but to date largely untestable, concepts (eg, speed conduction, increase ERP/repolarization ratio without prolonging repolarization).

These approaches should not be interpreted as providing a “quick fix.” However, the ability to prepare constructs and to apply them based on an understanding of arrhythmogenic mechanisms makes it highly likely that: (i) definitive answers—whether positive or negative—will be obtained to the questions we have regarding improvement of arrhythmic therapy, and (ii) we will understand why, mechanistically, an approach has succeeded, failed, or, as a worst case, been pro-arrhythmic. Negative answers will be as important as positive. Of signal importance is that as accurately and rapidly as possible we find the proper route, vector and/or platform, and construct for reducing the threat to the population of the arrhythmias of concern.

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The name of Doppler when we listen to the weather forecast, or read about the beginning of the universe (the Big Bang, the red shift), or when we study diagnostic reports on heart disease. Who was this man who gave us the tools to explore these divergent subjects?

Christian Doppler was born in November 1803 in Salzburg, Austria, and died in Venice, Italy, on March 17, 1853. He came from a family of master stonemasons and showed exceptional gifts for this craft. But because of his poor health, his father considered him suitable for the bookkeeping function of the family business. But it soon became apparent that Doppler had outstanding talents in mathematics. He was sent to the Polytechnic School in Vienna, but he disliked the instructions and called it “a one-sided education.” At the age of 21, he returned to his native Salzburg and finished his education while he supported himself by giving classes in mathematics and physics. He then returned for four years to the Polytechnic in Vienna. Like other great scientists, including Einstein, Doppler received many rejections in response to applications for positions, and finally was compelled to become a bookkeeper. Discouraged, he set his eye on America and in Munich, he discussed with the American consul the possibilities of finding a teaching position in America. He even sold his possessions to finance his journey. But while in Munich, he received two offers to teach in high schools in Switzerland or in Prague, which was then part of the Austrian Empire. These were not university positions, but involved high school teaching. Doppler chose the position in Prague, where he again encountered opposition and frustration, making him anxious to leave Prague. Like Einstein who failed to attain an appointment at the techni-


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cal high school in Zurich, Doppler had an unsuccessful interview for a position at the Polytechnic Institute in Vienna. Interviews with other institutions were equally unsuccessful. In Prague, he published a new optical instrument, called the distometer, for measuring distances and discussed the aberrations of light and sound in a rotating medium. It was at a meeting of the Natural Sciences Section in Prague that Christian Doppler postulated the theory that immortalized his name. He and his family left Prague after he was appointed Professor of Mathematics in a small Czechoslovakian town. Finally, he was elected a Full Member of the Imperial Academy of Science in Vienna and awarded an honorary doctorate from the philosophical faculty. He was then appointed to join the Polytechnic Institute in Vienna and finally, was authorized to found an institute of physics at the Imperial University in Vienna. There, a 20-year-old Augustinian monk, Johann Gregor Mendel, took a written and oral examination to study at the University of Vienna, but Doppler was not very impressed by his mathematical ability and Mendel was refused admission to the university. Mendel was finally admitted. He later laid the foundation of genetics as an abbot in a monastery. Doppler suffered from tuberculosis, which had spread to the larynx and made speaking increasingly difficult. In 1852, his health had so deteriorated that he took a six-month period of convalescence in Venice, where the climate was supposed to influence the course of tuberculosis. He died in 1853 and was buried in Venice. Aside from being plagued by ill health all his life, Doppler was continuously afraid of losing his livelihood, with good reasons as his career demonstrates. His friends called him modest, thrifty, and correct.

Like many physicists before and after him, he derived his inspiration for his principle from observations of natural phenomena. As he wrote, “We know from general experience that a ship of moderately deep draught which is steering toward the oncoming waves has to receive, in the same period of time, more waves and with a greater impact than one which is not moving or is even moving along in the direction of the waves. If this is valid for the waves of water, then why should it not also be applied with necessary modification to air and ether waves?” Doppler applied his principle first to astronomy. In his article on the "Col-
ored light of double stars and other constellations of the heavens” he argued that all stars emitted white light and that the color of some of the stars was due to their motion toward us or away from us. Actually this was an erroneous conclusion because on the approach of a star only a slight shift would be produced, but no change in color would take place. But the principle is correct; an apparent shift in the frequency of waves received by an observer depends on the relative motion between the observer and the source of the waves. This principle was immediately attacked and as recently as 1965 objections against his principle were published. Others opposed Doppler’s theory because of its simplicity. One of them, Petzval, used the argument that, “Without the application of differential equations, it is not possible to enter the realms of great science.” Obviously, his critics thought that great truth could not be found in a few lines and through an equation with only one unknown; at least one differential equation is necessary. In 1845, a Dutch scientist, Buys Ballot of Utrecht, confirmed Doppler’s principle on the railway between Utrecht and Amsterdam. Using a locomotive capable of attaining the at that time incredible speed of 40 mph, to pull an open cart in which horn players were riding, Ballot attempted to observe changes in the apparent pitch of the notes played by the musicians as they approached or receded. However, the experiment was performed in February and the musicians had trouble blowing their instruments because of the cold, and the project was postponed; in June of that year, the validity of Doppler’s theory was finally confirmed.

In cardiology, the principle was first utilized to detect cardiac motion and time the opening and closing of the cardiac valves. Satomura used a continuous ultrasound beam transmitted through the chest wall to the heart which, reflected from the heart structures underwent a frequency shift, or Doppler shift, of the transmitted sound; its magnitude and direction were based on the speed and direction of movements of the heart. The frequency of the reflected sound was proportional to the velocity of components of the target. Once the velocity of blood flow in the aorta could be recorded, the technique was adapted for measurement of cardiac output. The most important development in cardiac Doppler analysis was the introduction of pulse wave Doppler, which allows localization of flow velocity measurements to specific valves and chambers. It is based on the principle that blood flow in a small area within the heart can be recorded by the use of intermittent pulses of transmitted sound.

The receiver then listens for reflected
sound only at the end of the time interval required for the pulse to travel from the transducer to the area of interest and back. This way it is possible to localize murmur, determine orifice size from jet diameter, and measure pulmonary flow and pulmonary artery pressure.

Doppler’s principle has made it possible to determine the ejection fraction of the heart, one of the most valuable measurements in cardiology. It has to a large extent, together with echocardiography, replaced cardiac catheterization, particularly in children with congenital heart disease. The correlation between measured Doppler flow velocities and pressure gradients form the basis for assessment of valvular and vascular stenosis, prosthetic valves, and permitted estimation of chamber pressure. Simultaneous determination of velocity in several areas of the heart can also be performed. The digitally processed system displays velocity by color-coding. Current instrumentation allows for superimposition of color-coded velocity on the tomography image. Doppler’s principle is also applicable to the diagnosis of congenital malformations of the heart in utero.

Christian Doppler’s life shows again that scientific accomplishments do not guarantee personal happiness. One of the main reasons in Doppler’s case was ill health. Since his early youth, Doppler was plagued by progressive respiratory disease, due to tuberculosis, which involved the larynx. In addition, Doppler’s concept was new. Like many great discoveries, it was simple and it was direct. It was just this simplicity and directness, which caused other scientists of limited outlook to suspect his principle. Lesser scientists often judge a new discovery by its complexity, which they find attractive. Furthermore, Doppler’s discovery had no practical applicability. It took more than 100 years to make an impact on cosmology, meteorology, and medicine. As Einstein has said, “Ach der Mensch betrügt sich gern, nimmt die Schale für den Kern,” or translated freely “How man does fool himself, mistaking the outer shell for the inner truth.”

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Summaries of Ten Seminal Papers

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Highlights of the years by Ian Mudway, MD
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Remarkable examples of regeneration can be found throughout nature. Newts regrow whole limbs. A flatworm can form a complete flatworm from a small portion of itself. Regeneration of damaged tissue is essential for long human life as our bodies mend fractured bone, repair torn muscle and skin, and renew blood cells. It was long held as dogma that the adult human brain and heart were not capable of regenerating new neurons or cardiomyocytes. This belief was underscored by clinical experience. After a stroke, infarcted brain tissue appears permanently lost as demonstrated by decades of experience with brain imaging. In the heart, myocardial infarction (MI) is a common early insult leading to impaired cardiac function and clinical heart failure. Scar tissue replaces the infarcted region, not new myocardium. In the 1990s, breakthrough discoveries in hippocampal biology challenged the dogma that adult brains cannot regenerate. To revisit the regenerative potential of the adult heart, Beltrami et al conducted a thorough painstaking examination of human hearts with recent MI to search for evidence of mitosis. They found strong evidence that cell division does indeed occur in adult human hearts.

Thirteen hearts from patients who died 4 to 12 days after suffering a MI were harvested 7 to 17 hours after death. Samples were taken from the infarct border zone and a site distant to the infarct. Standard histological methods were used and sections were analyzed with confocal microscopy for the presence of Ki-67, a nucleolus component found in every cell cycle phase except G0 (resting state), and α-sarcomeric actin, expressed only by cardiomyocytes. Therefore, any cell staining positive to both Ki-67 and α-actin is a cardiomyocyte undergoing the process of cell division. Over 100,000 nuclei were analyzed in the infarct border and the normal region in each heart. The results are startling and provide much food for thought. In the infarct border, there was an 84-fold greater number of double labeled Ki-67/α-actin cells than in the comparable control region. When the site distant from the infarct was compared, a 28 times greater number of Ki-67/α-actin cells was seen. Using an antitubulin antibody to identify mitotic spindles, similar numbers of cardiomyocytes with visible evidence of mitosis were present (70-fold increase in infarct border and 24-fold increase in distant myocardium). To definitely prove completion of mitosis and formation of two daughter cells would require labeling studies in patients, unlikely with current technology.

The results provide strong evidence that the heart’s response to injury is cardiomyocyte proliferation to compensate for the lost cells. The presence of Ki-67/α-actin stained cells with mitotic spindles in the normal controls suggests that there maybe a continuous turnover of cardiomyocytes throughout life. As with all paradigm-shifting discoveries, more questions than answers were raised. What are the molecular signals that govern proliferation? What is the origin of the dividing cell? Are they differentiated cardiomyocytes that reenter the cell cycle? Are there resident cardiac stem cells? Do extracardiac stem cells home to the heart and proliferate into cardiomyocytes? Is regenerating activity in the infarct border zone a substrate for post-MI arrhythmias? It is clear that cardiac proliferation after a myocardial infarction is not a clinically meaningful process. However, this exciting research area will yield insights that may change tomorrow’s treatment of heart failure.

**Evidence that human cardiac myocytes divide after myocardial infarction**


Embryonic stem (ES) cells from mice have revolutionized biomedical science since their isolation in 1981. Manipulation of murine ES cells has allowed the creation of transgenic and knockout mice that have greatly expanded our understanding of development and gene function on an organism level. The fundamental property that makes ES cells unique is totipotency, the ability to give rise to any cell lineage in the body. In 1998, Thompson et al first described the generation of human ES cell lines derived from a blastocyst. The isolated human ES cells were shown to have the capacity to differentiate into the ectodermal, endodermal, and mesodermal lineages. Kehat et al were the first to demonstrate that human ES cells in culture can be differentiated into myocytes that possess characteristics that define cardiomyocytes.

In this important manuscript, the authors allowed human ES cells to aggregate and form embryonic bodies containing derivatives of all three germ layers. The embryonic bodies were plated on gelatin-coated dishes and were observed daily to assess the presence of spontaneous contractions. Contracting areas were mechanically dissected from the embryonic bodies and rigorously studied. By using reverse transcriptase polymerase chain reaction (RT-PCR), the contracting area cells were found to express cardiac specific genes such as troponin I, troponin T, the transcription factors GATA4 and Nkx2.5, as well as atrial and ventricular myosin light chains. Contracting area cells exhibited strong immunostaining to cardiac myosin heavy chain, troponin I, ANP, α-actinin and desmin. Contractile elements ranging from unorganized myofibrillar bundles to organized sarcomeres and Z bands could be appreciated by electron microscopy. Extracellular electrograms of the contracting area cells demonstrated depolarization and repolarization activity. Positive and negative chronotropic responses were observed with the application of the β-adrenergic agonist isoproterenol and the muscarinic agonist carbamylcholine. The authors conclusively showed that the contracting areas cells possessed the gene expression profile, ultrastructure, immunoreactivity, and functional properties of human cardiomyocytes.

The ability to reliably differentiate human ES cells into cardiomyocytes in the laboratory will be a powerful tool in understanding human cardiogenesis. Moreover, these findings raise the possibility of using human ES cells to achieve myocardial repair. Proof-of-concept studies in the mouse have suggested that embryonic cardiomyocytes can be useful for cardiac repair after injury. Many questions still need to be answered. The signals and events that promote human ES cell differentiation into cardiomyocytes are incompletely understood as only 8% of embryonic bodies exhibited contracting areas. By understanding these signals, human ES cells may be directed toward cardiomyocyte differentiation to increase the yield. The interaction between mature cardiomyocytes in a diseased heart and the ES cell–derived cardiomyocytes need to be understood as well as the processes that promote integration of the transplanted cardiomyocyte into the heart. Furthermore, issues related to rejection will have to be addressed with transplanted ES cell–derived cardiomyocytes before widespread clinical use.

Jean Henri Dunant receives the Nobel Peace Prize for his role in founding the International Committee of the Red Cross; New York becomes the first state to make automobile license plates compulsory; and German psychiatrist Alois Alzheimer describes his eponymous disease.
When gene therapy first entered medical scientific consciousness, clinical applications were focused on the cure of diseases resulting from defective or missing genes. By replacing deleterious genes with normal functional copies, disease can be halted or even reversed. Miake et al took a radical departure from this paradigm and showed that gene transfer can tweak existing cells to change their physiological role. The authors asked, “Can ventricular myocytes be engineered into pacemaker cells?”

Miake et al hypothesized that adult ventricular myocytes had the appropriate repertoire of ion channels for spontaneous pacemaker activity, but that this was normally repressed by the inward-rectifier potassium current \( I_{K1} \), encoded by the Kir2 gene family. \( I_{K1} \) is not found in nodal pacemaker cells, but is robust in adult atrial and ventricular myocytes, where it stabilizes a very negative resting potential and suppresses excitability. Because Kir2 potassium channel genes have a tetrameric structure, a dominant negative strategy to suppress \( I_{K1} \) is feasible with a nonfunctional Kir2.1 mutant, in this case Kir2.1AAA, which has 3 alanine substitutions in the pore region.

The dominant negative mutant was packaged with green fluorescent protein (GFP) into an adenoviral vector and introduced into the guinea pig left ventricle during transient cross-clamping of the great vessels. Gene transduction rates into ventricular myocytes were about 20% as seen by GFP expression and whole-cell recording of isolated GFP expressing myocytes had 80% suppression of \( I_{K1} \). The electrophysiology of the gene transduced myocytes fell into two categories: (i) no spontaneous activity, but prolonged elicited action potentials; or (ii) spontaneous activity remarkably similar to sinoatrial pacemaker cells. The myocytes with spontaneous activity had \( I_{K1} \) suppressed to a greater extent. The surface ECG of the transfected animals was fascinating. Half of the guinea pigs remained in sinus rhythm with QT prolongation. However, the other half had cardiac rhythms indicating spontaneous ventricular foci suggested by the broad QRS duration. The ventricular rhythms were noted to “march through” the sinus beats and at times faster than sinus rhythm.

Though the techniques are not clinically acceptable, this report clearly demonstrated for the first time that biological pacemakers are achievable. Furthermore, the findings shed insights into the electrophysiological makeup of ventricular myocytes. A particularly attractive aspect is that a biological pacemaker can be created from one’s own cardiac cells by ex vivo gene transfer. Ventricular myocytes can be harvested with a cardiac biotome, transduced with the gene of choice and reimplanted into the heart. Alternatively, the gene transfer vector can be injected directly into the myocardium. Miake et al’s findings triggered the race to develop a reliable biological pacemaker, with groups worldwide utilizing different viral vectors, cell-based delivery, and novel biomaterials. Obviously, many hurdles remain before clinical acceptance, especially regarding safety and reliability, and of course, proof that biological pacemakers are better than the current gold standard, the electronic pacemaker.
Heart regeneration in zebrafish

K. D. Poss, L. G. Wilson, M. T. Keating

Science. 2002;298:2188-2190

After a myocardial infarction, human hearts respond to the injury by extensive scarring with minimal regenerative potential. Replacement of myocardium with scar tissue has consequences for ventricular remodeling, cardiac function, and arrhythmia potential. Previous work has shown that the zebrafish (Danio rerio) is capable of regenerating fins, retina, and spinal cord. Poss and colleagues are the first to demonstrate that zebrafish can regenerate ventricular myocardium after acute injury.

Zebrafish have become a favored species in genetic research due to their ease of handling, fast generation times, and availability of simple genetic screening methods. Using adult zebrafish, the heart was exposed through a small skin incision and the ventricular apex excised with scissors, about 20% of the heart. Profuse bleeding from the ventricular cavity was stopped with a piece of laboratory paper tissue and a large clot of erythrocytes formed over the excision site. A survival rate of 90% was achieved when 20% of the ventricle was excised. Mortality rates increased when more than 20% of the ventricle was removed. The zebrafish were then followed for up to 60 days after surgery.

Within 2 to 4 days after ventricular apical amputation, fibrin began to replace the erythrocyte clot. The zebrafish during this time appeared sluggish, but by 1 week after amputation, they were indistinguishable from sham controls. Nine to 30 days after amputation, cardiac myofibers surrounded, penetrated, and eventually replaced the fibrin clot. By 60 days after amputation, the hearts that underwent ventricular apical amputation appeared grossly normal in size and shape. The zebrafish ventricle is composed of two myocardial layers, an outer compact layer, and an inner trabecular layer. The amputated hearts regenerated both myocardial layers and were indistinguishable on histological inspection. Using BrdU, a marker of DNA synthesis, Poss et al showed that the cardiomyocytes closest to the cut edge underwent cell division to replace the lost cardiomyocytes. A mitotic checkpoint kinase (mps1) is known to be required for zebrafish fin regeneration as well as cell proliferation. Using a conditional mps1 mutant zebrafish line, ventricular apical amputation resulted in scar formation rather than cardiac regeneration, similar to the human cardiac injury response.

Stem cell–based approaches to cardiac repair have gotten a lot of attention, but an alternative strategy is to stimulate the damaged heart to heal itself. Though the zebrafish heart is simpler in structure than mammalian hearts, elucidating the signals and pathways that direct injured hearts toward regeneration or scar formation may give insights into human cardiac biology and strategies for treatment. Amphibians have also been shown to have the capacity for cardiac regeneration. However, cardiac regeneration would be easier to study in the zebrafish because of its sequenced genome and the ease of conducting mutational studies. Poss et al have identified a species that possesses robust cardiac regeneration capacity after injury with well-established genetics. The great promise of understanding cardiac regeneration in the fish is that it may lead to therapeutics that can stimulate cardiac regeneration in the injured human heart. Let us hope that we can fish out these factors.

Birth of Leni Riefenstahl, the German film director who shot the controversial “Triumph of the Will” propaganda film at the 1934 Nuremberg Congress of the Nazi Party; Edward VII is crowned King of the United Kingdom; and the Carnegie Institution is founded in Washington DC, in support of scientific research.
Earlier work by these authors dispelled the long held belief that the adult human heart is incapable of cell division. The origins of the dividing cardiomyocytes were unclear. One intriguing possibility was a pool of resident cardiac stem cells that can be activated to proliferate and differentiate into cardiac cells. Beltrami et al demonstrate in this paper that adult mammalian hearts have such a defined pool of stem cells that can be isolated, cultured, and implanted into a myocardial infarct to regenerate myocardium and improve cardiac function.

In adult rats, Beltrami et al characterized cardiac stem cells by the cell surface marker profile (c-Kit+, Lin-, CD45-, CD34-). These cardiac stem cells can be isolated from rat heart preparation by flow cytometry using FACS or magnetic beads coated with c-Kit antibody. The isolated cells can be expanded in culture indefinitely and cloned. In laboratory cultures, the cardiac stem cells were able to differentiate into cardiomyocytes, smooth muscle cells as well as endothelial cells, however in immature forms. For example, the culture differentiated cardiomyocytes that expressed specific markers such as α-actin and cardiac myosin heavy chain, but exhibited disorganized structures rather than sarcomeres, and spontaneous contraction was absent. To test the ability of these cardiac resident stem cells in myocardial repair, adult rats with induced myocardial infarction received injections of stem cells labeled with BrdU along the infarct border. After 10 days, a thin regenerating band was seen that incompletely penetrated the infarct. After 20 days, the entire infarct demonstrated BrdU labeled cells and a significant increase in myocardial volume. The infarct size was significantly decreased with stem cell treatment (70% control vs 48% stem cell) and ejection fraction was improved (34% control vs 45% stem cell). The labeled resident stem cells gave rise to cardiomyocytes, smooth muscle cells, and endothelial cell in the infarct border zone. In contrast to the culture differentiated cells, the labeled cells differentiated in the rat heart morphologically appeared mature. Isolated cardiomyocytes derived from stem cells had similar contractile function as native cardiomyocytes when tested in vitro, unlike their cultured differentiated counterparts. The observed changes in vivo were not due to fusion of stem cells with native cardiac cells, as >99% of the cells examined were diploid, not tetraploid. More recently, these cardiac stem cells were delivered via the coronary circulation in rats with myocardial infarctions, with similar results.

Beltrami et al are the first group to have defined a population of resident cardiac stem cells that were clonogenic, multipotent, and able to participate in the formation of functional myocardium within a clinical relevant model. If similar populations of cardiac stem cells are present in humans, the implications for clinical applications are obvious. An interesting question is that if a resident pool of cardiac stem cells is present, why do these cells not repair the myocardium efficiently after injury? One potential answer may be that a critical number of cardiac stem cells is needed for meaningful repair. The authors do not comment on dose response, if any, of their cardiac stem cells. Three other populations of cardiac stem cells have been described since Beltrami et al. Stay tuned for more developments in this rapidly evolving area.

Adult cardiac stem cells are multipotent and support myocardial regeneration


Cell. 2003;114:763-776

2003/1903

King Edward VII is proclaimed Emperor of India; the Martha Washington Hotel, exclusively reserved for women, is founded in New York; and American frontierswoman Calamity Jane dies, aged 51
Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers


Circ Res. 2004;94:952-959

The electronic pacemaker is undoubtedly one of the major medical advances in history. Though highly successful, there is room for improvement, as electronic pacemakers have limited battery life, lack of autonomic response, and imply the presence of permanent hardware in the body. With regard to biological pacemakers, early efforts have focused on gene-based approaches utilizing different viral vectors. However, viral gene transfer raises questions concerning duration and magnitude of gene expression, the consequences of viral protein expression, carcinogenic, and infectious potential. With this paper, Potapova et al lay the foundation for using stem cell–based approaches to generate reliable cardiac pacemaking.

Human mesenchymal stem cells (hMSC) have several advantageous features making them attractive delivery vehicles. hMSC are readily available due to easy harvesting and can be maintained in culture. hMSC also possess local immunosuppressive properties that allow allogenic transplant without significant rejection, a feature that may ease clinical application. Potapova et al show that a robust \( I_f \) current is present in transfected hMSC with the mouse \( HCN2 \) gene by electroporation. Moreover, application of the \( \beta \)-adrenergic agonist isoproterenol induced a positive shift in \( I_f \) activation in the HCN2-hMSC. Acetylcholine, a muscarinic agonist, reversed the effects of isoproterenol. Therefore, HCN2-hMSC possesses the protein machinery required to respond to autonomic hormones.

A pacemaker cell must electrically couple to neighboring cardiomyocytes to pace, and the investigators elegantly showed that hMSC do indeed form functional gap junctions. In cocultures of HCN2-hMSC with canine ventricular myocytes, dual whole-cell recording of hMSC and myocyte pairs demonstrate electrical coupling. Furthermore, ventricular myocytes cocultured with HCN2-hMSC have more positive maximum diastolic potentials and faster spontaneous rates than myocytes cultured with hMSC expressing GFP. The pacemaking performance of HCN2-hMSC was very impressive with implanted dogs having significantly faster idioventricular rates originating from the implant site than controls. hMSC were easily identified histologically by their size and confirmed by positive vimentin and CD44 staining. Staining for Cx43 revealed that gap junctions formed between the hMSC and canine ventricular myocytes in vivo. No evidence of inflammation or rejection was seen, underscoring the immunoprivileged status of hMSC. More recent work by Plotnikov et al (Circulation 2007;116:706-713) has shown that HCN2-hMSC can provide reliable biological pacing for up to 6 weeks without rejection.

Potapova et al were the first to show the feasibility of a stem-cell–based approach to biological pacemaker development. hMSC appear to have many desirable features of a delivery platform that may allow for widespread clinical use. Unlike viral-based approaches where reliable expression can be challenging from subject to subject, a stem cell–based pacemaker can be verified for expression and performance prior to implantation. The immunoprivileged status of hMSC may allow for an inventory of ready-to-use biological pacemakers without significant rejection. Many questions need to be answered before clinical testing of a biological pacemaker to compete against current electronic pacemakers. This important work brings us one beat closer.
We have all been taught that the human heart is an end organ without any regenerative properties. Patients with failing hearts may receive a mechanical ventricular assist device or a heart transplant as treatment. Ventricular assist devices have a host of drawbacks such as infections, thromboembolic complications, and arrhythmias, limiting their chronic widespread use. Improved immunosuppressive regimens have made cardiac transplant a long-term solution for heart failure patients. However, there are simply not enough hearts available for the huge transplant demand. Advances in basic science over the past decade have initiated excited discussions on what used to be considered science fiction, “How to grow a new heart?” Laflamme and Murry have written an outstanding comprehensive review on the current state of progress in cardiac regeneration.

The underlying hypothesis is that heart failure can be reversed or prevented if new myocardium can be grown and integrated into diseased hearts. Early work toward cell-based cardiac repair utilized skeletal myoblasts injected into the heart; however, it became quickly clear that skeletal myoblasts remained skeletal, did not transdifferentiate into cardiomyocytes and did not electromechanically couple to the surrounding myocardium. Clinical transplantation studies provided some evidence that circulating cells had the ability to repopulate adult cardiac tissue, the most common case being a male patient receiving a female donor heart, with Y chromosome cells subsequently seen within the transplanted heart. Initial focus was on hematopoietic stem cells. The contribution of bone marrow stem cells to the healing myocardium was controversial, with several groups reporting conflicting results. Mesenchymal stem cells long thought to be permanent residents of the bone marrow stromal component have generated significant interest for therapeutic applications due to two fascinating properties. Mesenchymal stem cells appear to have local immunosuppressive properties that allow them to survive in allogenic settings and they appear to home to areas of injury. The discovery of resident myocardial progenitor cells in the adult heart changed what we understood about development. At last count, four separate populations of resident myocardial progenitor cells have been described. Embryonic stem cells have the greatest hope of generating an entire heart, but significant challenges remain on how to coax them toward cardiogenesis.

Tissue engineering will almost certainly play a major role in regenerative cardiac repair. The most common approaches utilize cell scaffolds, mechanical conditioned gels, and layered cellular sheets. One of the biggest challenges with tissue engineering is nutrient delivery, since diffusion alone can only supply to a depth 150 microns. Several obstacles also remain that limit widespread clinical applications. Cell delivery systems are less than ideal as the great majority of cells are lost in the circulation or leakage from injection site. Cell survival is a major problem, as most transplanted cells do not survive. Control of proliferation is difficult, as a delicate balance exists between cellular replacement and neoplasia.

The field is moving very rapidly but it is highly unlikely that we will learn how to mend broken hearts soon. Nonetheless, phase 1 clinical testing has been initiated for several stem cell–based therapies for ischemic heart disease and, thus far, they appear to be safe and well tolerated. The medical community will have to wait for larger randomized multicenter trial results to gauge therapeutic value.

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2005/1905

Tsar Nicholas II of Russia agrees to reintroduce an elected council, the Duma; Albert Einstein’s “miracle year,” which saw the four major publications that were to profoundly change the face of physics; and French novelist Jules Verne, author of “Twenty Thousand Leagues Under the Sea,” dies, aged 77
The human heart has limited regenerative capacity after a myocardial infarction (MI), a fact cardiologists are reminded of daily. Basic research has established the capability of stem cells to differentiate into cardiomyocytes. In animal models of myocardial injury, stem cell–based strategies have improved myocardial function. A crucial issue for any therapeutic is delivery to the target tissue. Histological examinations of postmortem cardiac tissue suggest that intravenously administered stem cells may preferentially localize to areas of injury. Kraitchman et al provide convincing data in a clinically relevant model of acute MI that mesenchymal stem cells (MSC) do indeed home, and furthermore stay, in the infarct region.

The study utilized two common cardiac imaging techniques, single photon emission computed tomography (SPECT)/CT and magnetic resonance imaging (MRI). Nontransmural MI was created in dogs by 90-minute balloon occlusion followed by reperfusion. Allogenic canine MSC dual-labeled with 111In oxine and Feridex was injected intravenously 3 days later. 111In oxine is used routinely to label leukocyte and its half-life (67.3 hours) allows for prolonged serial imaging by SPECT. In vitro assay with 111In oxine had no appreciable effect on MSC proliferation, viability or differentiation. SPECT/CT images were obtained on day of injection, 24 hours after injection, and up to 1 week afterwards. MRI images were obtained only with the final SPECT/CT scan. SPECT/CT permits high-resolution detection of the radiolabelled MSC with anatomic localization. Immediately after injection of labeled MSC, lung uptake predominated, with smaller amounts of uptake in the liver and kidney. Presumably, the relative large size of MSC (about 25 μm) may cause some difficulty traversing the pulmonary circulation. Twenty-four hours later, the radiodistribution was dramatically different as lung uptake is much lower and the predominant uptake is within the liver and spleen, suggesting redistribution to the reticuloendothelial system. The extracardiac distribution pattern was seen in both MI dogs and noninfarct controls. In the infarcted heart almost immediately after MSC injection, increased radiolabel was appreciated in the anterior apex. SPECT/CT imaging at 4 to 7 days after injection showed diffuse myocardial uptake corresponding to the anterior apical myocardial area, but not in normal myocardium. MRI imaging could not visualize the Feridex-labeled MSC due to the diffuse nature of the distribution. Postmortem histological examination confirmed the presence of the labeled MSC in the infarct and peri-infarct cardiac regions.

Kraitchman et al have provided the methods for noninvasive tracking of MSC inside the living body. Translation into humans should be straightforward as the scanners and labels used are approved by the US Food and Drug Administration and are available at most medical centers. Localizing and quantifying the number of MSC to the infarct region will be essential in determining the appropriate clinical dose amount and schedule. Interpretation of clinical data will be enhanced as outcomes can be related to the number of MSC targeted to the infarct area. Though validation studies will be required using human MSC, this work is a major step toward properly conducted stem cell trials pertaining to cardiac repair.

Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction


Circulation. 2005;112:1451-1461

An earthquake in India kills more than 20,000; the US Army begins work on the Panama Canal; and the FIFA (International Federation of Association Football) is created, still going strong a century later
ventricular tachycardia (VT) is unfortunately a common and often fatal complication of ischemic heart disease. Implantable cardiac defibrillators (ICD) have greatly improved survival. However, ICDs have their share of shortcomings, including inappropriate painful shocks, with their associated psychological consequences, as well as the fact that hardware is permanently present within the body. Current antiarrhythmic drugs are limited by incomplete arrhythmia suppression and toxicities, including proarrhythmic effects. Gene-based approaches to control arrhythmias are of great interest because they offer several distinct advantages. Expression vectors can be precisely delivered to the area of interest, such as the infarct border zone, to minimize systemic side effects with existing available technology (coronary catheterization or percutaneous endocardial injection). Unlike drugs that can only modulate ion channels and receptors expressed in the diseased cardiomyocyte, gene therapy is not limited by the existing protein repertoire, but can deliver any protein. The therapeutic protein can be an endogenous protein, a mutant, a chimera, or even a protein completely foreign to the cardiomyocyte.

Sasano et al gave the first demonstration of a gene therapy approach to effectively suppress postinfarction VT. Myocardial infarctions were created in pigs by balloon occlusion of the mid-left anterior descending artery (LAD) for 150 minutes. After 3 weeks of recovery, VT inducibility was assessed by programmed stimulation and monomorphic VT was inducible in all pigs tested. Adenovirus expressing a dominant negative version of the KCNH2 (hERG) potassium channel (G628S) was then locally infused into the mid-LAD with a catheter (the same site as for infarction balloon occlusion). KCNH2 potassium currents are involved in repolarization. Numerous drugs that block KCNH2 prolong the QT interval and are proarrhythmic. In all pigs treated with G628S, VT was no longer inducible. Two other groups receiving saline or adenovirus expressing the lacZ-reporter gene continued to have inducible VT. Sinus intracardiac electrograms in all pigs showed low amplitude fractionated electrical activation within the gene transfer zone. Surface 12-lead ECG showed no difference among the three groups of pigs, including with respect to the QT interval. However, monophasic action potential duration and the effective refractory period were increased only in the anterior septum (gene transfer zone), but not in other areas of the heart of the G628S pigs. Patch clamping of isolated myocytes from the anterior septum of the G628S animals also exhibited prolonged action potential durations. Furthermore, gene transfer did not appear to be proarrhythmic, as spontaneous ventricular arrhythmias were not observed in any of the pigs over the 4 weeks of study. To compare against current antiarrhythmics, 3 pigs with infarcts were treated with dofetilide, a known KCNH2-blocking drug. Unlike the G628S pigs, dofetilide increased the QT interval, prolonged the ERP globally, and the pigs still had inducible VT.

In this proof-of-concept study, Sasano et al were the first to demonstrate effective arrhythmia suppression with a gene transfer approach in a clinical relevant model. Sasano et al elegantly showed how local gene transfer via the coronary arteries is safe, effective, and can be a tailored therapeutic approach. Significant work lies ahead before clinical application is a reality. The adenovirus vector used in this study has short-lived expression of the order of 1 to 3 weeks. Long-term expression is required for clinical use. Stem cell–based gene delivery approaches are also promising.

Molecular ablation of ventricular tachycardia after myocardial infarction

T. Sasano, A. D. McDonald, K. Kikuchi, J. K. Donahue

Nat Med. 2006;11:1256-1258

The car manufacturing company Rolls-Royce Ltd is founded by Henry Royce and Charles Stewart Rolls; the first Victrola record player, a ponderous machine enclosed in a wooden cabinet, is manufactured in the USA; and SOS (later associated with the phrase Save Our Souls) becomes the first internationally recognized distress signal.
Theoretical impact of the injection of material into the myocardium: a finite element model simulation

S. T. Wall, J. C. Walker, K. E. Healy, M. B. Ratcliffe, J. M. Guccione

Circulation. 2006;114:2627-2635

Stem cell transplantation by direct injection into the myocardial infarction area has gained significant attention as a strategy to improve cardiac function and prevent clinical heart failure. Numerous preclinical experiments and several small clinical trials using stem cell-based therapies have shown small, but significant, improvement in cardiac function after treatment. However, convincing evidence that stem cell-derived cardiomyocytes working in concert with the native myocardium as the underlying reason for functional improvement has been absent. Cellular elements derived from implanted stem cells are found within the infarct area, but their numbers are quite small in comparison with the magnitude of cardiac functional improvement. Wall and colleagues were the first to question whether the improvement in cardiac function after stem cell injection into the infarct heart was due to the passive mechanical consequences of the injection rather than to the stem cells.

The experimental protocols used to inject stem cells into the heart may have significant mechanical consequences. The authors point out that recent rat experiments used 50 μL of fibrin gel with stem cells to inject into the left ventricle. The average heart mass of an adult rat is about 1 g, so a 50-μL injection amounts to 5% of total heart mass. Experiments in mice are even more exaggerated, as a 50-μL injection into the heart would correspond to 50% of heart mass (average adult mice heart mass is 100 mg).

In humans, in the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) trial, a 26-μL injection of stem cells was introduced into the infarct area. With the average human left ventricular wall volume being about 300 mL, this is over 8% of LV wall volume. Wall et al used a 216-element mesh computational model of a sheep left ventricle with an anterior apical infarct to calculate the effect of materials of varying stiffness and volume on wall stress and cardiac function. They looked at the effects of a single infarct border zone injection, multiple border zone injections, and injections into the infarct. They conclude that injections in the border zone decrease end-systolic fiber stress in proportion to the volume injected.

Injections in the infarct zone improved ejection fraction as well as the stroke volume/end-diastolic volume (SV/EDV) relationship, but did not affect the SV/EDP (end-diastolic pressure) relationship.

An obvious study limitation is the reliance of the results on the accuracy of the computational model in reflecting an infarcted left ventricle. Furthermore, the simulations only model the immediate consequence of an injection volume, the long-term consequences are not predicted by this model. Nonetheless, Wall et al were among the first to rigorously examine the passive properties of cardiac injections on function.

An intracardiac injection of a cellular therapeutic must be suspended in a solution or a biomaterial. The biomedical community has placed enormous emphasis on the cellular elements, but largely ignored the medium carrying the cells. Cell therapy efficacy must separately evaluate active cellular contributions and passive mechanical contributions. It is becoming quite clear that the future of cardiac repair will require a harmonious marriage of cellular technology with advanced biomaterials.

Mount Vesuvius erupts, devastating the city of Naples; Mahatma Gandhi adopts nonviolence as a form of political resistance in South Africa; and Norwegian playwright Henrik Ibsen dies, aged 78
Mending the Broken Heart

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selected by Ira S. Cohen*†, MD, PhD and Glenn R. Gaudette‡, PhD
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