

Mending the Broken Heart

Summaries of Ten Seminal Papers

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Evidence that human cardiac myocytes divide after myocardial infarction

A. P. Beltrami and others. *N Engl J Med.* 2001

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Human mesenchymal stem cells as a gene delivery system to create cardiac pacemakers

I. Potapova and others. *Cir Res.* 2004

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Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes

I. Kehat and others. *J Clin Invest.* 2001

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Regenerating the heart

M. A. Laflamme and C. E. Murry.
Nat Biotechnol. 2005

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Biological pacemaker created by gene transfer

J. Miale and others. *Nature.* 2002

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Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction

D. L. Kraitchman and others. *Circulation.* 2005

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Heart regeneration in zebrafish

K. D. Poss and others. *Science.* 2002

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Molecular ablation of ventricular tachycardia after myocardial infarction

T. Sasano and others. *Nat Med.* 2006

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Adult cardiac stem cells are multipotent and support myocardial regeneration

A. P. Beltrami and others. *Cell.* 2003

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Theoretical impact of the injection of material into the myocardium: a finite element model simulation

S. T. Wall and others. *Circulation.* 2006

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Evidence that human cardiac myocytes divide after myocardial infarction

A. P. Beltrami, K. Urbanek, J. Kajstura, S. M. Yan, N. Finato, R. Bussani, B. Nadal-Ginard, F. Silvestri, A. Leri, C. A. Beltrami, P. Anversa

N Engl J Med. 2001;344:1750-1757

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.

2001/1901

The US stock market crashes for the first time;
Wilhelm Conrad Röntgen, the discoverer of x-rays,
is awarded the Nobel Prize in Physics;
and Queen Victoria, Queen of England and
Empress of India, dies



Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes

I. Kehat, D. Kenyagin-Karsenti, M. Snir, H. Segev, M. Amit, A. Gepstein, E. Livne, O. Binah, J. Itskovitz-Eldor, L. Gepstein

J Clin Invest. 2001;108:407-414

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.

2001/1901

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

Biological pacemaker created by gene transfer

J. Miake, E. Marban, H. B. Nuss

Nature. 2002;419:132-133

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.

2002/1902

Mount Pelée (Montagne Pelée) volcano erupts in Martinique, spewing out a pyroclastic cloud that claimed more than 30 000 dead;

St Marks' Campanile collapses in Venice on 14th July—reconstruction was decided the same day by the Communal Council; and Indian mystic Swami Vivekananda dies, aged 39



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.

2002/1902

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

Adult cardiac stem cells are multipotent and support myocardial regeneration

A. P. Beltrami, L. Barlucchi, D. Torella, M. Baker, F. Limana, S. Chimenti, H. Kasahara, M. Rota, E. Musso, K. Urbanek, et al

Cell. 2003;114:763-776

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 clinically 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.

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

I. Potapova, A. Plotnikov, Z. Lu, P. Danilo Jr, V. Valiunas, J. Qu, S. Doronin, J. Zuckerman, I. N. Shlapakova, J. Gao, et al

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 allogeneic 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 β -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.

2004/1904

Theodore Roosevelt is reelected President of the USA; Ivan Petrovich Pavlov is awarded the Nobel Prize in Physiology or Medicine; and Birth of Umm Kulthum (Oum Kalsoum), who was to be unanimously celebrated as a singer in the Arab world

Regenerating the heart

M. A. Laflamme, C. E. Murry

Nat Biotechnol. 2005;23:845-856

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 allogeneic 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 I 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.

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



Dynamic imaging of allogeneic mesenchymal stem cells trafficking to myocardial infarction

D. L. Kraitchman, M. Tatsumi, W. D. Gilson, T. Ishimori, D. Kedziorek, P. Walczak, W. P. Segars, H. H. Chen, D. Fritzsche I. Izbudak, et al

Circulation. 2005;112:1451-1461

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 infarct 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.

2005/1905

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

Molecular ablation of ventricular tachycardia after myocardial infarction

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

Nat Med. 2006;11:1256-1258

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 clinically 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.

2006/1906

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 μ L 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- μ L injection amounts to 5% of total heart mass. Experiments in mice are even more exaggerated, as a 50- μ L 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-mL 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.

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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