Failing heart; remodel, replace or repair?

Terminal dönem kalp yetersizligi: Kalp nakli mi? Yeniden biçimlendirme mi? Yoksa onarım mı?

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ABSTRACT

In the era of proton-antiproton collisions, stem cell field has emerged as the newly recognized protons of regenerative medicine. Great interest and enthusiasm were depending on their behavioral difference such as self-renewal, clonogenicity and differentiation into functional progeny that may become vehicles for regenerative medicine. Although progress has evolved dramatically strategies using stem-cell-driven cardiac regeneration involve extremely complex and dynamic molecular mechanisms. Cell death in transplanted heart continues to be a significant issue. Every step from stem cell homing, and migration to retention, engraftment, survival and differentiation in cardiac cytotherapy deserves intense research for optimum results. Furthermore, regeneration of contractile tissue remains controversial for human studies and careful interpretation is warranted for modest benefit in clinical trials. Currently, the only realistic approach to replace the damaged cardiomyocytes is cardiac transplantation for patients with end-stage heart failure. Ultimately, the giant footsteps in cell-based cardiac repair can only be achieved by an enthusiastic but also skeptical teams adhering to good manufacturing practices. Better understanding of cell-fate decisions and functional properties in cardiac cytotherapy may change the erosion of initial enthusiasm and may open new prospects for cardiovascular medicine.
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Key words: Stem cell, cardiac regeneration, cell-based therapy, angiogenesis, bone marrow cells, plasticity

ÖZET

Proton-antiproton çarpışmalarının gündemde olduğu günümüzde kök hücreler onarımsal tıbbın yeni protonları olarak karşımıza çıkmaktadır. Konuyla ilgili büyük ilgi ve heyecan bu hücrelerin onarımsal tıbbın silahları olarak kullanılması olası kendini yenileme ve farklı hücrelere farklılaşabilme gibi davranışsal özellikleri nedeniyle gerçekleşmiştir. Konuyla ilgili çok önemli gelişmeler kaydedilirken kök hücre-aracılı kardiyak rejenerasyonun karmaşık ve dinamik moleküler mekanizmalar aracılığı ile olduğu da unutulmamalıdır. Örneğin transplante edilen hücrelerin hedef organdaki ölümü çözüm bekleyen önemli bir konudur. Amaçlanan sonuçları elde edebilmek için kök hücre migrasyonu ve hedef organa ulaşmasından engraftman, viabilitenin sürdürülmesi, differansiasyona kadar hücresel tedavideki her aşama için ciddi araştırmalara gereksinim sürmektedir. Ek olarak, insan çalışmalarında kasılan miyokard dokusunun rejenerasyonu tartışmalıdır ve olumlu klinik sonuçların yorumunda daha dikkatli yorumlara gereksinim vardır. Terminal dönem kalp yetmezliği bulunan hastalarda hasarlı kardiyomiyositlerin replasmanı açısından bakıldığında halen en gerçekçi çözüm kalp transplantasyonudur. Önümüzdeki süreçte, hücre aracılı kardiyak onarımda önemli bilimsel adımlar ancak yoğun ilgi ve bilginin yanı sıra şüpheci bilim insanlarını barındıran, iyi imalat uygulamalarını sürdüren takımlar tarafınca sağlanacağı gözlenmektedir. Transplante edilen organda hücre kaderini belirleyen mekanizmaların ve fonksiyonel özelliklerin daha iyi anlaşılması ile hücresel tedavi ile ilgili olarak erozyona uğramakta olan erken coşkunun tekrar kazanılması sağlanabilir ve kardiyovasküler tıpta uygulanan tedavi yöntemlerinde yeni kapılar açılabilir.
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Introduction

Rapidly proliferating human cardiomyocytes during fetal life exit the cell cycle soon after birth. Approximately 6-7 billion human cardiomyocytes contracting synchronously at birth would only decrease with aging process as a result of limited regenerative capacity of human myocardium. During aging, average loss of cardiomyocytes reach to 38 million per year in the left ventricle and 14 million per year in the right ventricle despite the lack of any injury due to cardiovascular disease (1).

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Unlike the blood, skin, and the gut epithelia, male hearts lose 1 g per year of myocardium during aging while women maintain relatively constant cardiomyocyte number. This loss is compensated by increase in cell size, called cellular hypertrophic response. However, this scenario is not valid for certain species. For example, adult zebra fish is able to regenerate myocardium without scarring even after 20% ventricular resection (2). In contrast, unfortunately, the rate of human cardiomyocyte death approaches 10% to 40% of the total cardiomyocyte population after an acute myocardial infarction (AMI) through necrosis and apoptosis. Only less than 0.01% of adult human cardiomyocytes are able to divide following AMI (3). Expectedly, the consequences of AMI are not benign. Insufficient cardiomyocyte cell number, subsequent adverse remodeling associated with fibrosis, cavitary dilation with wall thinning, increased wall stress, and scar expansion in the remote myocardium results in heart failure (HF) with an incidence of 25%. The prevalence of HF ranges from approximately 2% to 3% at age 65 to more than 80% in persons over 80 years of age (4). The incidence approaches 10 per 1000 population after age 65 (4). One-year life expectancy of patients with HF and New York Heart Association (NYHA) Class IV symptoms is approximately 50%. However, there have been many recent advances in the management of HF. As the prevalence of HF continues to increase as a result of aging population, pharmacological (β-adrenoreceptor blockers, angiotensin-converting enzyme inhibitors, angiotensin receptor blockers, aldosterone receptor blockers, vasodilators) and non-pharmacological (coronary revascularization, left ventricular reconstruction, mitral valve repair, cardiac resynchronization therapy with biventricular pacing, automatic implanted cardioverter-defibrillators, ventricular assist devices and cardiac transplantation) therapies continue to maintain better but still unsatisfactory outcome. However, apart from heart transplantation none of the approaches has the ability to replace damaged cardiomyocytes. Indeed, heart transplantation remains to be the gold standard long-term treatment for patients with refractory HF symptoms but is limited primarily by donor availability, immunologic rejection, infections and long-term failure of grafted heart.

Multiple animal studies and clinical trials on cell-based cardiac repair have been performed over the last decade (5). The consensus of the task force of the European Society of Cardiology suggested that the target diseases for myocardial repair should be AMI, chronic myocardial ischemia, and cardiomyopathy (6). Table 1 summarizes the other possible target cardiovascular diseases for cellular therapy. The features of ideal cell source for cardiovascular repair are listed in Table 2. As Table 3 shows, several types of stem and progenitor cell populations have been evaluated for cardiac repair. To date skeletal myoblasts, bone marrow-derived cells, endothelial progenitor cells (EPCs) and stem cell mobilization have undergone testing in phase 1 and 2 clinical trials. Our group has also investigated the efficacy and safety of autologous bone marrow-mononuclear cells in patients with critical limb ischemia (7), ischemic cardiomyopathy (8), and ungraftable coronary artery territories (unpublished data). However, the ideal cell source for cardiac cytotherapy remains to be defined. Between 2004 and 2007, the National Institutes of Health spent $8.41 billion on heart disease, and $2.46 billion on stem-cell research. Furthermore, estimated funding will be $4.23 billion, and $1.31 billion respectively for 2008 and 2009 (http://www.nih.gov/news/fundingresearchareas.htm). This review summarizes the milestones in cell-based cardiac repair and outlines the mechanisms based on experimental and clinical work.

Table 1. Targets for cellular therapy for cardiovascular diseases

Acute myocardial infarction*
Chronic myocardial ischemia*
Ischemic cardiomyopathy*
Dilated cardiomyopathy
Acute myocarditis
Biological cardiac pacemakers (supplementing the sinoatrial or atrioventricular nodes)
Heart valves
Critical limb ischemia
Tissue engineered vascular grafts

* Suggested targets by the consensus of the task force of the European Society of Cardiology (Data from reference 6)

Table 2. Characteristics of the ideal cell source for cardiovascular repair

Non-immunogenic, preferably autologous
Able to achieve adequate cell retention in vivo
Able to engraft in the target tissue; maintain survival (viability) and function in vivo
Able to differentiate into functional cardiomyocytes
Able to increase myocardial vascular network (endothelial cells and smooth muscle cells)
Stimulation of endogenous repair process
Reverse the process of adverse remodeling
Easily obtainable with reproducible protocols
Maintain donor/host electromechanical coupling and synchronous contractility

Historical landmarks

Rudolf Ludwig Karl Virchow, (1821-1902) the founder of cellular pathology, pioneered the modern concept of cell theory *("Omnis cellula e cellula")* and the stemness of each cell from another cell. His student, Julius Friedrich Cohnheim (1839-1884) studied the cells appearing in the wounds and concluded that they originate from the bloodstream, and, by implication, from bone marrow. After the first description of quantitative in vivo assay for hematopoietic stem cells (HSCs) by Till and McCulloch (9) initial attempts of injecting bone marrow cells into irradiated mice by Becker and co-workers (10) resulted in macroscopic spleen colonies in 1963. The investigators suggested that each colony arose from a single

Table 3. Different sources of cell-based therapies

Source	Abbreviation	Origin	Advantages	Disadvantages
Embryonic stem cells	ESCs	Inner cell masses of blastocysts from mammalian embryos	Pluripotent Able to differentiate into all cell types (fulfill the criteria of stemness) Highly expandable Unlimited supply Cardiomyogenic Electromechanical coupling Contractile and angiogenic capacity Long-term regeneration	Ethical and moral concerns Legal issues Tumorigenesis potential (teratoma or teratocarcinoma) Immunologic rejection Immune-suppressive therapy required Contamination with viruses or prions Arrhythmogenic potential No human clinical studies to date
Amniotic fluid-derived stem cells	AFSCs	Amniotic fluid and mesodermal lineages	Multipotent Ability to differentiate into ectodermal, Intermediate stage between ESCs and adult stem cells Doubling every 36 h	Tumorigenesis potential (teratoma or teratocarcinoma)
The umbilical cord/ placenta-derived stem/ progenitor cells	PSCs	Umbilical cord blood, Umbilical cord matrix (Wharton's jelly) Placenta	Pluripotent (cord blood) Multipotent (cord matrix) Low immunogenicity Availability and ease of procurement Rich source of HSCs Cryopreservation for future autotransplantation Absence of maternal/fetal risk Lower risk of viral contamination of the graft Teratoma formation unlikely	Less ethical concerns Delayed engraftment
Fetal/neonatal stem cells	FSCs	Fetal blood and bone marrow Fetal tissues	Multipotent Unlimited self-renewal capacity High differentiation potential	Ethical concerns Limited homing efficiency Slow and transient engraftment
Adult germline stem cells	MAGSCs	Testis	Multipotent ESC properties	Unable to differentiate into cardiomyocytes
Skeletal myoblasts (satellite cells)	SM	Adult skeletal muscle	Autologous No need for immunosuppression Contractile capacity even in fibrous scar Resistance to ischemia Less teratogenic Phase II studies ongoing	Unable to extravasate and migrate to ischemic areas Arrhythmogenic potential (failure to integrate electrically with surviving cardiomyocytes)
Bone marrow mononuclear cells	BMMNCs	Bone marrow	Autologous Easily accessible and obtainable Used in clinical trials in both AMI and HF settings No immune rejection No immune-suppressive therapy required No need for expansion (fresh methodology) Less ethical concerns	Modest benefit in clinical trials Non-homogenous Inflammation potential

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Peripheral blood mononuclear cells	PBMNCs	Blood	Autologous Used in clinical trials Reendothelialization Neovascularization Angiogenesis Paracrine signals Angiogenic Growth factors including VEGF-A, VEGF-B, (SDF)-1, and insulin-like growth factor-1	Modest benefit in clinical trials Inflammation potential
Hematopoietic stem cells	HSCs	Bone marrow, peripheral blood	Autologous Lymphoid and myeloid cell lineage High proliferation potential Long-term repopulation potential Less than 0.1% of HSCs are pluripotent Neovascularization Reduction of apoptosis Tested in clinical trials	Transdifferentiation into cardiomyocytes is controversial Cell fusion with host cardiomyocytes
Mesenchymal stromal cells	MSCs	Bone marrow, peripheral blood, adipose tissue, umbilical cord, placenta, connective tissues of the dermis, skeletal muscle gut, lung, liver, dental pulp, periodontal ligament	Autologous or allogeneic Multipotent Osteogenic, chondrogenic, and adipogenic Also differentiation potential into hepatocyte-like cells, neuronal, neuroglial cells, and cardiomyocyte lineages High proliferative capacity Low immunogenicity Both vascular and myocardial repair potential	Requires expansion Microcirculatory infarcts after intracoronary infusion Tumorigenesis potential (teratoma) Ossifications, calcification New cardiac sympathetic nerves leading to arrhythmogenicity Human clinical studies ongoing
Induced pluripotent stem cells	iPSCs	Reprogrammed human dermal fibroblasts	Autologous Pluripotent Less ethical concern	Tumorigenesis potential (teratoma) Virus-mediated transfection
Endothelial progenitor cells	EPCs	Bone marrow, vascular parenchyma, organ specific	Autologous source of endothelial cells Neovascularization Restoration of endothelial function Reduction of apoptosis Therapeutic angiogenesis Tested in clinical trials	
Circulating progenitor cells	CPCs	Peripheral blood	Autologous Neovascularization Tested in clinical trials	Requires stem cell mobilization
Cardiac stem cells	CSCs	Heart	Autologous Potential cells for cardiac self-repair Contractile capacity Ex-vivo expansion potential	No human clinical studies to date CSC function and frequency in HF patients remains to be defined

marrow cell. The stem-cell niche concept, a specialized microenvironment providing support and stimuli necessary to sustain self-renewal and programming was introduced in 1978 by Schofield (11). Further experimental examination of the gonads of *Drosophila melanogaster*, and *Caenorhabditis elegans* helped to improve our understanding the role of stem-cell niches in regulating stem cell behavior, tissue maintenance, and survival. Anatomically stem cell niche usually consists of the stem cell itself, stromal support cells, extracellular matrix proteins, and adjacent tissue vasculature providing control and balance function for self-renewal and differentiation.

Highly proliferative and pluripotent embryonic stem cells (ESCs) derived from the inner cell mass of blastocysts have been promising cell source but associated with ethical concerns and legal issues as well as potential side effects such as immune rejection and teratoma formation. Mouse embryonic stem cells were first isolated in 1981 (12, 13). The first derivation of human embryonic stem cell lines from the inner cell mass of a human primordial embryo was first achieved by Thomson et al. (14) from University of Wisconsin, in 1998. The first report of cardiomyocyte differentiation derived from human embryonic stem cells (ESC) appeared in 2001 by Kehat et al. (15) from the Bruce Rappaport Faculty of Medicine, Israel. Both mouse and human ESCs are also capable of differentiating into endothelial cells, vascular smooth muscle cells and fibroblasts. Concerns about destroying ex utero embryos may be prevented by derivation methods from single blastomeres.

In adults, bone marrow reservoir is the best established source of stem cells. Bone marrow microenvironment involves both differentiated and undifferentiated cells. Friedenstein et al. (16) at the University of Oxford, UK were the first to describe mesenchymal stromal cells (MSCs) derived from bone marrow. They demonstrated the feasibility of isolating and expanding MSCs ex vivo and their differentiation potential to osteogenic and hematopoietic tissues (16). Cardiomyocyte differentiation of MSCs by using a DNA demethylation agent, 5-azacytidine, was described by Makino et al. (17) from Japan in 1999. In contrary to nonadherent hematopoietic cells, MSCs are adherent to plastic culture and form colonies (colony-forming unit fibroblasts) under appropriate tissue culture conditions. More recently, the Mesenchymal and Tissue Stem Cell Committee of the International Society for Cellular Therapy (ISCT) proposed a set of minimal standards to define human MSCs (18): (1) adherence to plastic in standard culture conditions, (2) specific surface antigen (Ag) expression (≥95% of the MSC population must express CD105, CD73 and CD90, but lack hematopoietic markers such as CD34, CD45, CD14 or CD11b, CD79α or CD19 and HLA class II), (3) in vitro differentiation ability to osteoblasts, adipocytes, and chondroblasts. Currently, immunoselection methods have emerged aiming to isolate purified mesenchymal precursor cell population by using specific monoclonal antibodies. However, ideal conditions for MSC induction for cardiomyocyte differentiation still have not been determined.

In 1992, Marelli et al. (19) from McGill University, Montreal hypothesized that skeletal muscle satellite cells multiplied in vitro could be used for myocardial repair. In 1995, Chiu et al. (20) showed the differentiation of satellite cells into cardiac-like muscle cells in canine experimental model. In 1998, Taylor et al. (21) from Duke University, North Carolina, demonstrated improvement after autologous skeletal myoblast (SM) transplantation into cryoinfarcted myocardium in rabbit model.

The recognition of EPCs by Asahara et al. (22) from Tufts University, Boston in 1997 opened the door to a new era in vascular medicine. They isolated cells from human peripheral blood by magnetic bead selection and showed that EPCs differentiated into endothelial cells, incorporated into sites of active angiogenesis as a key factor for re-endothelialization. After this report, endothelial differentiation and tissue vascularization were no longer believed to take place exclusively in the embryonic development stage but in adults as well. Currently emerging evidence suggests that EPCs derive from the bone marrow and are recognized by their cell surface expression of the hematopoietic marker proteins CD133 and CD34 and the endothelial marker vascular endothelial growth factor receptor-2 (VEGFR2). Between 0.1 and 0.5% of $CD34^+$ cells from human bone marrow, express VEGFR2. However, there are additional bone marrow-derived cell populations (e.g., myeloid cells, "side population" cells, and mesenchymal cells) and non-bone marrow-derived cells, which can also give rise to EPCs (23).

Mechanisms in stem cell-based cardiac regeneration

In 2000, Li et al. (24) transplanted autologous porcine heart cells isolated and cultured from the interventricular septum at the time of AMI and showed improvement in cardiac function. However, engraftment and survival of differentiated adult cardiomyocytes within ischemic tissue was scarce, and high levels of cardiomyocyte death occur after transplantation (25). Perhaps one of the most exciting steps for scientific community in the development of cell-based cardiac therapies was the demonstration of cardiomyocyte transdifferentiation. Transdifferentiation can be defined as the unexpected transformation of one differentiated cell type into another. In April 2001, Orlic et al. (26) from New York Medical College first suggested cardiomyocyte transdifferentiation potential of BM cells when injected into infarcted mouse myocardium. Injection of lineage-negative, c-kit-positive male bone marrow cells in the peri-infarcted left ventricle of female transgenic mice resulted in myocardial regeneration by differentiation into the three cardiac cell types; mainly cardiomyocytes, smooth muscle cells and endothelial vascular cells. Accordingly, Kocher et al. (27) from Columbia University, New York, reported that injection of G-CSF-mobilized human $CD34^+$ cells into rats with AMI induced neoangiogenesis involving endothelium of both human and rat origin at 2^{nd} week post-LAD ligation. They also showed that the neoangiogenesis resulted in decreased apoptosis of hypertrophied myocytes in the peri-infarct region, reduction in collagen deposition and sustained improvement in cardiac function. Furthermore, in August 2001, Orlic et al. (28) reported that BM cell mobilization by G-CSF and stem cell factor resulted in a significant degree of tissue regeneration in the presence of an AMI in splenectomized mice model. Kamihata et al. (29) suggested that the potential mechanisms for improvement in regional blood flow and cardiac function were paracrine signals by angiogenic ligands (bFGF, VEGF, Ang-1) and cytokines (IL-1β and TNF-α) after injection of mixed populations of bone marrow cells in a swine model of AMI.

Another milestone in the cardiovascular biology was the detection of large numbers of nuclear mitotic divisions within the adult hearts (3, 30, 31) and subsequently identification of

replicating myocytes (32) and isolation of cardiac stem cells (CSCs) or resident myocardial progenitors (33). In 2003, Beltrami et al. (33) from New York Medical College first reported the existence of Lin^{-}, $c\text{-}kit^{POS}$ cells within adult myocardium of the rat. These multipotent cells were found in small clusters in the interstitia between well-differentiated myocytes with a higher density in the atria and the ventricular apex and can differentiate into endothelial cells, smooth muscle cells and functional cardiomyocytes. The possibility that these dividing cells are myocytes derived from an extra-cardiac source is suggested by investigations in sex-mismatched heart transplant patients (chimerism model) (34-37). Reinitiation of the cardiomyocyte cell division (cell cycle reprogramming) through cell cycle regulators is a new concept to stimulate intrinsic cardiomyocyte regeneration (38).

However, in 2004, skepticism about cardiomyocyte transdifferentiation from hematopoietic stem cells was in the agenda. In contrast to Orlic's findings, Murry et al. (39) from University of Washington, Seattle were unable to detect any transdifferentiation event (beta-galactosidase positive nucleus) into cardiomyocytes from hematopoietic stem cells although isolation and injection protocols from transgenic mice were similar with Orlic et al. Moreover, Nygren et al. (40) from Lund University, Sweden also reported transient engraftment of unfractionated bone marrow cells and HSCs within the infarcted myocardium but the main mechanism was cell fusion with host cardiomyocytes. Balsam et al. (41) from Stanford University reported that lineage-negative, c-kit-positive cells did not differentiate into cardiomyocytes but adopt only traditional hematopoietic fates. The investigators concluded that current clinical trials are premature and additional preclinical experimental data should be collected before moving to the clinical arena.

Efficient delivery methods for viable cell transplantation have paramount importance when significant rate of cell death early after transplantation is considered. Based on experimental work and clinical experience advantages and pitfalls of different delivery methods of stem/progenitor cells are listed in Table 4. Pro-survival strategies such as genetic modification of stem cells, anti-apoptotic proteins, extracellular matrix proteins, anti-inflammatory therapy, and erythropoietin for enhancement of cell survival in host myocardium are under intense research. Potential mechanisms to explain the beneficial effects of clinical cell transplantation are summarized in Table 5.

Clinical applications in stem-cell based cardiac regeneration

In parallel to discoveries in basic science and the promising results of experimental studies, clinical trials were initiated globally. The first cardiac operation combining coronary artery bypass surgery with cellular therapy for ischemic cardiomyopathy was reported by Menasche et al. in 2001 (42). They isolated SM from muscle biopsies and implanted into the infarct region in a 72-year-old man with left ventricular ejection fraction (LVEF) of 20% who had a transmural infarction. Five months later, there was evidence of contraction and viability in the grafted scar on echocardiography and positron emission tomography. The initial case report and the pilot study suggested that cell transplantation might have a potential to reverse extensive myocardial damage in the clinical setting but life-threatening arrhythmias in 4 of 10 patients resulted in major concerns with SMs. Further safety and feasibility pilot studies expanded rapidly. In 2001, Strauer et al. (43) reported the first successful autologous bone marrow mononuclear cell (ABMMNC) selective intracoronary transplantation 6 days after an anterior transmural infarction in a 46 year old man. The concept of intramyocardial implantation of ABMMNCs for ICMP guided by electromechanical mapping with a percutaneous catheter was conceived in 2003 (44-46). Subsequently, a number of research groups have reported the results of randomized clinical trials. The first randomized trial called BOOST trial (Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration) (47) was performed by Helmut Drexler's group in Hannover, Germany. Sixty patients were randomly assigned to either a control group (n=30) that received optimum post-infarction medical treatment, or a bone marrow cell group (n=30) that received optimum medical treatment, and intracoronary transfer of autologous bone marrow cells 4.8 days after percutaneous coronary intervention (PCI). The study demonstrated that cell-therapy enhanced LVEF (0.7% vs 6.7% improvement, p=0.0026), primarily in myocardial segments adjacent to the infarcted area. However, these effects were no longer significant at 18 months follow-up. Another multicenter trial, Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) (48) showed that intracoronary infusion of bone marrow cells after PCI resulted in improved left ventricular function at 4 months (5.5% vs. 3.0%, p=0.01) and reduction in combined clinical end point of death, recurrence of AMI, and any revascularization procedure at 1 year compared to placebo. The benefit was greatest in patients with poor left ventricular function. However, other groups, from Belgium and Norway, were unable to detect a difference in outcome between bone marrow cell group and controls in AMI setting (49, 50). Different cell isolation protocols, and cell viability and function prior to delivery may have contributed to heterogeneous clinical results of randomized trials. After recanalization of chronic coronary total occlusion intracoronary transplantation of circulating progenitor cells (CPCs), mobilized by granulocyte colony-stimulating factor (G-CSF) resulted in 7.2% improvement in LVEF and enhanced myocardial perfusion in a randomized, placebo-controlled, and double blinded study (51).

Intravenous infusion of stem cells or cytokine induced mobilization of BM-derived stem cells using G-CSF or granulocyte macrophage colony stimulating factor (GM-CSF) are systemic delivery methods. Intravenous infusion route of administration is an attractive, easy and noninvasive strategy for cell-therapy. The main disadvantage however, is that the homing of the injected cells within the target organ can be

Table 4. Route of delivery methods for cardiac cellular therapy

Route of delivery	Abbreviation	Advantages	Limitations
Systemic			
Intravenous infusion	IV	Easy Non-invasive	Limited myocardial homing Cell retention in lungs, liver, spleen and kidneys
Stem cell mobilization		Non-invasive	Mobilization of inflammatory
Granulocyte colony-stimulating factor	G-CSF	Safe	cells and mediators
Granulocyte macrophage colony stimulating factor	GM-CSF	BM cell mobilization Proliferation and differentiation	Increased restenosis rate
Stem cell factor	SCF	induction	has been prevented by
Flt3/flk2 ligand	FL	Better hematopoietic cell survival	drug eluting stents
Erythropoietin	EP	8 RCTs reported on G-CSF after AMI Better results in AMI patients with LV dysfunction	Modest benefit after MI in unselected patients (LVEF by 1.09%)
Local			
Cardiac percutaneous catheter based Selective intracoronary	IC	Non-invasive Most commonly used route in clinical trials First-pass delivery Better results in recently infarcted and reperfused myocardium (4-7 days after AMI)	Requires PCI for occluded coronary artery Limited cell homing Microcirculatory infarct potential
Endomyocardial	EM	Less invasive Determination of host myocardial viability before each injection Targeted delivery	Electromechanical mapping technique is required Duration of the procedure Perforation risk in patients with LV wall thinning
Transcoronary sinus retrograde	CSR	Non-invasive	Fluoroscopic guidance is required
Transcoronary vein intramyocardial	CVI	Less invasive	Appropriate positioning of the guiding catheter requires expertise
Intrapericardial	IP	Less invasive	Efficiency controversial
Surgical (via sternotomy or minimally invasive thoracoscopic procedures)			
Direct transepicardial intramyocardial	IM	Direct visualization Targeted delivery Eliminates transvascular cell migration Higher potential for myocardial cell retention Better results in chronic myocardial ischemia Used with off-pump, on-pump beating or arrested heart methods	Invasive (requires sternotomy, mini-thoracotomy, or video -assisted thoracoscopic surgery) May not be safe in AMI setting
Aortic root with distal aortic cross-clamp	AR-XC	Similar to selective intracoronary delivery	invasive

AMI-acute myocardial infarction, BM-bone marrow, LV-left ventricular, LVEF-left ventricular ejection fraction, PCI-percutaneous coronary intervention, RCT-randomized controlled trials

limited by the entrapment of cells by other organs being primarily the lungs or the spleen (52, 53). Recent meta-analysis including 8 randomized controlled trials demonstrated that, G-CSF therapy increased LV ejection fraction (EF) by 1.09% (95% CI: 0.21 to 2.38, p=0.10) in the setting of AMI (54). However, G-CSF may be potentially beneficial in patients with lower LVEF (<50%) at baseline and if given earlier (≤37 hours) after AMI/PCI (54). Potential complications cardiac cytotherapy are summarized in Table 6.

An important advancement in stem cell research has been accomplished in 2006 when Takahashi and Yamanaka demonstrated that fully differentiated somatic cells (mouse adult fibroblasts) can be reprogrammed to embryonic-like state which exhibit the morphology and growth properties of ESCs and express ESC marker genes (55). The investigators isolated four key pluripotency genes that were essential for the production of pluripotent stem cells; OCT-3/4, SOX2, c-MYC, and KLF-4 and coined the term "induced pluripotent stem cells" (iPSCs). One year later, another milestone was achieved by creating iPSCs from adult human cells independently by two research groups; Thompson's team at University of Wisconsin-Madison (56) and Yamanaka's team at Kyoto University, Japan (57). Yamanaka et al. (57) had successfully transformed human fibroblasts into pluripotent stem cells using the genes same as mouse with a retroviral system. Thomson et al. (56) used OCT4, SOX2, NANOG, and a different gene LIN28 using a lentiviral system. More recently, pluripotent stem cells from adult mouse liver and stomach cells were generated and cardiac cells were differentiated from mouse iPSCs.

Another promising area of investigation has been in vivo labeling and tracking the fate of transplanted cells (58). Currently available assessment and imaging modalities for cardiac cellular therapy are summarized in Table 7. Ideally, noninvasive in vivo imaging techniques should be safe, biocompatible, and nontoxic to both transplanted cells and the target organ, single-cell resolution, providing real-time visualization of injected cells either in the target area or throughout the body over relatively longer durations. In addition, false positive imaging should be eliminated. However, these targets are particularly difficult when cell division, and fusion, and particularly dynamic cell-to-cell interactions are considered. Thus, currently none of the single imaging approaches fulfill the ideal imaging criteria for continuous

Table 5. Potential mechanisms of cellular therapy for the injured heart

Neovascularization (vasculogenesis, angiogenesis, arteriogenesis)
Cardiac regeneration mediated by differentiation
Cell fusion
The paracrine hypothesis; secretion of growth factors, or cytokine-mediated effect
Amelioration of ventricular remodeling
Prevention of apoptosis in transitional zone and regional infarct expansion and promoting survival of tenuous cardiomyocytes
Modification of matrix remodeling with preservation of the elastic components of the myocardium
Transfer of mitochondria or mitochondrial DNA to cells with nonfunctioning mitochondria
Stimulation of endogenous stem cell niches (tissue-resident stem/progenitor cells)
Promoting re-entry of myocytes into the cell cycle

Table 6. Potential complications of cardiac cytotherapy

Myocardial infarction as a result of cell embolization
Intramyocardial calcification
Pulmonary emboli
Inappropriate electrophysiological coupling leading to arrhythmias
Immunologic rejection
Transmission of infection
Unregulated differentiation and tumorigenesis
Formation of cell aggregates leading to nodules of different tissues (ossifications, calcifications or callus formation)
Accelerated arteriosclerosis
Retinopathy
Increased cardiac scarring
Deterioration of cardiac function

Table 7. Assessment, labeling and imaging of cardiac cytotherapy

Labeling
Fluorescent proteins (green fluorescent protein-GFP)
Peptide tags
Organic fluorophores
Quantum dots (nanocrystals)
Assessment and Imaging
Electrophysiologic evaluation
Electrocardiography (ECG)
Holter monitoring
Electrophysiological mapping
Cardiac pressure measurements and pressure -volume loops
Echocardiography
Coronary angiography
Treadmill testing
Single-photon emission computed tomography (SPECT)
Positron emission tomography (PET)
Wide-field fluorescence microscopy
Confocal fluorescence microscopy
Multiphoton fluorescence microscopy
Fluorescence molecular tomography
Mesoscopic tomography
Bioluminescence imaging
Magnetic resonance imaging (MRI)
Multimodal imaging

assessment of stem-cell-driven cardiac regeneration. Multimodal imaging techniques may overcome most of the handicaps of currently available single imaging approaches.

Conclusion

Currently, close coordination and integrated approach between the basic scientists and the clinicians conducting clinical trials are missing. It is clear, however, that "we, the cardiovascular basic researchers, stem cell biologists, the clinicians, and the surgeons are the cells of the same organism; some differentiated, some undifferentiated, willingly or unwillingly should share the same nutrient source through a network and should act in harmony for survival." (A.R.A.)

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