TRANSLATIONAL PHYSIOLOGY |

Cardiac regeneration therapy: connections to cardiac physiology

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Submitted 1 August 2011; accepted in final form 22 September 2011

Takehara N, Matsubara H. Cardiac regeneration therapy: connections to cardiac physiology. Am J Physiol Heart Circ Physiol 301: H2169–H2180, 2011. First published September 30, 2011; doi:10.1152/ajpheart.00768.2011.—Without heart transplantation, a large number of patients with failing hearts worldwide face poor outcomes. By means of cardiomyocyte regeneration, cardiac regeneration therapy is emerging with great promise as a means for restoring loss of cardiac function. However, the limited success of clinical trials using bone marrow-derived cells and myoblasts with heterogeneous constituents, transplanted at a wide range of cell doses, has led to disagreement on the efficacy of cell therapy. It is therefore essential to reevaluate the evidence for the efficacy of cell-based cardiac regeneration therapy, focusing on targets, materials, and methodologies. Meanwhile, the revolutionary innovation of cardiac regeneration therapy is sorely needed to help the millions of people who suffer heart failure from acquired loss of cardiomyocytes. Cardiac regeneration has been used only in limited species or as a developing process in the rodent heart; now, the possibility of cardiomyocyte turnover in the human heart is being revisited. In the pursuit of this concept, the use of cardiac stem/progenitor stem cells in the cardiac niche must be focused to usher in a second era of cardiac regeneration therapy for the severely injured heart. In addition, tissue engineering and cellular reprogramming will advance the next era of treatment that will enable current cell-based therapy to progress to “real” cardiac regeneration therapy. Although many barriers remain, the prevention of refractory heart failure through cardiac regeneration is now becoming a realistic possibility.

This article is part of a collection on Physiological Basis of Cardiovascular Cell Therapies. Other articles appearing in this collection, as well as a full archive of all collections, can be found online at http://ajpheart.physiology.org/.

Introduction

Heart failure, resulting from various cardiovascular diseases, such as acute myocardial infarction (AMI), ischemic cardiomyopathy, and idiopathic cardiomyopathy (82), remains a critical public health issue worldwide. In the last two decades, although the therapeutic approach to cardiovascular diseases has reached a very high standard by means of coronary catheter intervention [particularly the drug-eluting stent (70)], surgical procedures [off-pump coronary artery bypass grafting (CABG) (75), mitral valve repair, and left ventricular (LV) reconstruction surgery (9)], and the permanent pacemaker with LV resynchronization device (113), several million people continue to suffer from refractory heart failure despite these modern therapies (17, 23, 40, 115).

Since the achievement of generating embryonic stem cells from each blastocyst in mice in 1981 (55) and in humans in 1998 (108), the experimental regeneration of rodent organs after birth has been encouraged in neurons (22), bones (18), the pancreas (33), retinas (111), blood vessels (5), and the heart (74). Although the heart is classically defined as a terminally differentiated organ, in limited cases endogenous cardiomyocyte regeneration has been used in the context of the adult chimeric heart in bone marrow and heart transplantation (25, 47, 81). Based on various theories of cardiac regeneration, translational cell-based therapy was started in the 1990s as an outgrowth of the autologous transplantation of human-derived marrow cells (119) and skeletal myoblast (SM) cells (63). However, consensus on the merits of cell-based therapy has still not been reached because of small heterogeneous clinical trials or the limited success of double-blind, randomized controlled trials. On the other hand, a new era of cardiac regeneration is emerging through the provision of cardiac stem/progenitor cells, the development of tissue engineering technology, and the ability of cellular reprogramming to contribute induced pluripotent stem (iPS) cells.
In this review, we summarize the current status of cell-based therapy using various cell types of the first generations (from 2000 to 2008) and discuss the clinical availability of cell-based therapy and its associated controversies for the treatment of heart failure. In addition, to gain pathophysiological insight, we examine the feasibility of cell-based cardiac regeneration therapy by focusing on the fate of engrafted cells in the host heart in several methods of transplantation, including tissue engineering.

Finally, we explore the new era of “real” cardiac regeneration in the novel field of stem cell research. The reader is referred to a recent review that offers deep insight into these topics (46).

Current Cell-Based Cardiac Regeneration Therapy

Unrestricted bone marrow-derived cells. At 3–5 days after myocardial infarction, the delivery of unrestricted bone marrow-derived cells extracted by Ficoll-Paque is usually accomplished through the transcoronary route after successful stent implantation (27, 98) (Fig. 1). The yielded cell number is on a huge scale (~3–5 × 10^9 cells/body); the emphasis is on the large number of cells rather than on other cell types, such as mesenchymal stem cells (MSCs) and cardiac stem cells (CSCs). The primary desired outcome is the preservation of LV contraction compared with placebo injection in the subacute phase of acute myocardial infarction (AMI) (8, 53, 119). In the Autologous Stem Cell Transplantation in Acute Myocardial Infarction (ASTAMI) (54) and Reinfusion of Enriched Progenitor Cells and Infarct Remodeling in Acute Myocardial Infarction (REPAIR-AMI) (86) trials, which were early and representative of randomized controlled trials, excellent feasibility and safety were achieved by the transcoronary delivery of very small cells, the majority of which were mononuclear cells. However, the preservation of LV function against ischemic damage did not show absolute significant positive changes (84). On the other hand, because patients with severe baseline LV dysfunction in the REPAIR-AMI trial have been significantly preserved in 7.5% of LV ejection fraction (LVEF) compared with the loss of LV function of placebo injection, transcoronary injection of unrestricted bone marrow-derived cells may have one of the application to myocardial ischemia with accompanying severe damage (26). Although a sustained benefit has been shown in the REPAIR-AMI trials over a long-term period (2 yr) (7) but not in the Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration (BOOST) trial in a 5-yr period (66), it is unclear whether the prolonged positive effect results from cell therapy since no significant improvement in LVEF was observed between the first period (4 mo) and latest period (2 yr).

AC133-positive cells, which are abundant in umbilical cord blood, are a more primitive and immature phenotype of unfractionated bone marrow-derived cells. Stamm et al. (98) reported on transepicardial cell therapy using marrow-derived AC133 cells concomitant with surgical revascularization (CABG) into the infarct zone of the hearts of patients suffering recent myocardial infarction without restriction of LV function (Fig. 1). Although there were no serious adverse events and the loss of LV function in cell-injection group was restored in 6.3% of LVEF compared with that in placebo group, mixed results were observed because the mean LVEFs (39%) in all groups were still preserved at baseline and each group contained patients who underwent mitral valve repair and LV...
reconstruction therapy (97). Currently, 142 eligible patients without indication for mitral valve repair, who have between 25 to 40% of LV function as evaluated by cardiac magnetic resonance imaging (MRI), are enrolled in a phase III trial that began in 2009 [Intramyocardial Transplantation of Bone Marrow Stem Cells in Addition to Coronary Artery Bypass Graft (CABG) Surgery (PERFECT) trial] (66a).

**Endothelial progenitor cells.** Endothelial progenitor cells (EPCs) are strict endothelial lineage-determined CD34-positive cells that are mobilized from bone marrow to peripheral circulation (5). Losordo et al. (52) reported on the endomyocardial injection of CD34-positive EPCs for chronic “no option” angina using the NOGA mapping catheter in phase I/IIa trials (Fig. 1). In that study, the clinical symptom of chest pain tended toward improvement as a result of EPC injection into viable myocardium; however, no restoration of LV function has been documented in patients with transplanted EPCs. In a current prospective, double-blind, randomized phase II study [A Double-blind, Prospective, Randomized, Placebo-controlled Study to Determine the Tolerability, Efficacy, Safety, and Dose Range of Intramyocardial Injections of G-CSF Mobilized Autologous CD34+ Cells for Reduction of Angina Episodes in Patients With Refractory Chronic Myocardial Ischemia (ACT34-CMI) trial; ClinicalTrials.gov; Identifier: NCT00300053] (51), 167 patients with refractory angina have received intramyocardial injections of 1 of 2 doses (1 × 10^6 or 5 × 10^6 cells/kg) of mobilized autologous CD34+ cells.

**Skeletal myoblasts.** In June 2000, SMs became the first cell type to be applied to ischemic cardiomyopathy by direct transendocardial injection concomitant with CABG (64). SMs are easily isolated from skeletal muscle and can be rapidly expanded ex vivo for autologous transplantation; however, SMs can never transdifferentiate into cardiomyocytes and are maintained as immature skeletal muscle cells in the host myocardium. Therefore, because SM has original electrical action potential even in the host myocardium, in the first nonrandomized phase I Myoblast Autologous Grafting in Ischemic Cardiomyopathy (MAGIC) trial, a high incidence of ventricular arrhythmia has been documented in SM-injected hearts with accompanying CABG (34) (Fig. 1). When moving forward to the randomized controlled phase II MAGIC trial, Menasche et al. (62) carefully coordinated the use of the implantable cardiac defibrillator to eliminate the risk of lethal arrhythmia for all patients. Although the phase II trial was well designed to evaluate the safety and efficacy outcome for ischemic cardiomyopathy patients with reduced LV function under considerations about patient eligibility (LVEF < 35%), the methodology (CABG excluded mitral valve repair and LV reconstruction surgery), and the model (dose escalation of SMs: low, 4 × 10^6 cells/body; and high, 8 × 10^6 cells/body), no evidence of amelioration of the loss of LV function has been observed in each of the SM injection groups compared with placebo group. However, Myocell, which is a pharmacologic preparation of SMs as “cell medicine” in good manufacturing practice grade, is presently ongoingly administered to patients with heart failure in phase II and phase III trials [A Multicenter Study to Assess the Safety and Cardiovascular Effects of Myocell Implantation by a Catheter Delivery System in Congestive Heart Failure Patients Post Myocardial Infarction(s) (MARVEL) trial] (16a).

**Mesenchymal stem cells.** Although the MSC is a representative somatic stem cell found in various organs, including stromal, adipose, and vascular niches, with a higher potential of proliferation and differentiation into the mesoderm lineage (79), its ability to differentiate into cardiomyocytes is not necessarily sufficient to repair the failing heart (92). As a cardioprotective paracrine mediator [such as IGF, hepatocyte growth factor (HGF), VEGF, and IL-6] (90, 91), the MSC is a better candidate than other cells in providing a less invasive allogeneic cell therapy for cardiac regeneration because of immune privilege (87) (Fig. 1). Clinically, two randomized controlled studies are ongoing to evaluate the potential of cardiac regeneration of transplanted bone marrow-derived MSCs using different strategies: transendocardial injection (110, 118) and transendocardial injection concomitant with CABG [Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS) trial (72a)], respectively.

**Cardio-derived progenitor/stem cells.** A damaged rodent heart, resulting in ischemic stress and genetic degeneration, cannot usually repair itself sufficiently to restore the loss of LV function by endogenous cardiac regeneration. On the other hand, animal studies have demonstrated postnatal heart regeneration through several strategies: screening of histological sections for incorporation of radiolabeled thymidine (95, 96) and genetic fate-mapping in transgenic mice (38). Therefore, the traditional theory that the adult mammalian heart might be a terminally differentiated organ has been extremely controversial for the past century (21). The law of cardiomyocyte turnover proposed by Bergmann et al. (16) provides elegant evidence showing that myocardial restructuring can endure for a lifetime, at the same time strongly suggesting the existence of the endogenous cardiac progenitor or stem cell.

Since Oh et al. (74) first reported in 2003 that cardio-derived Sca-1-positive cells highly conserved the potential of transdifferentiation into cardiomyocytes, several types of CSCs, which are defined by their cell surface antigens or proliferation properties, including islt-1 (mouse) (49, 121) and c-kit (mouse and human) (14, 15, 112) and/or sphere-forming abilities (65, 106, 107), were identified in adult rodent hearts between 2004 and 2007. In general, cardiac progenitor cells (CPCs), which are usually positive for c-kit antigen, are lineage-limited to cardiomyocytes, endothelial cells, and vascular smooth muscle cells. Furthermore, human CSCs (hCSCs), with their c-kit-negative sphere-forming phenotype-possessing ability for self renewal and mesodermal multilineage differentiation (not only for cardiomyocytes, endothelial cells, and vascular smooth muscle cells but also for adipocytes, osteoblasts, and chondroblasts), were reported in 2008 (103). In autologous transplantation, the clinical feasibility of cardiac progenitor/stem cells is usually achieved with less invasive methods obtained from the use of endomyocardial biopsy samples (94) (Fig. 1). Two trials have been ongoing since 2009: one in the United States, in which patients with ischemic cardiomyopathy are transplanted with cardiosphere-derived cells (CDCs) ex vivo expanded from endomyocardial biopsy samples [Cardiosphere-Derived Autologous Stem Cells to Reverse Ventricular Dysfunction (CADUCEUS) (21a)] and one in Japan [Autologous Human Cardiac-Derived Stem Cell to Treat Ischemic Cardiomyopathy (ALCADIA) (45a)]. In the CADUCEUS trial, CDCs containing a few c-kit-positive cells (~20%) are transplanted into a nonselected
ischemic area by transcoronary injection. In the ALCADIA trial, the other phenotypes of CDCs without c-kit-positive cells have been transplanted into a viable infarct area, defined as a late Gd-enhancement of cardiac MRI by surgical transmyocardial injection. On the other hand, CPCs, which are purified by c-kit antigen, must be surgically excised since large amounts of cardiac tissue are necessary for cell isolation. In the Cardiac Stem Cell Infusion in Patients with Ischemic Cardiomyopathy (SCICPIO) trial, CPCs obtained in the first CAbG from the right atrium appendage have been transplanted into post-CABG patients with heart failure by transcoronary injection (111a).

Ontology of Cardiac Regeneration Therapy: Target and Goal

Current cell therapy for various types of AMI and ischemic cardiomyopathy has had mixed results and has produced conflicting insights because of the widely varied and restrictive early clinical trials and nonstandardized clinical indications (84). To extend “real” cardiac regeneration therapy for saving the lives of patients with refractory heart failure, it is absolutely necessary to closely evaluate the indications, outcomes, and limitations of cardiac regeneration therapy.

Indications for cell-based cardiac regeneration therapy. Although the clinical application of cell-based therapy has been expanded in the first decade of the 21st century, there is still an incomplete consensus on guidelines and patient eligibility for cardiac regeneration therapy for refractory heart failure (13). To exclude allogeneic immune rejection in the donor heart, autologous cell therapies, which are easier and less invasive than extraction of donor tissue, have been initiated as a means of “cardioprotective” regeneration for patients with AMI in the clinical setting. Because embryonic stem cells, which function primarily as pluripotent cells, had no chance to be transplanted in acute phase of AMI, so cell-based therapy (the first generations) without pathophysiological cardiac regeneration by unrestricted bone marrow cells or peripheral blood cells containing a small number of EPCs could not clearly show us a milestone to restore the partial loss of LV function without poor outcome (10, 71). Therefore, the second generations of cardiac regeneration therapy, which is based on adjunctive cardiomyocyte regeneration, have aimed toward patients with severe ischemic cardiomyopathy in conjunction with low LVEF and poor prognosis (3, 115). The emerging cell types in second generations will be harnessed for their high propensity for cardiomyogenic differentiation. In these patients, it is often difficult to restore the loss of function through modern state-of-the-art therapies, not only multiple drug-eluting stent implantation (23) and cardiac resynchronization therapy (113) but also LV reconstruction surgery, which the Surgical Treatment for Ischemic Heart Failure (STICH) trial investigators clearly described (40). There is no eligibility criterion with respect to LV contraction (LVEF) for these therapies; meanwhile, patients with LVEF < 35% are never disqualified as candidates for adjunctive cardiac regeneration therapy.

Assessment of the efficacy of cell-based therapy. How should the pathophysiological effects of cell-based cardiac regeneration therapy be evaluated in clinical situations in vivo? Needless to say, an evaluation by any single methodology is difficult because cell-based therapy offers various benefits of not only cardiomyocyte regeneration but also cytokine release, apoptosis, and endogenous cardiac repair. Although the most standard strategy is a comparative study of the LVEF of eligible patients, even the methodology for evaluating the LVEF varies among clinical trials because of multimodalities [left ventriculography (86), echocardiography (62, 98), cardiac MRI (7, 119), and gated single-photon emission-computed tomography radionuclide imaging (41)]. In the meantime, the mechanisms of functional benefits cannot be directly shown in vivo by clinical laboratory methods. However, a reduction of the fibrosis area based on an increased number of cardiomyocytes might be evaluated because the myocardial scar is detectable as a hyperenhancement area by late Gd-enhancement of the cardiac MRI (43, 105). Radionuclide imaging, [123I]15-(p-iodophenyl)-3-(R,S)-methylpentadecanoic acid (BMIPP), and [18F]fluoro-deoxy-glucose (18FDG) with positron emission tomography are strongly useful for the representation of myocardial biological activity; however, the spatial resolution of radionuclide imaging is not of sufficiently high quality to determine the cell transplantation area before cell therapy and to assess the cardiac regeneration in the therapeutic area post-cell therapy. Overall, cardiac MRI might be the most suitable methodology for analyzing cardiac performance and in vivo pathohistology in cardiac regeneration therapy (82).

Injured hearts with LV dysfunction, especially in situations of dilated cardiomyopathy and ischemic cardiomyopathy, often cannot synchronize the wall motion between the septal wall and the lateral free wall (56, 76) (Fig. 2A). In their analysis of two-dimensional speckled-tracking technology by echocardiography, van Ramshorst et al. (114) reported that percutaneous intramyocardial injection of autologous unrestricted bone marrow cells improved the LVEF of ischemic cardiomyopathy patients with heart failure (from 23 to 27%), which related to reduced LV dysynchrony and increased global strain. Although electrical uncoupling by heterogeneous action potential provided from recipient cells is known as the origin of ectopic stimuli, it is still unclear whether the reversed LV dysynchrony is caused by electrical fusion between the host cardiomyocytes and transplanted cells.

Traceability of engrafted cell in vivo imaging. To investigate the mechanisms by which cell-based therapy brings functional benefit to the injured heart, the tracking of engrafted cells holds basic and inevitable applications for regenerative medicine. In experimental studies, although many techniques exist for cell tracking, such as membrane 1,1'-dioctadecyl-3,3,3’3’-tetramethyl indocarbocyanine perchlorate (DiI) staining (109), genetic labeling using reporter genes [green fluorescent protein, DsRed, X-gal stain for β-galactosidase (Lac-Z)] (28), the introduction of reporter genes under the control of a cardiac-specific promoter (α-myosin heavy chain) (44), and the use of xenografts for fluorescent in situ hybridization (31, 50, 103), these technologies are not feasible in humans. On the other hand, radiolabeling tracers, superparamagnetic iron nanoparticles (SPIO) (30), and the human ferritin heavy chain (20) have been reported as in vivo imaging methods in clinical trials of cardiac regeneration (Fig. 2B).

The tracing labels 18FDG (37), [111In]oxine (1), and [99mTc]hexamethylpropyleneamine have been employed to track engrafted cells (12). Unrestricted bone marrow cells (37), CD34-positive cells (32), and AC133-positive cells (32), which are radiolabeled by in vitro incubation for 30–60 min (indicated),
have been injected via the transcoronary route in the acute phase of AMI. Because the detectable time window depends on label half-time \((^{18}F\text{DG} < 20\ h, ^{99}m\text{Tc} < 24\ h)\), cellular in vivo imaging must be performed within 2 h (all cells) and \(\sim 24–72\ h\) (retention cells) after injection. Therefore, prolonged cell survival cannot be investigated in the host myocardium 4 wk after treatment. As reported by most investigators, a large number of radiolabeled engrafted cells (\(\sim 80\%\)) in the ischemic myocardium have been lost within 72 h after transcoronary route injection because of the washing away by the blood flow. Radiolabeled cell imaging in vivo clearly shows that solo cell-based therapy by transcoronary injection has been implicated in the limitation of cell survival (77).

Cell labeling with SPIO for visualization by MRI is a good application for engrafted cell tracking in longer time periods after cell transplantation (30) (Fig. 2, B and C). Needless to say, it is difficult to evaluate absolute values because SPIO released by dead cells are taken up by macrophages (2). Hence, an accelerated retention of SPIO-labeled cells by cell priming to enhance their survival efficacy is well recognized in the host myocardium compared with that of solo cell-based therapy. SPIO-labeled engrafted cells transplanted into the ischemic myocardium have been assessed only in experimental models; however, Zhu et al. (122) reported that magnetically labeled neural stem cells were administered to patients with brain trauma.

**Pathophysiological Fusion onto the Host Myocardium by Various Delivery Routes**

“Real” cardiac regeneration is not yet feasible with current cell-based therapy. A regenerative artificial myocardium has not yet been developed that can replace injured heart tissue through cardiomyocyte regeneration by pluripotent stem cells. Pathophysiological fusion between engrafted cells and the host myocardium is essential for the regenerative artificial myocardium to ensure that 1) a great number and long-term survival of the engrafted cells in the host heart, 2) the high rate of (trans)differentiation of the engrafted cells into cardiomyocytes, and 3) electrical coupling through the gap junction protein connexin (43, 85, 103). To actualize cardiac regeneration, three approaches have been conducted in cell-based therapy. Transvascular approaches to cardiovascular intervention offer the most beneficial and least invasive techniques in the current practice of cardiology. First, many cardiologists are adopting the transcoronary route to transplant stem/progenitor cells into an injured myocardium, and second, a few cardiologists are trying the transendocardial route using the cell-injectable catheter.
Finally, surgical direct injection through the epicardium is often considered the most trustworthy method concomitant with CABG (Fig. 3).

**Transcoronary injection.** In the first generations of clinical trials of cardiac regeneration, transcoronary injection of unrestricted bone marrow cells was performed in AMI patients through the infarct-related arteries, succeeded by revascularization via catheter intervention (8, 53, 54, 86, 119) (Fig. 1). This method, which adopted the standard balloon technique, is safer and less invasive; however, some concerns remain. In the case of larger cells (over 50 μm), capillary plugging by cell injection might lead to the slow (no)-reflow phenomenon in the infarct-related arteries (116) (Fig. 3A). Furthermore, transcoronary injection cannot control the therapeutic area restrictively in which the stem/progenitor cells should be engrafted because the injected cells might be flushed away to the coronary veins, reaching into not only the infarct border zone but also the scar zone or healthy myocardium (6, 21a, 111a). Unfortunately, the retention of engrafted cells in the host myocardium is limited to a small fraction of transplanted cells. Overall, transcoronary injection seems to be suitable for limited circumstances: small cells [unrestricted bone marrow cells, EPCs, and small-size CPCs (104, 111a)], a large cell number (>10^6 cells/body), and certain patients (with AMI, not experiencing ischemic and idiopathic cardiomyopathy).

**Transendocardial injection.** Transendocardial injection has usually been performed with the NOGA mapping catheter to identify the hibernating myocardium (51, 52) (Figs. 1 and 3B). This technique can be adapted to large cell types, such as SMs (16a) and MSCs (72a), and the therapeutic area can be adjusted by electrical mapping. However, the risk of cardiac tamponade is always involved when there is a thin layer of scar myocardium undetectable by needle depth. Furthermore, the NOGA mapping catheter remains an expensive device and requires a high level of skill of cell transplantation. Outside these issues, transendocardial injection is an option for cell-based cardiac regeneration therapy through direct intramyocardial delivery without open heart surgery.

**Transepicardial injection.** Transepicardial injection is a much more reliable method compared with transcoronary injection with respect to better retention of engrafted cells, and it is more suitable for ischemic cardiomyopathy patients who need adjunct stem cell therapy because it should be performed in conjunction with a surgical approach, usually CABG (62, 97) (Figs. 1 and 3B). However, a few concerns remain: 1) placebo control (no historical control) and 2) insufficient cell retention. An established placebo control is important to evaluate the efficacy of additional cell-based therapy because CABG can improve ~3–4% of LVEF of ischemic cardiomyopathy patients with low LV function (LVEF < 35%), as shown in the STICH trial (40). We and others (36, 45, 103) have shown poor retention (10–15%) in the host myocardium of SPIO-labeled cells that were transplanted by transepicardial injection, as visualized by MRI. Even if the transepicardial injection is superior to the transcoronary injection with respect to better retention of engrafted cells, solo cell-based therapy still does not result in sufficient retention of engrafted cells to organize the regenerative artificial myocardium because of the very poor environment due to oxidative stress, hypoxia, and inflammatory cytokines in ischemic myocardial tissue.

To realize the advantages (benefit) and disadvantages (risk) of cardiac regeneration therapy easily, we summarized the feasibility and various problems of cell-based cardiac regeneration therapy about cell sources, delivery routes, and assessment strategies (Fig. 4).

**The Road to “Real” Cardiac Biophysical Repair: a New Era of Cell-Based Technology**

What can we do to rescue injured hearts from refractory heart failure? Now, at the end of the first decade of cell-based cardiac regeneration therapy, we must quickly move forward to promote “real” cardiac repair by various biotechnological strategies: tissue engineering, the use of emerging stem cell types, and a novel approach to cell reprogramming.

**Tissue engineering and cell technology.** Various cytokines [IGF-1, HGF, VEGF, and basic FGF (bFGF)] are well recognized as enhancing the proliferation or differentiation of stem/progenitor cells. Tabata and Ikada (100) generated the biodegradable gelatin hydrogels that could incorporate the cytokines

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![Fig. 3](http://ajpheart.physiology.org/) The scheme of retention mechanism in each methods of solo cell-based therapy [1st generations: transcoronary injection (A) and transepicardial injection (B)] and hybrid cell therapy [2nd generations: transepicardial injection with biodegradable scaffold incorporated some cytokines (C) and cell sheet technology (D)]. ROS, reactive oxygen species; CM, cardiomyocyte; HGF, hepatocyte growth factor; bFGF, basic FGF.
and controlled release in vivo (Fig. 1). We generated emerging hCSCs obtained from endomyocardial biopsy samples using the identical technique as clonal cell isolation from the “cardiosphere” (106). To investigate the effect of hCSC transplantation with controlled release bFGF, we performed a preclinical trial in which we randomly assigned chronically ischemic pigs to receive placebo or bFGF hydrogel sheet implantation with or without hCSC transplantation (103). Our results showed that the hearts injected with hCSCs together with bFGF improved their cardiac function to a greater degree than the hearts that received hCSC injection alone. This occurred via the superior retention (35% of hCSC injection with bFGF vs. 13% of hCSC injection alone) of SPIO-labeled cells in the host myocardium and subsequent cardiovascular reconstruction, contributing to cardiomyocyte regeneration (Figs. 1, 3, and 4). Our findings suggest that this novel integrated strategy may have the potential for promoting functional repair in the severely injured heart. We are now moving forward to implement a phase I pilot trial (ALCADIA trial) to enroll ischemic cardiomyopathy patients with low LV function (LVEF < 35%) and concomitant refractory heart failure (45a).

Shimizu et al. (93) developed cell sheet technology, which was developed from temperature-responsive culture dishes (Fig. 1).
grafted with the temperature-responsive polymer poly(N-isopropyl-acrylamide), SMs (35), and MSCs (69). Sca1-positive CPC (58) sheets harvested by simple temperature changes can be layered to create cell-dense three-dimensional tissues. SMs that release some cytokines (mainly HGF) via the autocrine system have limited efficacy for LV functional repair through their cardioprotective effects (59), even though direct injection of SMs did not succeed in the MAGIC trial (62). Miyagawa et al. (68) reported that the SM cell sheets implanted on the epicardium of ischemic swine improved LV function, contributing to the paracrine effect of some cytokines (Figs. 1, 3D, and 4). Cell sheet technology might be a good candidate for the next era of cardiac regeneration therapy if it can supply the vascular networks in the cell sheets (89) and create the multiple layers of cell sheets (60). Fujita et al. (29) have evaluated the potential application of this combination cell therapy in four patients with severe ischemic cardiomyopathy who required implantation of a LV assist device.

Emerging stem cell and reprogramming technology. Takahashi et al. (101, 102) reported that iPS cells, which have multipotency to postnatal organs, can easily differentiate into beating cardiomyocytes in vitro. This innovative technology for nuclear reprogramming is enabled by the ectopic expression of four factors [Oct3/4, SRY-box containing gene 2 (Sox2), and Kruppel-like factor 4 (Klf4) with or without c-Myc] (72) and can pioneer the novel generation of cell-based cardiac regeneration therapy via autologous somatic cells with great potential for pluripotency. However, within the in vivo milieu, these have not yet reached clinical application for cardiovascular patients because of uncontrollable teratoma formation (67), the existence of primitive cardiomyocytes, and immunogenicity in the host myocardium posttransplantation (120). In order for iPS cells to have a role in ameliorating the injured heart, terminally differentiated iPS-derived cardiomyocytes are needed that would be immunologically compatible with the host myocardium (123). To this end, a subset of cardiomyocytes that is possibly including a type from among the cardiac stem/progenitor cells) should be sought with the “epigenetic memory” of cardiac fate, because human iPS cells derived from various somatic cells do not yet have sufficiently high quality to promote cardiac regeneration when accompanied by poor reprogramming (11, 42).

Cell reprogramming. In 1987, a primary gene of direct reprogramming was first reported as the MyoD gene, which generates cell-fate reprogramming by a single transcription factor (24). Weintraub et al. (48) demonstrated that expression of the MyoD gene is sufficient to convert fibroblasts and numerous other cell types into skeletal muscle cells, completely bypassing normal developmental lineage differentiation. In the two decades since 1987, Ieda et al. (39) demonstrated that cardiac fibroblasts were directly reprogrammed into the cardiomyocyte fate (induced cardiomyocyte) by three defined factors [Tbx5, GATA4, and myocyte enhancer factor 2C (Mef2C)] in mice. The induced cardiomyocytes seemed to be contractile cardiomyocytes that can be detected as transplanted cardiomyocytes in the injured host heart immediately after induction of three factors. Even though induced cardiomyocytes are not induced by a single master gene, the revolutionary area of cardiac regeneration is opened by this direct reprogramming technique, which might remove the risk of teratoma formation and immunogenicity. This could be the novel tool that functions as the master gene or cytokines for converting somatic cells to cardiomyocytes.

Summary

The first generations of cell-based cardiac regeneration therapy for AMI has terminated, and the door is now opening to the second generations of hybrid cell therapy for ischemic cardiomyopathy (in particular, dilated cardiomyopathy). Nuclear reprogramming technology will be an intriguing challenge for this new era (Fig. 5). At the same time, we must acknowledge that not only classical cell-based therapy but also advanced hybrid therapy (tissue engineering, cell sheets) and emerging cell types, together with novel insights, will provide superior, beneficial improvements to the standard medical therapy in cardiology. Evidence is needed from a clinical trial as follows: 1) a well-designed pilot trial to address safety and therapeutic mechanisms for testing specific hypotheses (phase I) and 2) a double-blind, randomized, controlled trial to objectively assess efficacy and safety (phase II) (13). By overcoming the many issues surrounding cell-based therapy, we will reach the milestone of “real” cardiac regeneration therapy for all patients with refractory heart disease.

GRANTS

N. Takehara and H. Matsubara were supported by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology (Japan) and by grants-in-aid from the Ministry of Health, Labor and Welfare (Japan).

DISCLOSURES

No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

N.T. approved final version of manuscript; H.M. edited and revised manuscript.

REFERENCES


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Published in: AJP-Heart Circ Physiol

Keywords: Cardiac Regeneration Therapy; Cell Therapy; Cardiomyocytes; Somatic Cells; Hybrid Cell Therapy

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