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Lost in Translation: What is Limiting Cardiomyoplasty and Can Tissue Engineering Help?

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Abstract

Heart failure accounts for more deaths in the United States than any other detrimental human pathology. Recently, repairing the heart after seemingly irreversible injury leading to heart failure appears to have come within reach. Cellular cardiomyoplasty, transplanting viable cell alternatives into the diseased myocardium, has emerged as a promising possible solution. Translating this approach from the laboratory to the clinic, however, has been met with several challenges, leaving many questions unanswered. This review assesses the state of investigation of several progenitor cell sources, including induced pluripotent stem cells, embryonic stem cells, bone marrow stem cells, adipose-derived adult stem cells, amniotic fluid stem cells, skeletal muscle progenitors, induced pluripotent stem cells and cardiac progenitors. Several current roadblocks to maximum success are discussed. These include understanding the need for cardiomyocyte differentiation, appreciating the role of paracrine factors, and addressing the low engraftment rates using current techniques. Tissue engineering strategies to address these obstacles and to help maximize cellular cardiomyoplasty success are reviewed.

Keywords

Progenitor cell; differentiation; paracrine factors; cell delivery; tissue engineering; myocardial infarction

INTRODUCTION

Heart failure has sustained its place as the leading cause of death in the United States over the past two decades and is characterized by the progression of electrical and mechanical dysfunction [1]. A major etiology of heart failure is myocardial infarction, which occurs when the demand for blood exceeds the perfusion of blood through the myocardium. Ultimately, necrosis, apoptosis, and fibrosis manifest as left ventricular (LV) remodeling and failure. The myocardium does not regenerate appreciably after injury, because of the limited pool of cardiac specific progenitor cells present and the inability of adult cardiomyocytes to extensively proliferate [2]. Except for heart transplantation, no treatments are able to fully restore cardiac function. Heart transplants, however, require a matched donor (heart size and blood group), and the number of hearts available is significantly lower than the number of patients who require this procedure [3]. Current pharmaceutical therapies have focused on restoring and enhancing inotropic function, reducing congestion, and

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decreasing adrenergic stimulation, but such approaches are not able to prevent remodeling and are associated with several negative side effects.

Recently, regenerative medicine has emerged as a strategy to repair myocardial damage after injury. This strategy, called cellular cardiomyoplasty, involves transplanting cells into injured myocardium to assist in the repair and restoration of myocardial function. In particular, stem cells have gained much attention over the past decade because of their ability to differentiate into cardiomyocytes as well as provide trophic factors to assist in the repair of an injured heart. Translating this approach into the clinical realm has proven to be difficult, however. Questions have arisen regarding cell source, stem cell fate once transplanted, and cell delivery strategies.

In this review, we discuss the current variety of stem cell sources and their therapeutic usefulness in cardiac repair. We review the current state of translation to man. Current roadblocks to full realization of the potential of cell replacement therapy are discussed with a highlight on tissue engineering, a promising new approach to address some of these obstacles.

THE SEARCH FOR THE OPTIMAL PROGENITOR CELL

In order to achieve successful clinical translation of cellular cardiomyoplasty, a cell capable of improving cardiac function without induction of adverse consequences must be identified. One elegantly simple solution would be exogenous cardiomyocytes. Several preclinical studies have been undertaken to show the potential of these cells in restoring cardiac function after infarction. In particular, Koh *et al.* demonstrated that AT-1 cardiomyocytes (derived by expressing an atrial natriuretic factor-simian virus 40 T antigen fusion gene) were able to survive up to four months and proliferate when injected into normal myocardium [4]. The ability of these cells to survive suggests that cardiomyocytes may provide a useful platform for cardiac therapy. Additional studies showed that injection of fetal ventricular cells into infarcted myocardium resulted in reduced scar formation and improved systolic pressure over eight weeks [5]. A study comparing fetal cardiomyocytes, smooth muscle cells, and fibroblasts concluded that the contractile potential of a cell (cardiomyocytes > smooth muscle cells > fibroblasts) determined the extent of improved muscle function in infarcted hearts [6]. Although these studies are promising, successful translation has been difficult given the inability of obtaining large numbers of primary cardiomyocytes (adult or neonatal) and immunological limitations.

The choice of progenitor cell becomes more challenging and encompasses more considerations when moving beyond cardiomyocytes. After the onset of myocardial infarction and other cardiomyopathies, fibroblast infiltration gives rise to collagen deposition [7]. This increase in fibrosis leads to changes in the mechanical properties of the muscle and usually is associated with reduced myocardial performance [8]. An ideal cell for cardiomyoplasty might reduce cardiac fibrosis and migrate to areas of myocardial damage to induce a beneficial effect. Other desirable characteristics might include the ability to promote endogenous cardiomyocyte proliferation or to differentiate into working cardiomyocytes. Thus, a suitable cell might provide a therapeutic scaffold for myocardial repair and preservation by stimulating endogenous cardiac progenitors to proliferate and migrate to areas of myocardial injury. Whatever the mechanism of benefit, a suitable cell would have to be free from significant adverse effects such as arrhythmias.

Unfortunately, no known cell exists which demonstrates all of these properties, and the notion of engineering suitable cells seems decades away. Several progenitor cells do provide many desirable traits, however. The selection is vast and continues to grow as new progenitor cells emerge. These cells may hold the key to successful cellular

cardiomyoplasty. Many progenitor cells have already been tested in preclinical and clinical studies and in most cases a beneficial effect was observed, at least transiently [9].

Skeletal Myoblasts

Skeletal myoblasts are precursor cells for skeletal muscle and are found in the basal lamina of muscle fibers. They are typically characterized by their expression of Pax 7 (quiescent) and MyoD (activated) [10]. Because of their contractile potential and resistance to ischemia, early efforts in cellular cardiomyoplasty involved the direct injection of skeletal myoblasts into injured hearts. Murry *et al.* demonstrated that skeletal myoblasts could engraft in infarcted myocardium, form myotubes, and mature into β -myosin heavy chain expressing muscle [11]. Additionally, this muscle could be induced to contract *ex vivo* and was able to convert into fatigue resistant, slow twitch fibers. There is still some debate whether these cells assume a cardiac-like phenotype *in vivo*. Reinecke *et al.* were not able to show transdifferentiation of skeletal myoblasts into cardiomyocytes once engrafted [12]. Other reports suggest partial transdifferentiation potential of skeletal myoblasts [13, 14]. One possible explanation for the disparity between reports is that skeletal myoblasts may fuse with surrounding myocardium, developing a hybrid phenotype [15, 16].

Regardless of the fate of the myoblast after *in vivo* intracardiac transplantation, improvements in myocardial performance have been demonstrated over four to five weeks in several studies. Discrepancies between reports, however, impart difficulties in trying to assess the mechanism of action by which myoblasts assist in myocardial repair. Additionally, myoblast require *ex vivo* expansion to increase cell number for autologous transplantation. Inherently, this delays the time until treatment can be administered and may contribute to the variability in cell behavior.

Cardiac Progenitors

There are several variations of cardiac progenitors that are currently being studied. Stem cell antigen-1 (Sca-1) has previously been shown to exist on tissue-specific progenitor cells [17, 18]. Sca-1+ cardiac progenitors have also been reported. These cells maintain the ability to proliferate *ex vivo* allowing for cell expansion. Also, these cells can be induced to differentiate into beating cardiomyocytes with the application of 5' azacytadine and oxytocin [19, 20]. Notably, beating frequency increases with isoproterenol treatment demonstrating the physiological responsiveness of cardiac Sca-1+ derived cardiac cells. When injected, these cells are able to home to the border zone of infarcted myocardium [19]. Nevertheless, the regenerative ability of cardiac Sca-1+ cells has yet to be determined.

Cardiac side population (CSP) cells have been isolated via the selection of cells which efflux Hoechst dyes. Such cells have a varied phenotypic profile in regards to the expression of Sca-1, c-kit, Abcg2, CD34 and CD31. High expression of Sca-1 and low expression of c-kit are observed typically [21, 22]. In this regard, CSP cells are similar to Sca-1+ cells. CSP cells have been shown to differentiate into a cardiac phenotype (Nkx2.5 and GATA4 positive) via co-culture with cardiomyocytes and demonstrate a high proclivity toward *ex vivo* proliferation [21–23]. The efficacy of these cells in an *in vivo* myocardial injury model has yet to be determined, although it has been demonstrated that the proliferation of CSP cells increases after MI and that these cells can be replenished by bone marrow derived progenitors during MI [24].

Another cardiac progenitor that has been isolated from the myocardium is characterized by its high expression of c-kit and negative expression for CD34 and CD45. These cells can be isolated from an enzymatically dissociated tissue lysate using c-kit specific antibodies and immuno-based cell sorting techniques. Upon isolation, 7–10% of cells express the cardiac

specific transcription factors Nkx2.5, GATA-4, and MEF-2 [25]. Further signs of differentiation are exhibited when c-kit⁺ cells are cultured in differentiation media. Although no distinct morphological signs are observed in regards to cardiac cell differentiation, many molecular resemblances emerge. Beltrami *et al.* injected BrdU-labeled c-kit⁺ cells into the border zone of infarcted myocardium. These cells engrafted, reduced infarct size and differentiated into cardiac muscle, smooth muscle and endothelial cells. Additionally, injection of c-kit⁺ cells enhances cardiac remodeling and improves myocardial performance [26].

Cardiospheres (CSph) are a heterogeneous progenitor cell population derived from an adult cardiac biopsy. Upon isolation and enzymatic dissociation, the cells are allowed to culture in suspension where they spontaneously form small clusters and differentially express markers such as c-kit, Sca-1, CD31, CD34 and CD105 [27, 28]. CSph can be observed to spontaneously differentiate into beating cardiac muscle, alone or as a co-culture with cardiomyocytes. When CSph are injected into infarcted hearts, a marked increase in fractional shortening and myogenesis is observed. Similar effects are also observed when enzymatically dissociated CSph (used to form single cells) are injected into infarcted myocardium [28]. The use of CSph-single cells offers the option of expanding the progenitor cell population, given the small number of cells initially isolated, and avoids using large cell clusters *in vivo*.

The presence of the LIM-homeodomain transcription factor islet-1 (Isl1) has been used as a marker to identify a progenitor cell population in the postnatal heart. These “cardioblast” are observed to primarily reside in areas of the second heart field (i.e. right ventricle, both atria, and the outflow tract). Although the number of Isl1⁺ cells substantially decreases after birth, they are able to propagate *ex vivo* when cultured on a cardiac mesenchymal feeder layer. Isl1⁺ cardioblast are positive for Nkx2.5 and GATA4 but fail to express both Sca-1 and c-kit indicating they are phenotypically distinct from other reported cardiac progenitors [29]. Interestingly, Isl1⁺ cardioblasts are able to differentiate spontaneously into functional cardiomyocytes when co-cultured with neonatal cardiomyocytes. These cardioblast-derived cardiomyocytes express cardiac structural proteins, exhibit calcium transients, and have the ability to undergo excitation contraction coupling. The role of Isl1⁺ cardioblasts in myocardial repair after injury has yet to be determined.

Although cardiac progenitors show a high proclivity for cardiac differentiation, it is difficult to isolate large numbers of cells. Therefore, *ex vivo* expansion is typically performed to allow for a suitable graft. Cardiac progenitor cells would benefit from methods to decrease the delay from cell isolation to cell transplantation.

Bone Marrow Stem Cells

Bone marrow stem cells (BMSC) include both mesenchymal and hematopoietic cell types. Both have been used extensively as a cell therapy for myocardial infarction. Mesenchymal stem cell (MSCs) are adult progenitor cells that have the potential to differentiate into tissues from the mesoderm [30]. These include fibroblast, muscle, bone, tendon, ligament, and adipose tissues. Such cells are characterized by their expression of SH2 (type III TGF receptor), SH3, SH4 (ecto-5'-nucleotidase), and STRO-1 [31] and by their lack of expression of CD45 and CD34. These cells have also been shown to successfully differentiate into cardiomyocytes *in vitro* [32]. In landmark papers by Orlic and his co-workers, they showed that Lin⁻, c-kit⁺ BMSC differentiated into premature cardiomyocytes, endothelial cells, and smooth muscle cells after injection into infarcted myocardium. Functional assessments of infarcted hearts with BMSC grafts also revealed improvement in several hemodynamic measures [33, 34]. Other reports showed lesser functional improvements, and hematopoietic stem cell differentiation into cardiomyocytes was not

observed [35, 36]. Such discrepancies question the necessity of cardiomyocyte presence to induce cardiac repair and leave open the degree to which these cells can impact cardiac function. When human MSCs are injected into normal mouse myocardium they attain a “cardiaclike” phenotype [37] as tested by the expression of several cardiac related markers. Unfortunately, few studies have shown well-defined cardiomyogenic differentiation of MSCs delivered to infarcted hearts [9, 38–40]. In addition, these studies indicate a need for *ex vivo* expansion of MSCs before implantation. Other fractions of the bone marrow do not require such action and can be readily used as an autologous graft. Nevertheless, most studies do report improvements in remodeling, hemodynamic measures, and mechanical parameters upon delivery of MSCs. One theory to explain such improvements without substantial cardiomyocyte repopulation is that MSCs secrete paracrine factors that act on host cells in a beneficial manner [41, 42].

Some benefit of BMSC transplantation in myocardial repair seems clear. Nevertheless, the extent and mechanism of repair are still uncertain. In addition, unfractionated BMSCs represent a heterogeneous cell population. The use of BMSCs for cellular cardiomyoplasty may benefit from advancements in bioprocessing to identify different marrow populations with enhanced cardiomyogenic or angiogenic potential.

Adipose-Derived Mesenchymal Stem Cells

MSCs are not only found in the bone marrow but can be found in several tissues throughout the body. In particular, cells isolated from the aspirates of patients undergoing liposuction have demonstrated mesenchymal-like properties and offer an alternative to bone marrow-derived MSCs [43]. Adipose-derived stem cells (ADSC) express similar markers expressed by MSCs such as CD105, CD29, CD44 and CD90. One difference appears in the expression of the VLA-VCAM-1 receptor-ligand pair. ADSCs express Very Late Antigen (VLA) but fail to express Vascular Cell Adhesion Molecule-1 (VCAM-1), while MSCs express VCAM-1 but not VLA. Such differences in adhesion molecule/integrin expression may account for the distinct differences in tissue localization [44]. There appears to be 500 times more ADSCs per gram of fat than MSCs per gram of marrow thus possibly eliminating the need for *ex vivo* scale up for cellular cardiomyoplasty. In addition, ADSCs differentiate into a α actinin and β -myosin heavy chain expressing cardiac phenotype with the use of 5-azacytidine, co-culture with neonatal cardiomyocytes, or spontaneously under defined culture conditions [45–47]. Observations indicate that cardiomyocytes derived from ADSCs can generate action potentials and respond to pharmacological stimuli such as isoproterenol [47]. ADSCs injected into the LV of a cryoinjured mouse myocardial infarct model determined that these cells engraft and differentiate into Nkx2.5-, troponin I-, and myosin heavy chain-expressing cells. These cells were not shown to integrate with healthy myocardium, however [48]. Other studies have shown the angiogenic potential of ADSCs upon enrichment of the CD34+/CD31- population [49] and the beneficial effect these cells have on global myocardial function after LV chamber injection [50]. Also, ADSCs secrete paracrine factors that may contribute to tissue repair after injury [51].

The ability of ADSCs to differentiate into cardiomyocytes, secrete paracrine factors, provide functional augmentation after myocardial infarction, and not require *ex vivo* expansion suggests these cells represent an important advancement in the search for useful cell sources. An understanding of the mechanism by which these cells contribute to cardiac repair is lacking, however.

Amniotic Fluid Stem Cells

Amniotic fluid stem cells (AFSC) represent a multipotent cell population isolated from amniocentesis specimens. Although cultures of amniocentesis contain a heterogeneous

population of cells with diverse potency, AFSC can be isolated and enriched by using immune selection for the c-kit receptor. Upon isolation, AFSC express markers characteristic of mesenchymal, neural, and embryonic stem cells. These include the expression of CD90, CD44, CD105, CD29, CD73, Oct4, and SSEA4. AFSCs fail to express the hematopoietic markers CD45, CD34 and CD133 [52]. AFSCs differentiate into a cardiomyogenic phenotype in the presence of neonatal cardiomyocytes as evidenced by the expression of Nkx2.5, cardiac troponin I, GATA-4, and MLC-2v, but cell fusion with host myocytes has not been excluded. When AFSCs are injected into infarcted pig hearts, they differentiate into endothelial, fibroblast, and smooth muscle phenotypes, but no cardiogenic phenotypes are observed [53]. Human AFSCs were acutely rejected when used in normal or immunosuppressed rat myocardial infarct models, however [54].

AFSC represent another abundant cell source. The efficacy of AFSCs in cardiac repair is still ill defined, however. In order for further progress, more studies will have to be performed to investigate issues of cell integration, rejection, and efficacy for myocardial repair.

Embryonic Stem Cells

Embryonic stem cells (ESCs) are another cell type which has garnered attention lately because of their relative availability, expansion capabilities, and proven cardiomyocyte differentiation. ESCs are isolated from the inner cell mass of a developing blastocyst, are defined by their ability to differentiate into tissues from all three germ layers, and express pluripotency markers such as OCT 3/4 and Tra 1-81 [55–58]. Mouse and human embryonic stem cells have been shown to successfully differentiate into cardiomyocytes through the formation of embryoid bodies [59] or co-culture with the visceral endoderm-like cell line END-2 [60, 61]. ESCs have also been extensively tested *in vivo* for their potential for differentiation into cardiomyocytes [62]. Limitations of undifferentiated ESCs include the formation of teratomas and susceptibility to ischemia after delivery [63]. Both Swijnenburg *et al.* [64] and Nussbaum *et al.* [65] report significant teratoma formation after ESC transplantation into infarcted myocardium. To avoid teratoma formation, investigators are starting to differentiate cells into cardiomyocytes before transplantation. Differentiated mouse ESC-derived cardiomyocytes have shown arrhythmic potential *in vitro* [42], however. In addition, ESC-derived cardiomyocytes continued to display susceptibility to ischemia [63].

The use of undifferentiated ESCs risks teratoma formation and low graft viability. Addressing these issues has resulted in many investigators differentiating ESCs into cardiomyocytes before implantation. This approach focuses on remuscularization of damaged heart, although other mechanisms may play a significant role. Additionally, integration of these grafts with host myocardium is poor and may result in unwanted arrhythmias. The issue of rejection is also a concern.

Induced Pluripotent Stem Cells

Induced pluripotent stem (iPS) cells represent a major breakthrough in regenerative medicine and involve the trans-differentiation of an unipotent or multipotent cell into a pluripotent state. Typical approaches to inducing pluripotency include retroviral transduction of somatic cells with four transcription factors: octamer-binding transcription factor 3/4 (oct 3/4), SRY-related high-mobility-group-box protein-2 (Sox2), Myc, and Kruppel-like factor-4 (Klf4) [66–68], although variations on which factors can be used or excluded are being explored [69–71]. Additional methods intended to avoid genomic integration of the expression vectors have been undertaken and include use of adenoviral vectors and plasmid transfection [72, 73]. iPS cell formation suffers from low induction

efficiencies, however. Also, retroviral-based transduction has resulted in tumor formation in chimeric animals [74]. Recently, it was demonstrated that functional cardiomyocytes could be derived from iPS cells [75]. After induced pluripotency, cardiomyocytes were derived through embryoid body formation. iPS cell-derived cardiomyocytes expressed many cardiac markers and displayed cardiomyocyte-specific action potentials.

Despite several obstacles, iPS cells hold potential in providing an autologous cell therapy without the ethical or immunological concerns surrounding the use of ESCs. There are still many questions unanswered concerning iPS cells (mechanism of induced pluripotency, safety, and similarity to ESCs), and thus their potential for myocardial repair has yet to be explored.

In summary, several progenitor cell populations have been used or marked for use for myocardial repair. In most cases, the delivery and engraftment of cells to infarcted myocardium leads to improvements in function after injury. Such improvements seem transient, indicating the goal of myocardial “regeneration” has transitioned into myocardial “preservation” marked by reduced fibrosis, attenuated remodeling, and improved myocardial perfusion. Although several studies have shown viable muscular grafts, the mechanism by which these grafts induce myocardial improvement is still lacking. Given the similarity in outcome with the progenitor cells used for cellular cardiomyoplasty, how do we choose which is the best? Recently, van der Bogt *et al.* reported a direct comparison of how mononuclear cells (fresh unfractionated bone marrow), MSCs, skeletal myoblasts, and fibroblasts acted as mediators for infarct repair [76]. The authors conclude that mononuclear cells provide a superior regime for cardiac repair and preservation compared to the other cell types given their capacity for long survival. Such comparisons between cell types were lacking previously in the field and are likely necessary in the future to determine if an optimal cell type exists. The transient nature of cellular cardiomyoplasty also raises the question as to whether only one cell type is needed or if multiple cell doses would be required to have an extended effect [77, 78]. As discussed below, some of these cell sources have been used in humans, and the results have not always paralleled the preclinical outcomes, raising another level of complexity in choosing the right cell.

TRANSLATION TO MAN

Many phase I human clinical trials indicate that cellular cardiomyoplasty is safe and feasible. Efficacy reports from larger controlled trials reveal transient and somewhat limited effects for primary endpoints (Table 1). Thus far, autologous bone marrow derived stem cells, skeletal myoblast, MSCs, and circulating blood-derived progenitor cells have been used in human clinical trials.

Phase I Clinical Trial Results

Many of the first human clinical trials for cellular cardiomyoplasty were small and non-randomized. In 1999, Hamano and colleagues [79] directly injected bone marrow mononuclear cells (BMMC) into the ischemic area of patients undergoing coronary artery bypass graft (CABG) surgery. Results indicated that three out of five patients obtained increased blood perfusion in the area where the cells were grafted. This increase in blood flow was persistent after a one-year follow-up. Strauer *et al.* [80] demonstrated that intracoronary infusion was a feasible approach for the delivery of autologous BMMC. In this study, cells were injected 5–9 days after percutaneous transluminal coronary angioplasty and resulted in a significant reduction of 18% in end systolic volume and a 12% increase in stroke volume index at a three-month follow-up. This was the first human study to not only consider alternate delivery strategies for cellular cardiomyoplasty but also the time of delivery after acute myocardial infarction (AMI), showing less invasive procedures could be

used to deliver cells. The contribution of time of delivery to improved cardiac function was not tested, but it was thought that waiting until after the inflammatory response has subdued would allow for enhanced cell engraftment. Such an idea has been validated in animal studies [81].

Additional studies followed, including the TOPCARE-AMI (Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction) [82, 83] clinical trial that delivered either circulating blood-derived progenitor cells (CPC) or BMMC to patients diagnosed with acute myocardial infarction (AMI). Cells were delivered an average of 4.3 days after AMI and resulted in reduced end systolic volume and in increased LV ejection fraction, coronary flow reserve, and myocardial viability. Although both cells types gave rise to improved functional outcomes, there were no differences observed between the two cell types used. Given the angiogenic potential of both cell types, the similarity in results seems likely to arise from each cell type's ability to promote neovascularization, endothelial cell migration, and proliferation. This conclusion is similar to many preclinical studies discussed above. Supporting this idea, neovascularization was observed when Stamm and colleagues [84] directly injected BMMC enriched for AC133+ cells into the border zone of infarcted myocardium. After a three-month follow-up, patients receiving AC133+ enriched cell treatment had increased ejection fraction and perfusion in infarcted segments of the heart. Similar results were seen by Tse *et al.* [85], Perin *et al.* [86], and Patel *et al.* [87].

In 2004, the BOOST (Bone Marrow Transfer to Enhance ST-Elevation Infarct Regeneration) clinical trial represented the first randomized, controlled and blinded human clinical trial for cellular cardiomyoplasty [88]. Sixty patients were randomly assigned to a control or BMMC transplant groups. BMMC-treated patients were injected, via intracoronary infusion, five days after percutaneous coronary intervention. After six months, cardiac magnetic resonance imaging revealed BMMC-treated patients had a significant increase in LV ejection fraction from baseline value after infarct and as compared to controls. Additionally, systolic wall motion in the border zone increased over this period. There were no occurrences of major adverse events after infusion of BMMC.

Autologous skeletal myoblasts have also been used in human clinical trials for cellular cardiomyoplasty. In an initial feasibility study, $\sim 871 \times 10^6$ skeletal myoblasts were injected directly into 37 sites in 10 patients undergoing CABG [89]. At an average follow-up of 11 months, most patients had increased ejection fraction and improved systolic thickening, but an increase in arrhythmias was observed in four patients. A similar result was observed when Pagani and colleagues [90] directly injected autologous skeletal myoblasts into five patients undergoing implantation of a left ventricular assist device. Of the five patients treated, four developed cardiac arrhythmias.

Phase II and III Clinical Trials

After the demonstration of feasibility in several smaller human clinical trials, larger, randomized, controlled clinical trials were initiated. In 2005, Assmus and colleagues [91] expanded upon their previous investigation using both autologous BMMC and blood derived progenitor cells. Patients were randomized into control, BMMC and blood progenitor cell groups. Following a three month follow-up, patients were entered into a crossover phase whereby patients initially designated into the BMMC group were given blood progenitors and vice versa. Patients in the control group were randomized into either BMMC or blood progenitor groups at cross over. In general, BMMC performed better than CPCs as demonstrated by significant improvements in LV ejection fraction (4% by MRI) over control at three months. This observation was further confirmed at crossover as patients given BMMC at the three-month follow-up examination also showed increased improvements in LV ejection fraction. Additionally, Schachinger and colleagues [92–94]

demonstrated similar trends of LV ejection fraction (4% over placebo) improvement at four months with intracoronary delivery of autologous BMMC. Interestingly, they found that the degree of improvement was correlated to the time of cell delivery after reperfusion therapy and the extent of impaired cardiac function at enrollment.

Not all larger clinical trials, however, have shown comparable improvements in cardiac function after cell treatment. Lunde and colleagues [95] delivered autologous BMMC via intracoronary injection and showed no change in LV ejection fraction or infarct size versus control at a six month follow-up. This might be ascribed to differences in cell preparation [96] and number of delivered cells in the Autologous Stem Cell Transplantation in Acute Myocardial Infarction (ASTAMI) trial.

The US registry of federally and privately supported clinical trials, Clinicaltrials.gov, reports several ongoing studies aimed at cellular cardiomyoplasty. The majority of these trials use autologous BMMC as the cell source. Several trials, however, are taking new approaches to cardiac cell therapy. For instance, the Combination Stem Cell Therapy for Utilization and Rescue of Infarcted Myocardium (MESENDO) trial is attempting to use an autologous mixture of two cell sources (BMMCs and MSCs in equal proportions); one which would promote neovascularization and the other would promote cardiac remuscularization. In addition, the Study of Allogeneic Mesenchymal Precursor Cells (MPCs) in Subjects with Recent Acute Myocardial Infarction is attempting to determine the suitability of an allogeneic progenitor cell source. This work and others could prove beneficial to optimizing cellular cardiomyoplasty in the future.

In summary, of the two major cell types used in clinical trials, only one has emerged as a viable option. BMMCs appear to have a beneficial effect on myocardial function while the threat of adverse arrhythmias precludes the use of skeletal myoblasts. Unfortunately, the extent of repair with BMMCs appear less robust than that reported in preclinical studies using the same or a similar cell source. The lack of expansion beyond the use of BMMCs in clinical trials has provided a significant roadblock to the progression of cellular cardiomyoplasty. Are there other cells which could supply even greater benefit? The answer is likely to be yes, but there are roadblocks to be addressed Fig. (1).

TRANSLATIONAL ROADBLOCKS

Translational Roadblock #1: No Optimum Cell has been Identified

The progression of clinical cellular cardiomyoplasty appears to be moving forward, but as noted above, the extent of repair in humans is limited when compared to preclinical models. One reason for this is that the cell source pool is limited. Cell sources are constrained by immunological rejection and the need for large numbers. Autologous sources address the issue of rejection but prevent “off-the-shelf” availability of cell therapies for efficient treatment. In addition, patients likely to be treated with autologous cell cardiomyoplasty may be the same ones that have deficiencies in source number or efficacy. Ongoing clinical trials continue to utilize previously suggested cell sources in their experimental design, [97] although the number of cell sources used in preclinical studies is vast. This disconnect will likely have to be addressed.

Translational Roadblock #2: Low Engraftments Rates

The rate of cell engraftment is a factor in determining the initial outcomes and may explain the lack of durable results seen in some studies. Assmus and colleagues and Schachinger and colleagues delivered 200×10^6 and 236×10^6 cells, respectively and observed modest improvements in LV ejection fraction compared to placebo [91, 93]. Lunde and colleagues

however, reported no change in LV ejection fraction with the delivery of 68×10^6 cells [95]. This suggests cell number may play an important role in the repair of myocardial damage.

Engraftment rates can be influenced in a number of ways. One of which is the cell delivery method. Typical approaches to deliver cells include intravenous (IV) injection, intracoronary (IC) injection, retrograde venous intracoronary (RIC) infusion, and intramyocardial (IM) injection. Although IV injection offers the advantage of being minimally invasive, there is low cell engraftment (< 1%) into the injured area [98, 99]. IC injection and RIC infusion provide more localized delivery of cells, resulting in improved but still limited cell engraftment (3–6%) [98, 100]. IM injection offers direct localization of cells to the injured area, but engraftment (6–12%) is limited by leakage out of the injection sites and cellular washout into the native venous shunts [99]. Moreover, this technique results in inhomogeneous cell delivery with emerging cell clusters within the myocardial scar [82]. This could act as a substrate for adverse electrical remodeling [101]. In a study comparing the relative efficiency of cell delivery by intramyocardial (IM), intracoronary (IC), and interstitial retrograde coronary venous (IRV) delivery, each method resulted in only modest engraftment [100]. Specifically, IM injection resulted in 11% engraftment, while IC and IRV injections resulted in 2.6% and 3.2% engraftment, respectively. Similar studies performed by Freyman *et al.* [98] showed that 14 days after IC infusion, engraftment was 6%, and this delivery procedure was also accompanied by reduced coronary blood flow and subsequent myocardial injury. The low engraftment rates reported with conventional delivery strategies may not allow for optimal reparative ability from individual cells. Therefore, methods aimed at improving engraftment may help bring cellular cardiomyoplasty to its full potential.

Translational Roadblock #3: Understanding the Mechanism of Repair

Just how cells might fix hearts is not understood, and this lack of understanding slows the design of future trials. Competing hypotheses include stem cell differentiation into cardiac or vascular cells and the secretion of beneficial trophic factors to modulate endogenous functions.

Do Cardiomyocytes Matter?

ESCs, MSCs, and skeletal myoblasts have been extensively tested *in vivo* for their potential for differentiation into myocytes. For these myocytes to be functional, they would have to be electrically integrated and be able to produce sufficient force to explain the observed preclinical and clinical results. As of yet, it is unclear if any of these cell types meets these criteria.

Early studies involving the injection of undifferentiated ESCs into infarcted hearts reported enhanced cardiac function after cell transplantation with evidence that these cells engraft and differentiate into cardiomyocytes [62]. In addition, there was also evidence of ESC differentiation into vascular smooth muscle and endothelial cells. These cells were also able to attenuate apoptosis and adverse cardiac remodeling [102]. The uncertainty about teratoma formation, however, has led many investigators to initially differentiate ESCs into cardiomyocytes before transplantation. Early investigations by Min *et al.* [103] showed that delivery of a modest number of ESC-derived cardiomyocytes could improve cardiac function after infarction. Engraftment was calculated to be 7.3% after direct injection of cells and was complemented with improvements in ventricular function and myocardial remodeling after six weeks. These results appeared to be maintained out to thirty-two weeks, suggesting a potential long term benefit [104]. Human ESC-derived cardiomyocytes were also shown to engraft into healthy myocardium with no teratoma formation by four weeks [105]. Additionally, the cardiomyogenic grafts exhibited a substantial proliferative capacity

and an ability to interact with host myocardium through the formation of vascular beds. Other studies which delivered human ESC-derived cardiomyocyte grafts into infarcted myocardium accompanied by pro-survival factors reported improved cardiac function and remodeling [63]. Improvements in cardiac function, ventricular wall remodeling, and remuscularization were observed at four weeks after cell delivery. Despite evidence of remuscularization and neovascularization, it is yet to be established that these grafts are functional and contribute to the improvements in cardiac function observed.

A similar circumstance is the case for MSCs. MSCs have also demonstrated cardiomyogenic differentiation potential *in vivo*. Human MSCs injected into healthy murine heart upregulated their expression of cardiac proteins out to 40 days after delivery [37]. Unfortunately, the percent engraftment was extremely low with only 0.44% MSCs detected after only four days. Min *et al.* [42] showed that human MSCs had a beneficial effect on myocardial perfusion in a pig model and that MSCs could differentiate into a cardiac α -myosin heavy chain and troponin I expressing cell phenotype. This effect was enhanced when human MSCs were co-transplanted with fetal cardiomyocytes. Dai *et al.* [9] studied the short and long term effects of MSC therapy on infarcted myocardium. These studies revealed that allogeneic MSCs could differentiate to express cardiac-specific proteins, that MSC therapy resulted in improved cardiac function, but that the effect of MSCs on global cardiac function was transient. Moreover, the number of MSCs expressing cardiac proteins decreased over time. Contrary to these results, Amado *et al.* [106] reported improved cardiac function with reduced scar formation in Yorkshire pigs undergoing myocardial infarction but without MSC differentiation into a cardiac-expressing phenotype. Unfortunately, none of these studies have investigated the function of MSC-derived cardiac gene expressing cells. Thus, even if MSCs can differentiate toward the cardiac lineage, it is unclear that they can make myocytes with sufficient contractile properties to explain the observed effects of cell transplantation.

More evidence that factors other than myocyte differentiation may be important comes from the experiments with skeletal muscle progenitors. The transdifferentiation potential of skeletal myoblasts into cardiomyocytes is unclear. In one study, Reinecke *et al.* reported that skeletal myoblasts are unable to transdifferentiate into cardiomyocytes after delivery to the myocardium [12]. Skeletal myoblasts differentiated into mature skeletal muscle but failed to co-express a cardiomyogenic phenotype. On the other hand, Horackova *et al.* reported that over time engrafted skeletal myoblasts downregulated their expression of skeletal muscle markers and partially transdifferentiated into a cardiac phenotype through the expression of cardiac troponin T in addition to other markers [13]. Additionally, Invernici and colleagues [14] reported that upon treatment with retinoic acid, human skeletal myoblasts would differentiate into spontaneously beating cells which expressed cardiomyogenic markers. These differentiated skeletal myoblast also demonstrated efficacy toward improved cardiac function after they were injected into infarcted myocardium. Since skeletal myoblasts are capable of mediating myocardial improvement but may have a limited ability to differentiate into cardiac myocytes and since they do not electrically couple with native cardiac cells, it seems unlikely that their ability to improve outcomes is the sole result of generation of new, functional cardiac myocytes.

In summary, although many studies have focused on cardiomyocyte differentiation, the function of these myocytes is unclear, and engraftment and differentiation rates seem too low to explain the full effect of exogenous cell transplants. Therefore, it seems likely that, despite the original idea of regenerating myocardium, other mechanisms are at work with current therapeutic strategies.

Trophic Factors – The Paracrine Hypothesis

It now seems possible that the main effect of improved cardiac function after cell delivery results from secreted factors that preserve native cells, induce neovascularization, or attract resident stem cells [107]. *In vitro* studies with MSCs show that they secrete paracrine factors under hypoxic and normoxic conditions. MSC-conditioned media can attenuate fibroblast proliferation [108], induce electrical remodeling of cardiomyocytes [109], stimulate endothelial cell proliferation and activation [110, 111], and inhibit apoptosis [112, 113]. These results provide a basis for possible paracrine mechanisms by which stem cells may repair and/or preserve myocardial function after AMI.

Potential paracrine factors mediating these effects include VEGF, FGF basic, SDF-1 α , IGF-1 and secreted frizzled related protein [114]. Studies using only conditioned media from Akt-MSCs confirmed that paracrine factors can mediate myocardial repair [115]. Pro-survival cocktails have also been used with the intention of prolonging engraftment of progenitor cells in ischemic conditions [63]. Results show improved engraftment and survivability after graft delivery. Additionally, Korf-Klingebiel *et al.* [116] recently described human bone marrow cells as rich sources for pro-angiogenic and cytoprotective factors. This suggests that current clinical trials which have focused on the use of autologous bone marrow progenitor cells may promote myocardial repair via a paracrine pathway. ESC-derived cardiomyocytes have also been shown to secrete beneficial paracrine factors [117].

These studies and others suggest that exogenous cells secrete factors that affect the host tissue. This observation may explain why so many different cell types can mediate repair and why differentiation seems poorly correlated to functional improvement. Also, it would suggest that the field would seem to be at an implementation bifurcation point, having to choose between understanding and refining the paracrine effect with or without cells or moving on to identify cells with more potential to generate cardiac myocytes.

TISSUE ENGINEERING FOR ADDRESSING THE ROADBLOCKS

Tissue engineering may help address the obstacles noted above. Tissue engineering involves the restoration, maintenance, or enhancement of tissue and organ function. Initial tissue engineering treatment options for heart failure involved acellular synthetic materials which surrounded the ventricle to prevent ventricular dilation [118]. Cellularized scaffolds have been constructed as alternative delivery and graft solutions to cardiomyoplasty. Tissue engineered approaches include neonatal rat ventricular cells embedded in gelatin mesh (Gelfoam®) [119] and skeletal myoblasts suspended in fibrin glue [120]. Zimmerman *et al.* [121] have demonstrated electrical integration with host myocardium in addition to improved myocardial performance and remodeling after application of engineered heart tissue (EHT). EHTs are created by combining neonatal cardiomyocytes and collagen and achieved spontaneous contraction while in culture. Currently fibroblasts [122, 123], skeletal myoblasts [120], embryonic stem cells [124, 125], cardiomyocytes [121, 126–131] and BMSCs [132–135] have been used in conjunction with a variety of biomaterials to form “cardiac patches” (Table 2). Some groups have also used acellular biodegradable materials as cardiac grafts and have seen improvements in remodeling and cardiac function [136] in preclinical studies. Recently, we demonstrated that tissue engineered constructs could be used to efficiently deliver MSC to infarcted myocardium. This method of cell delivery leads to engraftment of 23% of transplanted cells, exceeding reported rates for other delivery techniques [137]. Possible explanations for this increased delivery include that exogenously applied cells remained localized to the infarct area increasing opportunities for engraftment. The technique had the further advantage of delivering cells in a relatively homogenous manner as compared to the next most efficacious delivery technique, direct injection. Another benefit of tissue engineered constructs that may prove useful is that materials have

been shown to induce differentiation or modulate cell function [138, 139]. Therefore, tissue engineering is likely to direct progenitor cell fate more efficiently through the combination of biomaterials, bioactive factors, and physical forces. This would provide more controllable methods for optimization of cell source in cellular cardiomyoplasty. Unfortunately, cellularized constructs are restricted in size due to diffusion limitations. In order to sustain cell viability with tissue engineered constructs, methods to induce angiogenesis and cell survival within cardiac patches will need to be explored. Other considerations will involve optimizing construct size and delivery.

Tissue engineering, in conjunction with bioreactors and biomimetics, can be used to expand and prepare cells and tissues and offers the ability to develop and test tissue function *in vitro* in a controlled manner [140]. Bioreactors are culture devices and schemes used for scalable cell and tissue production. Bioreactors can be used to reduce time between cell isolation and cell transplantation and to promote progenitor cell differentiation. Biomimetics is a functional system which serves to mimic a biological process. This allows for an *in vitro* test situation for various strategies. Currently, investigators are taking steps to optimize cardiac tissue formation through the combination of cells, biomaterials, and electrical or mechanical stimulation [141–144]. For instance, cardiac organoids have been formed with the intent to develop a working biological model of the left ventricle. This tissue engineered model was shown to contract, develop a small pressure and even eject fluid. This model was responsive to cryoinjury [143], a technique commonly used in animal models to induce myocardial infarction. Bioreactors and biomimetics may allow researchers to develop systems to rapidly optimize cell bioprocessing and cardiomyoplasty.

The versatility of tissue engineering also extends to making chemical modifications to biomaterials for the attachment of proteins, immunosuppressive, or biochemical agents. Such techniques can provide localized bulk delivery of paracrine factors [145, 146] or other molecules which may directly benefit the myocardium or act to enhance engraftment and reduce rejection after cell transplantation. Local delivery of defined factors may also help elucidate the role of these factors in cardiac repair and help overcome roadblocks in understanding the mechanism by which cellular cardiomyoplasty is effective. The rate of release of these factors can also be modulated to prolong their effects or provide a temporal augmentation to the repair process. Furthermore, by using their inherent mechanical properties, the material may also be tailored to provide mechanical support for the ailing heart either transiently, using biodegradable materials, or permanently in the case of non-degradable substrates. Therefore, tissue engineering may offer higher engraftment rates, improved differentiation, maintenance of progenitor cells, an ability to tailor and sustain the release of various paracrine factors, and assistance in the understanding of cardiac repair via cellular cardiomyoplasty, helping to address the major roadblocks identified above Fig. (2).

SUMMARY

The roadblocks preventing clinical success for cellular cardiomyoplasty have proved more formidable than first expected. Although this therapy is a viable option for the treatment of ischemic cardiomyopathy, there are many questions which still need to be addressed. In particular, issues related to optimizing cell source, understanding the mechanism of repair, and enhancing engraftment need to be optimized before maximally successful transition from the laboratory to the clinic will be possible. The use of autologous cells appears to be the safest route, yet obtaining large numbers of cells needed for transplantation limits the cell source pool. Also, there is a lack of understanding the mechanism by which progenitor cells can repair injured myocardium. Enhancing cell engraftment may also play a critical role in enhancing clinical outcomes. Tissue engineering may offer solutions to current problems by allowing for *ex vivo* cell expansion and differentiation, suitable test models, cell

and paracrine factor delivery systems, and replacement tissue, which may bring us a step closer to making cardiomyoplasty a therapeutic option for heart disease patients.

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REFERENCES

1. American Heart Association. 2008. www.americanheart.org
2. Soonpaa MH, Field LJ. Assessment of cardiomyocyte DNA synthesis in normal and injured adult mouse hearts. *Am J Physiol*. 1997; 272:H220–H226. [PubMed: 9038941]
3. Garrity ER, Moore J, Mulligan MS, Shearon TH, Zucker MJ, Murray S. Heart and lung transplantation in the United States, 1996–2005. *Am J Transplant*. 2007; 7:1390–1403. [PubMed: 17428287]
4. Koh GY, Soonpaa MH, Klug MG, Field LJ. Long-term survival of AT-1 cardiomyocyte grafts in syngeneic myocardium. *Am J Physiol*. 1993; 264:H1727–H1733. [PubMed: 8498586]
5. Li RK, Jia ZQ, Weisel RD, et al. Cardiomyocyte transplantation improves heart function. *Ann Thorac Surg*. 1996; 62:654–660. discussion 60–1. [PubMed: 8783989]
6. Sakai T, Li RK, Weisel RD, et al. Fetal cell transplantation: a comparison of three cell types. *J Thorac Cardiovasc Surg*. 1999; 118:715–724. [PubMed: 10504639]
7. Gupta KB, Ratcliffe MB, Fallert MA, Edmunds LH Jr, Bogen DK. Changes in passive mechanical stiffness of myocardial tissue with aneurysm formation. *Circulation*. 1994; 89:2315–2326. [PubMed: 8181158]
8. Holmes JW, Borg TK, Covell JW. Structure and mechanics of healing myocardial infarcts. *Annu Rev Biomed Eng*. 2005; 7:223–253. [PubMed: 16004571]
9. Dai W, Hale SL, Martin BJ, et al. Allogeneic mesenchymal stem cell transplantation in postinfarcted rat myocardium: short- and long-term effects. *Circulation*. 2005; 112:214–223. [PubMed: 15998673]
10. Zammit PS, Partridge TA, Yablonka-Reuveni Z. The skeletal muscle satellite cell: the stem cell that came in from the cold. *J Histo-chem Cytochem*. 2006; 54:1177–1191.
11. Murry CE, Wiseman RW, Schwartz SM, Hauschka SD. Skeletal myoblast transplantation for repair of myocardial necrosis. *J Clin Invest*. 1996; 98:2512–2523. [PubMed: 8958214]
12. Reinecke H, Poppa V, Murry CE. Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol*. 2002; 34:241–249. [PubMed: 11851363]
13. Horackova M, Arora R, Chen R, et al. Cell transplantation for treatment of acute myocardial infarction: unique capacity for repair by skeletal muscle satellite cells. *Am J Physiol Heart Circ Physiol*. 2004; 287:H1599–H1608. [PubMed: 15165986]
14. Invernici G, Cristini S, Madeddu P, et al. Human adult skeletal muscle stem cells differentiate into cardiomyocyte phenotype *in vitro*. *Exp Cell Res*. 2008; 314(2):366–376. [PubMed: 17888423]
15. Pajcini KV, Pomerantz JH, Alkan O, Doyonnas R, Blau HM. Myoblasts and macrophages share molecular components that contribute to cell-cell fusion. *J Cell Biol*. 2008; 180:1005–1019. [PubMed: 18332221]
16. Horsley V, Pavlath GK. Forming a multinucleated cell: molecules that regulate myoblast fusion. *Cells Tissues Organs*. 2004; 176:67–78. [PubMed: 14745236]
17. Cherqui S, Kurian SM, Schussler O, Hewel JA, Yates JR 3rd, Salomon DR. Isolation and angiogenesis by endothelial progenitors in the fetal liver. *Stem Cells*. 2006; 24:44–54. [PubMed: 16099996]
18. Parise G, McKinnell IW, Rudnicki MA. Muscle satellite cell and atypical myogenic progenitor response following exercise. *Muscle Nerve*. 2008; 37:611–619. [PubMed: 18351585]

19. Oh H, Bradfute SB, Gallardo TD, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci USA*. 2003; 100:12313–12318. [PubMed: 14530411]
20. Matsuura K, Nagai T, Nishigaki N, et al. Adult cardiac Sca-1-positive cells differentiate into beating cardiomyocytes. *J Biol Chem*. 2004; 279:11384–11391. [PubMed: 14702342]
21. Martin CM, Meeson AP, Robertson SM, et al. Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev Biol*. 2004; 265:262–275. [PubMed: 14697368]
22. Hierlihy AM, Seale P, Lobe CG, Rudnicki MA, Megeney LA. The post-natal heart contains a myocardial stem cell population. *FEBS Lett*. 2002; 530:239–243. [PubMed: 12387899]
23. Pfister O, Mouquet F, Jain M, et al. CD31- but Not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. *Circ Res*. 2005; 97:52–61. [PubMed: 15947249]
24. Mouquet F, Pfister O, Jain M, et al. Restoration of cardiac progenitor cells after myocardial infarction by self-proliferation and selective homing of bone marrow-derived stem cells. *Circ Res*. 2005; 97:1090–1092. [PubMed: 16269652]
25. Beltrami AP, Barlucchi L, Torella D, et al. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell*. 2003; 114:763–776. [PubMed: 14505575]
26. Dawn B, Stein AB, Urbanek K, et al. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc Natl Acad Sci USA*. 2005; 102:3766–3771. [PubMed: 15734798]
27. Messina E, De Angelis L, Frati G, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res*. 2004; 95:911–921. [PubMed: 15472116]
28. Barile L, Messina E, Giacomello A, Marban E. Endogenous cardiac stem cells. *Prog Cardiovasc Dis*. 2007; 50:31–48. [PubMed: 17631436]
29. Laugwitz KL, Moretti A, Lam J, et al. Postnatal isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature*. 2005; 433:647–653. [PubMed: 15703750]
30. Pittenger MF, Mackay AM, Beck SC, et al. Multilineage potential of adult human mesenchymal stem cells. *Science*. 1999; 284:143–147. [PubMed: 10102814]
31. CA Dennis JE. Bone Marrow Mesenchymal Stem Cells. *Stem Cells Handbook*. :107–117.
32. Shim WS, Jiang S, Wong P, et al. *Ex vivo* differentiation of human adult bone marrow stem cells into cardiomyocyte-like cells. *Bio-chem Biophys Res Commun*. 2004; 324:481–488.
33. Orlic D, Kajstura J, Chimenti S, Bodine DM, Leri A, Anversa P. Transplanted adult bone marrow cells repair myocardial infarcts in mice. *Ann N Y Acad Sci*. 2001; 938:221–229. discussion 9–30. [PubMed: 11458511]
34. Orlic D, Kajstura J, Chimenti S, et al. Bone marrow cells regenerate infarcted myocardium. *Nature*. 2001; 410:701–705. [PubMed: 11287958]
35. Murry CE, Soonpaa MH, Reinecke H, et al. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. 2004; 428:664–668. [PubMed: 15034593]
36. Balsam LB, Wagers AJ, Christensen JL, Kofidis T, Weissman IL, Robbins RC. Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. *Nature*. 2004; 428:668–673. [PubMed: 15034594]
37. Toma C, Pittenger MF, Cahill KS, Byrne BJ, Kessler PD. Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart. *Circulation*. 2002; 105:93–98. [PubMed: 11772882]
38. Mangi AA, Noiseux N, Kong D, et al. Mesenchymal stem cells modified with Akt prevent remodeling and restore performance of infarcted hearts. *Nat Med*. 2003; 9:1195–1201. [PubMed: 12910262]
39. Jiang W, Ma A, Wang T, et al. Homing and differentiation of mesenchymal stem cells delivered intravenously to ischemic myocardium *in vivo*: a time-series study. *Pflugers Arch*. 2006 Aug 17.
40. Kudo M, Wang Y, Wani MA, Xu M, Ayub A, Ashraf M. Implantation of bone marrow stem cells reduces the infarction and fibrosis in ischemic mouse heart. *J Mol Cell Cardiol*. 2003; 35:1113–1119. [PubMed: 12967634]

41. Tang YL, Zhao Q, Zhang YC, et al. Autologous mesenchymal stem cell transplantation induce VEGF and neovascularization in ischemic myocardium. *Regul Pept.* 2004; 117:3–10. [PubMed: 14687695]
42. Min JY, Sullivan MF, Yang Y, et al. Significant improvement of heart function by cotransplantation of human mesenchymal stem cells and fetal cardiomyocytes in postinfarcted pigs. *Ann Thorac Surg.* 2002; 74:1568–1575. [PubMed: 12440610]
43. Zuk PA, Zhu M, Mizuno H, et al. Multilineage cells from human adipose tissue: implications for cell-based therapies. *Tissue Eng.* 2001; 7:211–228. [PubMed: 11304456]
44. Zuk PA, Zhu M, Ashjian P, et al. Human adipose tissue is a source of multipotent stem cells. *Mol Biol Cell.* 2002; 13:4279–4295. [PubMed: 12475952]
45. Rangappa S, Fen C, Lee EH, Bongso A, Sim EK. Transformation of adult mesenchymal stem cells isolated from the fatty tissue into cardiomyocytes. *Ann Thorac Surg.* 2003; 75:775–779. [PubMed: 12645692]
46. Gaustad KG, Boquest AC, Anderson BE, Gerdes AM, Collas P. Differentiation of human adipose tissue stem cells using extracts of rat cardiomyocytes. *Biochem Biophys Res Commun.* 2004; 314:420–427. [PubMed: 14733922]
47. Planat-Benard V, Menard C, Andre M, et al. Spontaneous cardio-myocyte differentiation from adipose tissue stroma cells. *Circ Res.* 2004; 94:223–229. [PubMed: 14656930]
48. Strem BM, Zhu M, Alfonso Z, et al. Expression of cardiomyocytic markers on adipose tissue-derived cells in a murine model of acute myocardial injury. *Cytotherapy.* 2005; 7:282–291. [PubMed: 16081355]
49. Miranville A, Heeschen C, Sengenes C, Curat CA, Busse R, Bou-loumie A. Improvement of postnatal neovascularization by human adipose tissue-derived stem cells. *Circulation.* 2004; 110:349–355. [PubMed: 15238461]
50. Schenke-Layland K, Strem BM, Jordan MC, et al. Adipose tissue-derived cells improve cardiac function following myocardial infarction. *J Surg Res.* 2009; 153:217–223. [PubMed: 18694573]
51. Rehman J, Traktuev D, Li J, et al. Secretion of angiogenic and antiapoptotic factors by human adipose stromal cells. *Circulation.* 2004; 109:1292–1298. [PubMed: 14993122]
52. De Coppi P, Bartsch G Jr, et al. Isolation of amniotic stem cell lines with potential for therapy. *Nat Biotechnol.* 2007; 25:100–106. [PubMed: 17206138]
53. Sartore S, Lenzi M, Angelini A, et al. Amniotic mesenchymal cells autotransplanted in a porcine model of cardiac ischemia do not differentiate to cardiogenic phenotypes. *Eur J Cardiothorac Surg.* 2005; 28:677–684. [PubMed: 16188450]
54. Chiavegato A, Bollini S, Pozzobon M, et al. Human amniotic fluid-derived stem cells are rejected after transplantation in the myocardium of normal, ischemic, immuno-suppressed or immuno-deficient rat. *J Mol Cell Cardiol.* 2007; 42:746–759. [PubMed: 17300799]
55. Zeng X, Miura T, Luo Y, et al. Properties of pluripotent human embryonic stem cells BG01 and BG02. *Stem Cells.* 2004; 22:292–312. [PubMed: 15153607]
56. Itskovitz-Eldor J, Schuldiner M, Karsenti D, et al. Differentiation of human embryonic stem cells into embryoid bodies compromising the three embryonic germ layers. *Mol Med.* 2000; 6:88–95. [PubMed: 10859025]
57. Thomson JA, Itskovitz-Eldor J, Shapiro SS, et al. Embryonic stem cell lines derived from human blastocysts. *Science.* 1998; 282:1145–1147. [PubMed: 9804556]
58. Mitalipova M, Calhoun J, Shin S, et al. Human embryonic stem cell lines derived from discarded embryos. *Stem Cells.* 2003; 21:521–526. [PubMed: 12968106]
59. Kehat I, Gepstein L. Human embryonic stem cells for myocardial regeneration. *Heart Fail Rev.* 2003; 8:229–236. [PubMed: 12878831]
60. Mummery C, Ward-van Oostwaard D, Doevendans P, et al. Differentiation of human embryonic stem cells to cardiomyocytes: role of coculture with visceral endoderm-like cells. *Circulation.* 2003; 107:2733–2740. [PubMed: 12742992]
61. Mummery C, Ward D, van den Brink CE, et al. Cardiomyocyte differentiation of mouse and human embryonic stem cells. *J Anat.* 2002; 200:233–242. [PubMed: 12033727]

62. Singla DK, Hacker TA, Ma L, et al. Transplantation of embryonic stem cells into the infarcted mouse heart: formation of multiple cell types. *J Mol Cell Cardiol.* 2006; 40:195–200. [PubMed: 16288779]
63. Laflamme MA, Chen KY, Naumova AV, et al. Cardiomyocytes derived from human embryonic stem cells in pro-survival factors enhance function of infarcted rat hearts. *Nat Biotechnol.* 2007; 25:1015–1024. [PubMed: 17721512]
64. Swijnenburg RJ, Tanaka M, Vogel H, et al. Embryonic stem cell immunogenicity increases upon differentiation after transplantation into ischemic myocardium. *Circulation.* 2005; 112:1166–1172. [PubMed: 16159810]
65. Nussbaum J, Minami E, Laflamme MA, et al. Transplantation of undifferentiated murine embryonic stem cells in the heart: teratoma formation and immune response. *FASEB J.* 2007; 21:1345–1357. [PubMed: 17284483]
66. Takahashi K, Tanabe K, Ohnuki M, et al. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. *Cell.* 2007; 131:861–872. [PubMed: 18035408]
67. Takahashi K, Yamanaka S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell.* 2006; 126:663–676. [PubMed: 16904174]
68. Lowry WE, Richter L, Yachechko R, et al. Generation of human induced pluripotent stem cells from dermal fibroblasts. *Proc Natl Acad Sci USA.* 2008; 105:2883–2888. [PubMed: 18287077]
69. Shi Y, Despons C, Do JT, Hahm HS, Scholer HR, Ding S. Induction of pluripotent stem cells from mouse embryonic fibroblasts by Oct4 and Klf4 with small-molecule compounds. *Cell Stem Cell.* 2008; 3:568–574. [PubMed: 18983970]
70. Huangfu D, Osafune K, Maehr R, et al. Induction of pluripotent stem cells from primary human fibroblasts with only Oct4 and Sox2. *Nat Biotechnol.* 2008; 26:1269–1275. [PubMed: 18849973]
71. Nakagawa M, Koyanagi M, Tanabe K, et al. Generation of induced pluripotent stem cells without Myc from mouse and human fibroblasts. *Nat Biotechnol.* 2008; 26:101–106. [PubMed: 18059259]
72. Stadtfeld M, Nagaya M, Utikal J, Weir G, Hochedlinger K. Induced pluripotent stem cells generated without viral integration. *Science.* 2008; 322:945–949. [PubMed: 18818365]
73. Okita K, Nakagawa M, Hyenjong H, Ichisaka T, Yamanaka S. Generation of mouse induced pluripotent stem cells without viral vectors. *Science.* 2008; 322:949–953. [PubMed: 18845712]
74. Okita K, Ichisaka T, Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature.* 2007; 448:313–317. [PubMed: 17554338]
75. Zhang J, Wilson GF, Soerens AG, et al. Functional cardiomyocytes derived from human induced pluripotent stem cells. *Circ Res.* 2009; 104:e30–e41. [PubMed: 19213953]
76. van der Bogt KE, Sheikh AY, Schrepfer S, et al. Comparison of different adult stem cell types for treatment of myocardial ischemia. *Circulation.* 2008; 118:S121–S129. [PubMed: 18824743]
77. Poh KK, Sperry E, Young RG, Freyman T, Barringhaus KG, Thompson CA. Repeated direct endomyocardial transplantation of allogeneic mesenchymal stem cells: safety of a high dose, "off-the-shelf", cellular cardiomyoplasty strategy. *Int J Cardiol.* 2007; 117:360–364. [PubMed: 16889857]
78. Diederichsen AC, Moller JE, Thayssen P, et al. Effect of repeated intracoronary injection of bone marrow cells in patients with ischaemic heart failure the Danish stem cell study--congestive heart failure trial (DanCell-CHF). *Eur J Heart Fail.* 2008; 10:661–667. [PubMed: 18555742]
79. Hamano K, Nishida M, Hirata K, et al. Local implantation of autologous bone marrow cells for therapeutic angiogenesis in patients with ischemic heart disease: clinical trial and preliminary results. *Jpn Circ J.* 2001; 65:845–847. [PubMed: 11548889]
80. Strauer BE, Brehm M, Zeus T, et al. Repair of infarcted myocardium by autologous intracoronary mononuclear bone marrow cell transplantation in humans. *Circulation.* 2002; 106:1913–1918. [PubMed: 12370212]
81. Hu X, Wang J, Chen J, et al. Optimal temporal delivery of bone marrow mesenchymal stem cells in rats with myocardial infarction. *Eur J Cardiothorac Surg.* 2007; 31:438–443. [PubMed: 17239611]
82. Assmus B, Schachinger V, Teupe C, et al. Transplantation of Progenitor Cells and Regeneration Enhancement in Acute Myocardial Infarction (TOPCARE-AMI). *Circulation.* 2002; 106:3009–3017. [PubMed: 12473544]

83. Schachinger V, Assmus B, Britten MB, et al. Transplantation of progenitor cells and regeneration enhancement in acute myocardial infarction: final one-year results of the TOPCARE-AMI Trial. *J Am Coll Cardiol*. 2004; 44:1690–1699. [PubMed: 15489105]
84. Stamm C, Westphal B, Kleine HD, et al. Autologous bone-marrow stem-cell transplantation for myocardial regeneration. *Lancet*. 2003; 361:45–46. [PubMed: 12517467]
85. Tse HF, Kwong YL, Chan JK, Lo G, Ho CL, Lau CP. Angiogenesis in ischaemic myocardium by intramyocardial autologous bone marrow mononuclear cell implantation. *Lancet*. 2003; 361:47–49. [PubMed: 12517468]
86. Perin EC, Dohmann HF, Borojevic R, et al. Transendocardial, autologous bone marrow cell transplantation for severe, chronic ischemic heart failure. *Circulation*. 2003; 107:2294–2302. [PubMed: 12707230]
87. Patel AN, Geffner L, Vina RF, et al. Surgical treatment for congestive heart failure with autologous adult stem cell transplantation: a prospective randomized study. *J Thorac Cardiovasc Surg*. 2005; 130:1631–1638. [PubMed: 16308009]
88. Wollert KC, Meyer GP, Lotz J, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet*. 2004; 364:141–148. [PubMed: 15246726]
89. Menasche P, Hagege AA, Vilquin JT, et al. Autologous skeletal myoblast transplantation for severe postinfarction left ventricular dysfunction. *J Am Coll Cardiol*. 2003; 41:1078–1083. [PubMed: 12679204]
90. Pagani FD, DerSimonian H, Zawadzka A, et al. Autologous skeletal myoblasts transplanted to ischemia-damaged myocardium in humans. Histological analysis of cell survival and differentiation. *J Am Coll Cardiol*. 2003; 41:879–888. [PubMed: 12628737]
91. Assmus B, Honold J, Schachinger V, et al. Transcoronary transplantation of progenitor cells after myocardial infarction. *N Engl J Med*. 2006; 355:1222–1232. [PubMed: 16990385]
92. Marenzi G, Bartorelli AL. Improved clinical outcome after intra-coronary administration of bone marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. *Eur Heart J*. 2007; 28:2172–2173. author reply 3–4. [PubMed: 17675308]
93. Schachinger V, Erbs S, Elsasser A, et al. Intracoronary bone marrow-derived progenitor cells in acute myocardial infarction. *N Engl J Med*. 2006; 355:1210–1221. [PubMed: 16990384]
94. Schachinger V, Erbs S, Elsasser A, et al. Improved clinical outcome after intracoronary administration of bone-marrow-derived progenitor cells in acute myocardial infarction: final 1-year results of the REPAIR-AMI trial. *Eur Heart J*. 2006; 27:2775–2783. [PubMed: 17098754]
95. Lunde K, Solheim S, Aakhus S, et al. Intracoronary injection of mononuclear bone marrow cells in acute myocardial infarction. *N Engl J Med*. 2006; 355:1199–1209. [PubMed: 16990383]
96. Egeland T, Brinchmann JE. The REPAIR-AMI and ASTAMI trials: cell isolation procedures. *Eur Heart J*. 2007; 28:2174–2175. [PubMed: 17673446]
97. NIH ClinicalTrials.gov: 2008. <http://clinicaltrials.gov>
98. Freyman T, Polin G, Osman H, et al. A quantitative, randomized study evaluating three methods of mesenchymal stem cell delivery following myocardial infarction. *Eur Heart J*. 2006; 27:1114–1122. [PubMed: 16510464]
99. Barbash IM, Chouraqui P, Baron J, et al. Systemic delivery of bone marrow-derived mesenchymal stem cells to the infarcted myocardium: feasibility, cell migration, and body distribution. *Circulation*. 2003; 108:863–868. [PubMed: 12900340]
100. Park TH, Nagueh SF, Khoury DS, et al. Impact of myocardial structure and function postinfarction on diastolic strain measurements: implications for assessment of myocardial viability. *Am J Physiol Heart Circ Physiol*. 2006; 290:H724–H731. [PubMed: 16183729]
101. Fukushima S, Coppen SR, Lee J, et al. Choice of cell-delivery route for skeletal myoblast transplantation for treating post-infarction chronic heart failure in rat. *PLoS ONE*. 2008; 3:e3071. [PubMed: 18728781]
102. Singla DK, Lyons GE, Kamp TJ. Transplanted embryonic stem cells following mouse myocardial infarction inhibit apoptosis and cardiac remodeling. *Am J Physiol Heart Circ Physiol*. 2007; 293:H1308–H1314. [PubMed: 17416601]

103. Min JY, Yang Y, Converso KL, et al. Transplantation of embryonic stem cells improves cardiac function in postinfarcted rats. *J Appl Physiol.* 2002; 92:288–296. [PubMed: 11744672]
104. Min JY, Yang Y, Sullivan MF, et al. Long-term improvement of cardiac function in rats after infarction by transplantation of embryonic stem cells. *J Thorac Cardiovasc Surg.* 2003; 125:361–369. [PubMed: 12579106]
105. Laflamme MA, Gold J, Xu C, et al. Formation of human myocardium in the rat heart from human embryonic stem cells. *Am J Pathol.* 2005; 167:663–671. [PubMed: 16127147]
106. Amado LC, Saliaris AP, Schuleri KH, et al. Cardiac repair with intramyocardial injection of allogeneic mesenchymal stem cells after myocardial infarction. *Proc Natl Acad Sci USA.* 2005; 102:11474–11479. [PubMed: 16061805]
107. Tang YL, Zhao Q, Qin X, et al. Paracrine action enhances the effects of autologous mesenchymal stem cell transplantation on vascular regeneration in rat model of myocardial infarction. *Ann Thorac Surg.* 2005; 80:229–236. discussion 36–7. [PubMed: 15975372]
108. Ohnishi S, Sumiyoshi H, Kitamura S, Nagaya N. Mesenchymal stem cells attenuate cardiac fibroblast proliferation and collagen synthesis through paracrine actions. *FEBS Lett.* 2007; 581:3961–3966. [PubMed: 17662720]
109. Chang MG, Tung L, Sekar RB, et al. Proarrhythmic potential of mesenchymal stem cell transplantation revealed in an *in vitro* co-culture model. *Circulation.* 2006; 113:1832–1841. [PubMed: 16606790]
110. Takahashi M, Li TS, Suzuki R, et al. Cytokines produced by bone marrow cells can contribute to functional improvement of the in-farcted heart by protecting cardiomyocytes from ischemic injury. *Am J Physiol Heart Circ Physiol.* 2006; 291:H886–H893. [PubMed: 16603697]
111. Ladage D, Brixius K, Steingen C, et al. Mesenchymal stem cells induce endothelial activation via paracrine mechanisms. *Endothelium.* 2007; 14:53–63.
112. Li W, Ma N, Ong LL, et al. Bcl-2 engineered MSCs inhibited apoptosis and improved heart function. *Stem Cells.* 2007; 25:2118–2127. [PubMed: 17478584]
113. Xu M, Uemura R, Dai Y, Wang Y, Pasha Z, Ashraf M. *In vitro* and *in vivo* effects of bone marrow stem cells on cardiac structure and function. *J Mol Cell Cardiol.* 2007; 42:441–448. [PubMed: 17187821]
114. Mirotsou M, Zhang Z, Deb A, et al. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell-released paracrine factor mediating myocardial survival and repair. *Proc Natl Acad Sci USA.* 2007; 104:1643–1648. [PubMed: 17251350]
115. Gneccchi M, He H, Noiseux N, et al. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. *FASEB J.* 2006; 20:661–669. [PubMed: 16581974]
116. Korf-Klingebiel M, Kempf T, Sauer T, et al. Bone marrow cells are a rich source of growth factors and cytokines: implications for cell therapy trials after myocardial infarction. *Eur Heart J.* 2008; 29:2851–2858. [PubMed: 18953051]
117. Ebelt H, Jungblut M, Zhang Y, et al. Cellular cardiomyoplasty: improvement of left ventricular function correlates with the release of cardioactive cytokines. *Stem Cells.* 2007; 25:236–244. [PubMed: 16973829]
118. Blom AS, Mukherjee R, Pilla JJ, et al. Cardiac support device modifies left ventricular geometry and myocardial structure after myocardial infarction. *Circulation.* 2005; 112:1274–1283. [PubMed: 16129812]
119. Li RK, Jia ZQ, Weisel RD, Mickle DA, Choi A, Yau TM. Survival and function of bioengineered cardiac grafts. *Circulation.* 1999; 100:II63–II69. [PubMed: 10567280]
120. Christman KL, Fok HH, Sievers RE, Fang Q, Lee RJ. Fibrin glue alone and skeletal myoblasts in a fibrin scaffold preserve cardiac function after myocardial infarction. *Tissue Eng.* 2004; 10:403–409. [PubMed: 15165457]
121. Zimmermann WH, Melnychenko I, Wasmeier G, et al. Engineered heart tissue grafts improve systolic and diastolic function in infarcted rat hearts. *Nat Med.* 2006; 12:452–458. [PubMed: 16582915]

122. Kellar RS, Landeen LK, Shepherd BR, Naughton GK, Ratcliffe A, Williams SK. Scaffold-based three-dimensional human fibroblast culture provides a structural matrix that supports angiogenesis in infarcted heart tissue. *Circulation*. 2001; 104:2063–2068. [PubMed: 11673347]
123. Kellar RS, Shepherd BR, Larson DF, Naughton GK, Williams SK. Cardiac patch constructed from human fibroblasts attenuates reduction in cardiac function after acute infarct. *Tissue Eng*. 2005; 11:1678–1687. [PubMed: 16411813]
124. Kofidis T, de Bruin JL, Hoyt G, et al. Myocardial restoration with embryonic stem cell bioartificial tissue transplantation. *J Heart Lung Transplant*. 2005; 24:737–744. [PubMed: 15949735]
125. Kofidis T, de Bruin JL, Hoyt G, et al. Injectable bioartificial myocardial tissue for large-scale intramural cell transfer and functional recovery of injured heart muscle. *J Thorac Cardiovasc Surg*. 2004; 128:571–578. [PubMed: 15457158]
126. Zimmermann WH, Eschenhagen T. Cardiac tissue engineering for replacement therapy. *Heart Fail Rev*. 2003; 8:259–269. [PubMed: 12878835]
127. Carrier RL, Papadaki M, Rupnick M, et al. Cardiac tissue engineering: cell seeding, cultivation parameters, and tissue construct characterization. *Biotechnol Bioeng*. 1999; 64:580–589. [PubMed: 10404238]
128. Shimizu T, Yamato M, Kikuchi A, Okano T. Two-dimensional manipulation of cardiac myocyte sheets utilizing temperature-responsive culture dishes augments the pulsatile amplitude. *Tissue Eng*. 2001; 7:141–151. [PubMed: 11304450]
129. Memon IA, Sawa Y, Fukushima N, et al. Repair of impaired myocardium by means of implantation of engineered autologous myoblast sheets. *J Thorac Cardiovasc Surg*. 2005; 130:1333–1341. [PubMed: 16256786]
130. Leor J, Aboulafia-Etzion S, Dar A, et al. Bioengineered cardiac grafts: A new approach to repair the infarcted myocardium? *Circulation*. 2000; 102:III56–III61. [PubMed: 11082363]
131. Miyagawa S, Sawa Y, Sakakida S, et al. Tissue cardiomyoplasty using bioengineered contractile cardiomyocyte sheets to repair damaged myocardium: their integration with recipient myocardium. *Transplantation*. 2005; 80:1586–1595. [PubMed: 16371930]
132. Liu J, Hu Q, Wang Z, et al. Autologous stem cell transplantation for myocardial repair. *Am J Physiol Heart Circ Physiol*. 2004; 287:H501–H511. [PubMed: 15277194]
133. Miyahara Y, Nagaya N, Kataoka M, et al. Monolayered mesenchymal stem cells repair scarred myocardium after myocardial infarction. *Nat Med*. 2006; 12:459–465. [PubMed: 16582917]
134. Zhang G, Wang X, Wang Z, Zhang J, Suggs L. A PEGylated fibrin patch for mesenchymal stem cell delivery. *Tissue Eng*. 2006; 12:9–19. [PubMed: 16499438]
135. Wei HJ, Chen CH, Lee WY, et al. Bioengineered cardiac patch constructed from multilayered mesenchymal stem cells for myocardial repair. *Biomaterials*. 2008; 29:3547–3556. [PubMed: 18538386]
136. Gaballa MA, Sunkomat JN, Thai H, Morkin E, Ewy G, Goldman S. Grafting an acellular 3-dimensional collagen scaffold onto a non-transmural infarcted myocardium induces neo-angiogenesis and reduces cardiac remodeling. *J Heart Lung Transplant*. 2006; 25:946–954. [PubMed: 16890116]
137. Simpson D, Liu H, Fan TH, Nerem R, Dudley SC Jr. A tissue engineering approach to progenitor cell delivery results in significant cell engraftment and improved myocardial remodeling. *Stem Cells*. 2007; 25:2350–2357. [PubMed: 17525236]
138. Engel E, Martinez E, Mills CA, Funes M, Planell JA, Samitier J. Mesenchymal stem cell differentiation on microstructured poly (methyl methacrylate) substrates. *Ann Anat*. 2009; 191:136–144. [PubMed: 19008086]
139. Xie J, Willerth SM, Li X, et al. The differentiation of embryonic stem cells seeded on electrospun nanofibers into neural lineages. *Biomaterials*. 2009; 30:354–362. [PubMed: 18930315]
140. Grayson WL, Martens TP, Eng GM, Radisic M, Vunjak-Novakovic G. Biomimetic approach to tissue engineering. *Semin Cell Dev Biol*. 2008 [Epub ahead of print].
141. Radisic M, Park H, Gerecht S, Cannizzaro C, Langer R, Vunjak-Novakovic G. Biomimetic approach to cardiac tissue engineering. *Philos Trans R Soc Lond B Biol Sci*. 2007; 362:1357–1368. [PubMed: 17594967]

142. Radisic M, Park H, Shing H, et al. Functional assembly of engineered myocardium by electrical stimulation of cardiac myocytes cultured on scaffolds. *Proc Natl Acad Sci USA*. 2004; 101:18129–18134. [PubMed: 15604141]
143. Lee EJ, Kim do E, Azeloglu EU, Costa KD. Engineered cardiac organoid chambers: toward a functional biological model ventricle. *Tissue Eng Part A*. 2008; 14:215–225. [PubMed: 18333774]
144. Zimmermann WH, Schneiderbanger K, Schubert P, et al. Tissue engineering of a differentiated cardiac muscle construct. *Circ Res*. 2002; 90:223–230. [PubMed: 11834716]
145. Davis ME, Hsieh PC, Takahashi T, et al. Local myocardial insulinlike growth factor 1 (IGF-1) delivery with biotinylated peptide nanofibers improves cell therapy for myocardial infarction. *Proc Natl Acad Sci USA*. 2006; 103:8155–8160. [PubMed: 16698918]
146. Kutschka I, Chen IY, Kofidis T, et al. Collagen matrices enhance survival of transplanted cardiomyoblasts and contribute to functional improvement of ischemic rat hearts. *Circulation*. 2006; 114:1167–1173. [PubMed: 16820568]

Fig. (1). Translational roadblocks which are preventing the successful application of cellular cardiomyoplasty in the clinic include determining an optimal cell source, increasing engraftment rates, and understanding the mechanism of stem cell repair.

Fig. (2).

Tissue engineering principles are likely to help overcome roadblocks that are slowing the progress of cellular cardiomyoplasty. Bioreactors and biomimetics can be used to create and test functional tissue engineered constructs. These constructs can act to modulate cell function and enhance delivery of cells or paracrine factors.

Table 1

Major Human Clinical Studies Involving Stem Cell Therapy for Heart Failure

Study	Primary End Point	Cells Used	Randomized Controlled	# Cells	# Patients	Time After PCI	Delivery	Results
Hamono <i>et al</i> [79]	Myocardial Perfusion	BMMC	N	50×10 ⁶ – 100×10 ⁶	5		Direct injection	60% Efficacy
Strauer <i>et al</i> [80]	LVEF %	BMMC	N	9×10 ⁶ – 28×10 ⁶	20	5–9d	Intracoronary (IC)	Increased LVEF (not significant), decreased ESV
Assmus <i>et al</i> [82]	LVEF %	CPC / BMMC	N	10×10 ⁶	20	4.3±1.5d	IC	Increased LVEF%, ESV and myocardial viability. No difference between CPC and BMMC
Stamm <i>et al</i> [84]	LVEF% and Perfusion	AC133+ BMMC	N	--	6		Direct injection	Increased LVEF% and blood perfusion through heart
Menasche <i>et al</i> [89]	LVEF %	Skeletal Myoblast	N	500–1150	10		Direct injection	Increased LVEF% and systolic thickening. Increased risk of arrhythmia
Tse <i>et al</i> [85]	LVEF %	BMMC	N	--			Trans-Endocardial injection	No significant increase in LVEF%, increased wall thickening and motion
Patel <i>et al</i> [87]	LVEF %	CD34+ BMMC	N	--	20		Direct injection	Increased LVEF% and LVEDV
Wollert <i>et al</i> [88]	LVEF %	BMMC	Y	--	60		IC	Increased LVEF% and decreased infarct size
Perin <i>et al</i> [86]	LVEF %	BMMC	N	25.5×10 ⁶	14		Trans-Endocardial injection	Increased LVEF% and decreased ESV. Results decline by 4 months
Assmus <i>et al</i> [91]	LVEF%	BMMC	Y	200×10 ⁶	92		IC	Increased LVEF%
Lunde <i>et al</i> [95]	LVEF%	BMMC	Y	68×10 ⁶	100	4–8d	IC	No Change in LVEF % or infarct size
Schachinger <i>et al</i> [93]	LVEF%	BMMC	Y	236×10 ⁶	204	3–7d	IC	Increased LVEF%, ESV. The more time after PCI the better the result

ESV - End systolic volume, LVEDV – Left ventricular end diastolic volume, PCI – Percutaneous coronary intervention, LVEF – Left ventricular ejection fraction, CPC – Circulating blood-derived progenitor cell, BMMC – Bone marrow mononuclear cell

Table 2

Major Preclinical Studies Involving Tissue Engineering for Myocardial Repair

Study	Cell Type (Seeding Density)	Construct Type	Animal Model	Immune Status	Time of Measurements	Results of Outcome Measures	TE Controls
Christman <i>et al</i> [120]	Skeletal Myoblast (5×10^6 / construct)	Fibrin Glue	Female SD Rats	IC	ECHO 1 weeks and 5 weeks Histology 5 weeks	Improved FS% and LV AWTh	Fibrin Glue only
Li <i>et al</i> [119]	Rat Fetal Ventricular Cells (4×10^7 / mL)	Gelatin	Male Lewis Rats	IC	5 weeks	Little effect on cardiac function, formed junctions with host myocardium	Acellular Gelatin
Zimmerman <i>et al</i> [121]	Rat Neonatal Heart Cells (2.5×10^6 / construct)	Collagen Type I	Male Wistar Rats	IS	4 weeks <i>in vivo</i> histology 2 weeks all other measures	Electrical integration with host myocardium, improved LVDD, LVEDD, FS%, max LV volume, tau (relaxation index) LVEDP and LVEDV	Formaldehyde fixed, Non-cardiomyocyte construct
Leor <i>et al</i> [130]	Rat Fetal Heart Cells (3×10^5 / construct)	Alginate	Female SD Rats	IC	5-7 days after MI and 65 days after implantation	Improvement in FS%, LVIDs and LVIDd	None
Kellar <i>et al</i> [122, 123]	Human Dermal Fibroblast (N/A)	Vieryl Mesh	Female Mice	SCID	2 weeks	Increased overall survival, increased microvessel formation, improved EF, preload recruitable stroke work, and volume at end-systole	Non-viable construct
Miyagawa <i>et al</i> [131]	Rat Neonatal Cardiomyocytes (1×10^6 / sheet)	N/A	Male Lewis Rats	IC	ECHO 2, 4, 8 weeks; Histology 2, 8 weeks, Electrophysiology	Improved LV AWTh, vessel density, FS%, EF electrical communication with host myocardium	Fibroblast Sheet, collagen membrane
Kofidis <i>et al</i> [124]	Mouse Embryonic Stem Cells (2.5×10^6 / mL)	Collagen Type I	Rat	Athymic Nude	2 weeks	Improved LV AWTh and FS%	Acellular Collagen
Kofidis <i>et al</i> [125]	Mouse Embryonic Stem Cells (1×10^6 / 50uL)	Matrigel	BALB/c Mice	IC	2 weeks after in situ injection	Increased graft/scar ratio, improved FS%	Matrigel only
Miyahara <i>et al</i> [133]	Rat Mesenchymal Stem Cells (5×10^5 / sheet)	N/A	Male SD Rats	IC	ECHO, Hemodynamics 4 and 8 weeks; Histology 1-4 weeks	MSC differentiation within host, improved LVDD, FS%, LV AWTh, +/- dP/dt, and LVEDP	Fibroblast sheet
Gaballa <i>et al</i> [136]	N/A	Collagen Foam	Male Fischer Rats	IC	6 weeks	No change in Hemodynamics, increase vascular density, improved cardiac remodeling	None
Simpson <i>et al</i> [137]	Human Mesenchymal Stem Cells (1×10^6)	Collagen Type I	Male CDF Rats	IC	4 weeks	Decreased adverse myocardial remodeling, increased myofibroblast presence	Non-viable construct

Study	Cell Type (Seeding Density)	Construct Type	Animal Model	Immune Status	Time of Measurements	Results of Outcome Measures	TE Controls
Wei <i>et al</i> [135]	Rat Mesenchymal Stem Cells (1.5 × 10 ⁶ /sheets)	Acellular bovine pericardia	Lewis Rats	IC	12 weeks	Improved FS%, LVEDP and LVESP, increased neovessel formation	None

FS% - Percent fractional shortening, AWTh - Anterior wall thickness, LVDD - Left ventricular diastolic diameter, LVESP - Left ventricular end systolic pressure, LVEDP - Left ventricular end diastolic pressure, LVEDV - Left ventricular end diastolic volume, IC - Immuno-competent, IS - Immuno-suppressed, ECHO - Echocardiography, LVIdd - Left ventricular internal diameter at diastole, LVIDs - Left ventricular internal diameter at systole, SCID - Severe combined immunodeficiency, dP/dt - Change in pressure over time