HHS Public Access

Author manuscript

Cardiovasc Pathol. Author manuscript; available in PMC 2016 May 01.

Published in final edited form as:

Cardiovasc Pathol. 2015 ; 24(3): 133–140. doi:10.1016/j.carpath.2015.02.004.

The winding road to regenerating the human heart

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Abstract

Regenerating the human heart is a challenge that has engaged researchers and clinicians around the globe for nearly a century. From the repair of the first septal defect in 1953, followed by the first successful heart transplant in 1967, and later to the first infusion of bone-marrow derived cells to the human myocardium in 2002, significant progress has been made in heart repair. Yet, chronic heart failure remains a leading pathological burden worldwide. Why has regenerating the human heart been such a challenge, and how close are we to achieving clinically relevant regeneration? Exciting progress has been made to establish cell transplantation techniques in recent years, and new pre-clinical studies in large animal models have shed light on the promises and challenges that lie ahead. In this review, we will discuss the history of cell therapy approaches and provide an overview of clinical trials using cell transplantation for heart regeneration. Focusing on the delivery of human stem cell-derived cardiomyocytes, current experimental strategies in the field will be discussed as well as their clinical translation potential. Although the human heart has not been regenerated yet, decades of experimental progress have guided us onto a promising pathway.

Summary—Exciting progress has been made in recent years to establish clinical cell transplantation techniques, and new pre-clinical studies in large animal models have shed light on the promises and challenges that lie ahead. Although the human heart has not been regenerated yet, decades of experimental progress in pre-clinical and clinical trials have guided us onto a promising pathway.

Keywords

myocardial infarction; heart regeneration; cell transplantation; stem cell-derived cardiomyocytes

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1.0 Introduction

A myocardial infarction transforms healthy and contractile myocardium into an akinetic, fibrotic tissue, resulting in a heart that cannot pump blood at full capacity. As the heart is one of the least regenerative organs in the body, this often leads to the development of chronic heart failure – a disease with a 50% survival rate over 5 years [1]. Current treatment options are limited and consist primarily of palliative drugs, organ replacement by heart transplant (available to < 0.1% of heart failure patients), or mechanical assist devices (with complications related to infection, thrombosis, and power supply). While these available treatments have greatly impacted the trajectory of patient health after a myocardial infarction, ischemic heart disease remains the number one cause of death and disability worldwide [2].

In recent years, the field of heart regeneration has emerged from a far-fetched notion to the forefront of cardiac research. Heart regeneration is an interdisciplinary field with the goal of restoring functional myocardium after cardiac injury [3]. Approaches to repair the injured heart have been widespread and include cell transplantation, gene therapy, stimulating innate repair pathways, direct cellular reprogramming, cardiac tissue engineering, and biomaterial delivery. The most established strategy for heart repair has been the delivery of exogenous cells. Nearly every cell type imaginable has been transplanted into the damaged myocardium, from skeletal myoblasts to pluripotent stem cells and their derivatives. It is an exciting but challenging time for physicians, scientists, and engineers in the field – we now have over a decade of experience in clinical trials contributing to heart regeneration research, and there are several promising pre-clinical strategies emerging as contenders to our current clinical approaches.

In this review, we provide an overview of the clinical trial progression using cell therapy to regenerate the heart after ischemic injury, and discuss strengths and limitations of these trials. We will then discuss current experimental strategies designed to improve upon what we have learned in these clinical trials, focusing on the advancements in stem cell-derived cardiomyocyte transplantation and the clinical translatability of this approach for heart repair.

2.0 Cell Therapy Clinical Trials for Heart Repair

Approximately 1 billion cardiomyocytes are lost during a myocardial infarction (MI) [3]. As the adult human heart has an extremely limited regenerative capacity, this damaged myocardial tissue is replaced by fibrotic scar. There is increasing evidence of the slow cardiomyocyte turnover rate during normal organ growth and development (reviewed in [4]), and following myocardial injury [5], however this turnover accounts for a low percentage of cells. Up to 3% of pre-existing cardiomyocytes near the injury region undergo cell division while most DNA replication occurs without cytokinesis as a hypertrophic response, and there is minimal contribution from progenitor cells [5]. As a result, the innate generation of *de novo* cardiomyocytes post-infarction falls orders of magnitude short of meaningful regeneration.

Exogenous cell transplantation aims to repair damaged myocardial tissue by delivering cells that either act via paracrine-mediated effects or by providing *de novo* cardiomyocytes that directly contribute to force production. Towards this goal, numerous clinical trials have been conducted using cell types including skeletal myoblasts, bone marrow-derived hematopoietic cells, mesenchymal stem cells (aka marrow stromal cells), adipose-derived cells, endothelial progenitor cells, and cardiac-derived cells (reviewed in [6-9]). A schematic overview of the derivation, delivery mode, and proposed mechanism of action for the major groups of cell therapies is provided in Figure 1. An ideal cell type for replacing damaged myocardial tissue would have contractile and electrophysiological properties, the ability to survive and integrate into an ischemic area, proliferation potential, and the ability to elicit a paracrine effect to stimulate endogenous regeneration (e.g. vascularization; discussed in detail in [9, 10]). Despite the plethora of cell types tested in clinical trials to date, none have met all of these expectations. The type of cell used for transplantation inherently places restrictions on important variables that may affect the success of cell therapy, making it difficult to directly compare results across trials. These include the delivery mode (intracoronary catheter, transendocardial catheter, or epicardial catheter delivery compared to epicardial delivery in tissue patches or hydrogels), the availability of autologous or allogenic cells, and the timing of cell delivery dependent on the need for *in vitro* cell expansion (i.e. mesenchymal stem cells require extensive *in vitro* expansion, while unfractionated bone marrow cells may be delivered the same day of isolation).

The field has made tremendous progress in terms of establishing clinical trial design, delivery techniques, and demonstrating safety, however the clinical benefits have been modest at best. This indicates that there is room for improvement on our cell source. The two major cell sources used in the clinics thus far have been bone marrow-derived cells and cardiac explant-derived cells, which are discussed below.

2.1 Bone Marrow-Derived Cells

Following closely behind the first major wave of clinical trials in the field using skeletal myoblasts [11], bone marrow-derived cells paved the way for intracoronary cell therapy in the heart, transitioning quickly into the clinic despite the scarcity of published evidence supporting their role in heart regeneration at the time [12, 13].

2.1.1 Bone Marrow-Derived Mononuclear Cell Derivatives—Most bone marrow-derived cell transplantation trials in the heart have used an unfractionated subpopulation called bone marrow mononuclear cells (BMMNCs) (reviewed in [14]). Referring to BMMNCs as a stem cell preparation is a misnomer, because true stem cells comprise well below 0.1% of the total mononuclear cell population. Unfractionated BMMNCs principally consist of a heterogeneous population of hematopoietic cells including monocytes, committed myeloid progenitor cells and lymphocytes, and a small population of hematopoietic and mesenchymal stem cells [9, 15].

Intracoronary transplantation of BMMNCs into patients with acute MI was first reported in 2002 [13], and while this trial has been discredited for ethics violations, it was followed by a flurry of more rigorously performed studies. Most of these early BMMNC studies enrolled

acute-MI patients with ST-segment elevation and a baseline ejection fraction of 40-50%, and reported functional improvement after treatment. One such study was the BOOST trial [16], in which autologous BMMNCs (characterized as <1% $CD34^+$) were isolated from patients and delivered by intracoronary infusion to the infarct-related artery the same day. No serious adverse events were reported in either group, and cardiac MRI at 6 months indicated a significant increase in left ventricular (LV) ejection fraction after cell treatment (compared to placebo control), providing evidence that intracoronary infusion of BMMNCs improves systolic function in acute MI patients. In longer term follow up studies, however, the control group showed a "catch-up" period of recovery, such that benefits of BMMNCs could no longer be demonstrated [17]. Results from the REPAIR-AMI trial [18] further supported efficacy for BMMNCs, reporting a 5.5% increase in LV ejection fraction at 4 months after intracoronary infusion of BMMNCs compared to a 3.0% improvement in controls. While the results of this study were hindered by the use of quantitative LV angiography to assess function as opposed to cardiac MRI, the enrollment of over 200 patients made this the largest BMMNC trial at the time and set the standard for expected systolic improvement, albeit a modest increase, after cell therapy. The same group reported that functional improvement persists up to 5 years post-treatment in a subset of patients who were enrolled in the TOP-CARE-AMI trial [19-21], which compared the benefits of BMMNCs to those of autologous circulating progenitor cells isolated from venous blood.

Despite these and other studies reporting functional improvement after BMMNC treatment (reviewed in [22]), larger trials employing greater degrees of randomization, placebo controls, and blinding conducted in the years following have not replicated these results. The Cardiovascular Cell Therapy Research Network (CCTRN) was designed to facilitate cell-based therapies in the United States [23] and sponsored the FOCUS-CCTRN trial [24], which was one of the first trials to target patients with chronic LV dysfunction who had not qualified for revascularization therapy post-MI. Enrolled patients had a mean baseline ejection fraction of 30-32% and New York Heart Association (NYHA) class of 2 or 3, and while there was no improvement in the primary endpoints of LV end systolic volume (LVSEV) or maximal oxygen consumption, there was a small yet statistically significant 1.4% improvement in LV ejection fraction over baseline at 6 months. The CCTRN also sponsored the TIME and LateTIME trials to assess the influence of BMMNC delivery timing on LV function [25-27]. Each of these double-blinded and placebo-controlled trials enrolled successfully reperfused MI patients and delivered 150×10^6 autologous BMMNCs by intracoronary perfusion either at day 3 or 7 (TIME) or at 2-3 weeks (LateTIME) after MI. Neither study detected any functional benefit by cardiac MRI at 6 months after cell treatment, regardless of delivery timing. Similar in cell dose and design, the SWISS-AMI study [28] compared BMMNC delivery at days 5-7 to delivery at weeks 3-4 after post-MI reperfusion, and again detected no improvement in LV ejection fraction at 4 months. Collectively, these studies challenge the earlier reports of functional improvement, but they differ in using a double-blinded study design and in targeting patients with significantly worse baseline cardiac function (for example, the median ejection fraction of SWISS-AMI patients was 37%). It seems unlikely to us that this difference in baseline cardiac function underlies the difference, however, because the REPAIR-AMI trial found that the patients with the worst ejection fractions showed the greatest improvement with treatment. Results

are eagerly awaited from the 3,000 patient enrollment, multicenter phase 3 trial (the BAMI trial), which is currently underway in Europe, as results will help clear up some of the conflicting results in the field (clinical trial identifier NCT01569178 [29]).

2.1.2 Mesenchymal Stem Cells—Numerous trials have been conducted using mesenchymal stem cells (MSCs) purified from bone marrow, which are adult cells characterized for their osteogenic, chondrogenic, and adiopogenic differentiation potential [30, 31]. Less than 0.01% of the cells isolated from bone marrow are considered MSCs [32, 33], therefore obtaining clinically-relevant cell numbers requires *ex vivo* expansion.

The first clinical trial investigating the intracoronary injection of MSCs reported an improvement in LV ejection fraction and increased myocardial perfusion 3 months after treatment [33], echoing the results reported using BMMNCs at the time. A few studies have directly compared the safety profile and efficacy of BMMNCs to MSCs, including the TAC-HFT trial [34]. In this study, chronic MI patients received a transendocardial injection of BMMNCs (harvested the day of implant) or MSCs (expanded 4-6 weeks *in vitro* prior to implantation). While there was no difference between groups in the 1-year serious adverse event rate or LV ejection fraction, patients receiving MSCs showed increased regional function by strain analysis and an improvement in exercise capacity. These studies used autologous MSCs; however a limitation of this approach is that autologous MSCs require a significant expansion period between the time of bone marrow aspiration and implantation. The POSEIDON trial was designed to address this and to directly compare the safety and efficacy of autologous MSCs to allogeneic MSCs [35]. Chronic MI patients received a dose of 20, 100, or 200 million autologous or allogeneic MSCs, injected into the myocardium via a transendocardial catheter, and the study concluded that neither cell source stimulated a significant adverse immune response. Curiously, there was an inverse dose response in terms of improved ejection fraction and reversed LV remodeling, with more improvement with the 20 million cell dose than the 200 million cell dose.

Up until this point, studies focused their efforts on testing cells in their native MSC state, but the C-CURE trial took a unique approach by treating autologous MSCs with a cytokine cocktail prior to transplantation [36]. Guided by NOGA electromechanical mapping, these cytokine-stimulated MSCs were transplanted transendocardially into chronic heart failure patients an average of 1540 days post-MI. Cell-treated patients showed an absolute improvement of 7% in their ejection fraction and enhanced exercise capacity, compared to no improvement in controls. This is a surprisingly large treatment effect, given the long duration post-infarction in these patients. As previously reviewed [37], most MSC studies have demonstrated that the cells die off within a week or two post-transplantation with little direct cardiac differentiation. Mechanisms of benefit in this trial could not be determined, but animal studies suggest it is likely a paracrine action.

2.1.3 Comments on Bone Marrow-Derived Cell Therapy—Through the successful completion of numerous Phase I clinical trials, bone-marrow derived cell therapies have established an important feasibility and safety baseline for delivering cells into the myocardium [14, 38]. While there have been a few reports of significant functional improvement, for the most part these therapies have resulted in a modest reduction in scar

size after infarction with little (at best) improvement in systolic function. Because the majority of transplanted cells die off within a few weeks [39] and there is no solid evidence of cardiogenic potential, all benefits are believed to be paracrine-mediated (Fig. 1B) [3]. Therapies using BMMNCs and MSCs suggest that intervention by cell therapy can change the trajectory of wound healing and the inflammatory response after an infarction, but with no long-term improvement in global heart function or long-term engraftment, these therapies are not truly regenerating the heart.

2.2 Cardiac-Derived Cells

The most recent addition to the clinical trials has been cardiac-derived cells (CDCs), which are derived from myocardial biopsies and grown as explants in culture to obtain an autologous, cardiac-derived cell population. Studies in rodents have supported their potential to be a more effective cell source than BMMNCs and MSCs [40], and CDCs were originally postulated to be progenitor cells capable of forming new cardiomyocytes. However, most investigators now think that these cells, like bone marrow cells, show minimal long term engraftment or cardiac differentiation and instead work principally through paracrine signaling pathways. The three leading trials using cardiac-derived cells to date are described below.

2.2.1 The SCIPO Trial—The first trial using cardiac-derived cells focused on cells expressing the surface antigen c-kit, which were first isolated and characterized in the rat [41]. Similar to bone marrow-derived cells, initial animal studies suggested that c-kit$^+$ cells gave rise to cardiomyocytes, however lineage tracing studies have determined these cells show minimal long-term engraftment and only extremely low rates of cardiac differentiation in the adult heart [42-44]. The SCIPIO (cardiac Stem Cell Infusion in Patients with Ischemic CardiOmyopathy) trial enrolled 33 heart failure patients with chronic MI (mean ejection fraction of 27.5% at baseline), who underwent a right atrial appendage biopsy during coronary bypass surgery. This atrial tissue was used to isolate a putative cardiac progenitor cell that expressed the surface antigen c-kit and was negative for hematopoietic lineage markers. After four months of *in vitro* expansion, 0.5-1 million cells were injected via the coronary arteries perfusing the ischemic myocardium of 20 patients, while 13 patients remained as controls. Analysis of heart function by 3D echocardiography or cardiac MRI showed an 8.2% and a 12.3% improvement in LV ejection fraction at 4 and 12 months respectively, and, somewhat surprisingly, a reduction in infarct size in a subset of patients [45]. Readers should know, however, that as of this writing, results from this study have been flagged with an “expression of concern” by the editors of the Lancet relating to an ongoing investigation pertaining to data integrity [46].

2.2.2 The CADUCEUS Trial—The next trial of cardiac-derived cells involved “cardiosphere-derived cells”, a mesenchymal cell population obtained by explant culture of endomyocardial biopsies, followed by transient growth as cellular spheroids [47]. Cardiosphere-derived cells are heterogeneous by surface markers, but are primarily CD105$^+$/CD45$^-$. In the Phase I CADUCEUS trial (CArdiosphere-Derived aUtologous stem CElls to reverse ventricULar dySfunction) [48, 49], patients with a mean LV ejection fraction of 39% undergoing primary angioplasty 2-4 weeks after MI had a right ventricular biopsy removed

to expand autologous cells. After a 1-3 month expansion period, 25 million cardiosphere-derived cells were delivered as an intracoronary infusion into the infarct-related artery. Although the primary endpoint was safety, cardiac MRI at 6 and 12 months after cell delivery revealed a reduction in infarct size (identified as a reduced region of delayed gadolinium enhancement) and an increase in viable myocardium. Although there was no significant change in global ejection fraction, cell-treated patients showed improved regional systolic wall thickening that was maintained from 4 months to 1 year after treatment. The authors interpreted the increase in viable myocardium seen by MRI as regeneration, but pathological hypertrophy of pre-existing cardiomyocytes cannot be ruled out as an alternate explanation. Although not statistically significant, cell-treated patients experienced higher levels of non-sustained ventricular tachycardia and serious adverse events at the 1-year follow-up, which will require closer evaluation in future trials.

2.2.3 The ALCADIA Trial—The ALCADIA (AutoLogous human CArdiac-Derived stem cell to treat Ischemic cArdiomyopathy) trial is ongoing at the time of this review (clinical trial identifier NCT00981006), and takes a combined cell therapy and controlled growth factor-release approach that was first established in a porcine model of chronic MI [50]. The trial enrolled advanced heart failure patients with LV ejection fraction of 15-45% and NYHA class of 3 or 4, with primary endpoints of 1-year safety and secondary endpoints of assessing functional improvement by echocardiography and MRI, NYHA class, and exercise capacity at 6 months. At the time of coronary artery bypass grafting, patients received a transepicardial injection of autologous $CD105^{+}CD90^{+}$ cardiac-derived cells grown from an endocardial biopsy (0.5 million cells/kg patient body weight). Injection sites were subsequently covered with a biodegradable gelatin sheet that was loaded with basic fibroblast growth factor (bFGF) by incubation with bFGF prior to implantation. Although there were only six patients enrolled and no controls at the time, preliminary reports suggest an increase in ejection fraction, a decrease in infarct size, and an increase in patient aerobic exercise capacity [51]. If successful in larger-enrollment trials, this dual cell delivery and biomaterials method may promote a shift in clinical approaches in the future towards the combined use of cell and drug delivery, and is a progressive approach that deserves more attention in the pre-clinical and clinical setting. Of course, sorting the effects of the cells from the growth factor delivery will require additional control groups where one of the combined factors is omitted.

2.2.4 Comments on Cardiac-Derived Cell Therapy—Taken together, the achievements made with cardiac-derived cells support some advantages over previous bone marrow-derived cell therapies. The need for *ex vivo* cell expansion of CDCs has provided insight to a later post-MI delivery timeline, and it is promising that cell delivery into mature infarct scars 1-4 months post-MI has resulted in detectable improvements in clinical endpoints (primarily a reduction in infarct size). How such a reduction in scar volume is achieved remains mysterious, because scar size is typically quite stable by 3 months post-MI. We speculate that the cells may reactivate innate immune mechanisms, particularly related to the macrophage. It is important to note that long-term cell retention is almost nil with both CDCs and BMMNCs, so any benefits must require only the transient presence of the cell. Although double-blinded studies with cardiac-derived cells have been precluded by

the need for myocardial biopsy, such trials in bone marrow-derived cell therapy have demonstrated the importance of using proper controls, and this will be necessary in moving forward with larger-scale trials. Since some groups are now moving toward allogeneic CDCs, it should be feasible to have placebo-controlled trials and to test these cells in acute MI patients [52].

3.0 Pluripotent Stem Cell-Derived Cardiomyocyte Delivery

Considering the modest benefits from heart regeneration clinical trials to date, there has been some debate over cell source – is there a more potent cell type to use for transplantation into the heart? D*e novo* cardiomyocytes meet many of the desired characteristics outlined earlier, but finding a reliable cell source for cardiomyocytes was precluded until the last decade. Methods to derive cardiomyocytes from human pluripotent stem cells (hPSCs) have progressed tremendously since the first report of mouse embryonic stem cell (ESC)-cardiomyocyte derivation [53], and there are now several efficient protocols to achieve cardiac differentiation that mimic developmental pathways (reviewed in [54]). These differentiation advancements have brought hPSC-cardiomyocytes to the forefront as a promising next-generation cell source, and their transplantation into the heart has been studied extensively in pre-clinical experiments. The leading strategy for cell delivery has been the intramyocardial injection of dispersed cardiomyocytes, which mirrors the delivery methods established in many cardiac- and bone marrow-derived cell transplantation clinical trials. Using this approach, various groups have demonstrated that hPSC-cardiomyocytes engraft in the infarct region of numerous animal models and result in an increase in cardiac function (reviewed in [55]).

In contrast to bone marrow derivatives and cardiac-derived cells, human cardiomyocytes give stable, long-term grafts in infarcted hearts [56]. Inherent cell properties give transplanted cardiomyocytes the capability to electrically integrate with the host tissue, which is a prerequisite for synchronous contraction with the host myocardium. The fluorescent calcium reporter protein GCaMP3 [57] has been a useful tool to study the gap junction coupling between graft and host tissue, and genetically modified human embryonic stem cell (hESC)-cardiomyocytes expressing GCaMP3 have been found to electrically integrate with ischemia/reperfusion injured rat hearts (Coulombe and Gerbin et al, in revision) as well as in cryoinjured guinea pig hearts [58, 59]. Unlike previous cell transplantation studies that are paracrine-driven, this electrical coupling indicates that the engrafted cardiomyocytes are electrically integrating with the host myocardium and are directly contributing to force generation (Fig. 1B). Indeed, improvements in systolic function have been reported in various injury models after hPSC-cardiomyocyte transplantation ([60-62]).

Successful studies demonstrating long-term cardiomyocyte engraftment and functional integration in rodents have motivated the translation of this approach into a non-human primate injury model [63]. Pig-tailed macaques (*Macaca nemestrina*) received an ischemia/reperfusion injury by inflating a balloon catheter into the distal left anterior descending coronary artery for 90 min followed by reperfusion. Two weeks later, after initiating immunosuppression, 1 billion hESC-derived cardiomyocytes were transplanted through

transepicardial injections into the infarcted myocardial wall. This study was the first to demonstrate large-scale myocardial remuscularization (Fig. 2A), and large cardiomyocyte grafts were found in the infarct region 3 months after transplantation. Engrafted human cardiomyocytes demonstrated *in vivo* maturation from 14 days to 84 days, as indicated by an increase in cell diameter, sarcomere alignment, and myofibril content (Fig. 2B). Grafts were perfused by the host vasculature, which was shown by the presence of $CD31^+$ endothelial cells in the GFP^+ graft and further supported by 3D rendered microcomputed tomography to visualize vessels within the graft region (Fig. 2C-D). Furthermore, GCaMP3 fluorescence imaging showed that engrafted cardiomyocytes were electromechanically coupled to the host (Fig. 2E), as had been previously demonstrated in rodents. A notable concern from these studies, however, was the detection of non-fatal ventricular arrhythmias in the cardiomyocyte-engrafted hearts. These arrhythmias were not observed in mice, rats or guinea pigs [58], demonstrating the importance of using relevant large animal models. The ventricular arrhythmias will need to be managed for safe translation of human cardiomyocytes to the clinic.

4.0 Translating hPSC-Cardiomyocyte Delivery to the Clinic

While pre-clinical therapies with hPSC-derived cardiomyocytes have shown promise and are progressing quickly, questions regarding cardiomyocyte engraftment, phenotype, and large-scale production must be addressed in order to promote successful translation from bench to bedside. Firstly, cell survival after transplantation is low regardless of the cell type and injury model used. Despite the improved engraftment after adopting ‘pro-survival’ cell treatments prior to implantation [60], current methods are not sufficient to achieve high cell retention long-term. The use of tissue engineering approaches such as the implantation of cell sheets, epicardial patches, or cardiomyocytes delivered in biomaterials may help increase the engraftment rate (reviewed in [64-66]), although the development of minimally-invasive delivery techniques will be important for clinical translation.

Secondly, the optimal maturation state of cardiomyocytes for transplantation is not fully understood. Previous studies suggest that an intermediary maturation state may be ideal: mature adult cardiomyocytes do not survive transplantation [67], immature hESC-cardiomyocytes have automaticity, slow conduction and may be pro-arrhythmic [63], and mesodermal cardiac progenitor cells do not outperform definitive cardiomyocytes in terms of engraftment or efficacy (Fernandes, Chong et al, in Press). Current *in vitro* approaches may mature cardiomyocytes from the ‘early fetal’ state typically achieved after differentiation into a late-fetal or neonatal stage. While *in vitro* maturation to an adult phenotype will be difficult, as indicated above it also is undesirable for transplantation purposes (reviewed in [68]). Experiments that directly compare the engraftment of aged hPSC-cardiomyocytes to the current standard are needed, as well as studies designed to track implanted cells and characterize their maturation *in vivo*.

Lastly, one of the major hurdles for successful translation of hPSC-cardiomyocytes into the clinic is developing an “off-the-shelf” cardiomyocyte cell product: will these come from hESCs or from human induced pluripotent stem cells (hiPSCs)? The biggest advantage of using hiPSCs is their ability to provide an autologous cell source, but unfortunately this is

also one the major limitations when it comes to clinical and financial feasibility. The process of obtaining patient-specific somatic cells, reprogramming to iPSCs, differentiating into cardiomyocytes (perhaps requiring individual protocol optimization), and performing quality control would take over four months [9]. This precludes their use in an acute or subacute MI setting, and conversely, we have shown that hPSC-derived cardiomyocytes have no beneficial effect on cardiac function when delivered into chronic myocardial infarcts in rodents [56, 59]. An additional limitation of the autologous hiPSC approach is the cost. At present there are very expensive quality control experiments that are required before the release of a product derived from pluripotent stem cells, and doing this for each patient is cost-prohibitive unless the regulatory path is changed. There is also an inherent risk of residual undifferentiated stem cells giving rise to teratoma formation, and proper quality control measures must be taken to minimize this risk [69].

Most pre-clinical models thus far have used hESC-cardiomyocytes, although immunosuppression after an allogeneic hESC-cardiomyocyte implantation is a potential downfall of the therapy and requires more research to elucidate proper immunosuppression strategies that address the differential immunogenicity and rejection of autologous vs allogenic cells (reviewed in [70]). Research strategies to engineer HLA-homozygous hESC subclones or “universal donor cells” that are HLA class 1-negative will be useful in addressing this problem, and exciting progress has been made on this front in recent pre-clinical work [71]. Regardless of cell source, it remains unclear if this number of cells can be mass-produced for clinical use in a way that is financially manageable and biologically controlled. Cardiomyocyte production will need to be scaled up significantly to meet the current demand, although the development of methods to increase cardiomyocyte proliferation for *in vitro* scale-up may help alleviate this concern.

5.0 Concluding Thoughts

As the field of cell-based cardiac repair has matured there has been a natural shift from basic studies towards more clinical and translational goals. Nevertheless, it is important to continue with studies focusing on the underlying biology of heart regeneration; understanding the biological mechanisms of cardiac repair will be critical in the field's success regardless of therapeutic approach used. Simply put, unless we understand the mechanisms through which cell therapies work, there is no way to rationally improve upon them. A promising alternative approach to heart regeneration is stimulating endogenous repair after injury, which takes advantage of cues learned from regeneration experiments in lower vertebrates and the discovery of the mammalian regenerative window after birth (reviewed in [72]). Although this approach is far from the clinic, exciting progress has been made to identify factors that promote cardiomyocyte dedifferentiation and proliferation after injury, including the overexpression of cyclins [73-75], FGF and NRG signaling pathways [76, 77], Notch signaling [78, 79], and microRNAs [80]. Lessons learned here will provide important insight to the cell therapy field and may guide the development of dual gene and cell delivery therapies.

There are multiple parameters to consider when working towards clinically meaningful regeneration including functional recovery, attenuation of fibrosis, preventing adverse

remodeling, cardiomyocyte proliferation (and subsequent increase in viable myocardium), and the maturation of regenerated cardiomyocytes. Because an ideal therapy would be suitable for patients with recent cardiac injury or with established heart failure, pre-clinical studies will require careful evaluation of how to translate benefits to a more chronically affected patient population. The approaches discussed in this review were limited to ischemic heart disease; however the advancements in the field will have broad implications for other heart failure patients, such as those suffering from dilated cardiomyopathy, hypertrophic cardiomyopathy, or congenital heart disease. Better understanding the biology governing the heart's response to injury and to regenerative cues will provide insight to better direct gene therapy, drug delivery, and tissue engineering approaches that target non-ischemic heart disease.

In conclusion, the past few decades of heart regeneration research have been exciting and informative. Considerable progress has been made to establish cell transplantation techniques with bone marrow-derived cells and cardiac-derived cells, and hPSC-derived cardiomyocytes have been well established in pre-clinical studies as a promising cell type moving forward. While many challenges lie ahead before successfully regenerating the human heart, we are optimistic that the field is moving forward on a promising path.

Acknowledgments

This work was supported in part by NIH grants R01 HL084642 (CEM), P01HL094374 (CEM), P01 GM81619 (CEM), and U01 HL100405 (CEM), T32EB001650 (KAG), and the National Science Foundation Graduate Research Fellowship Program under grant numbers DGE-1256082 and DGE-0718124 (KAG).

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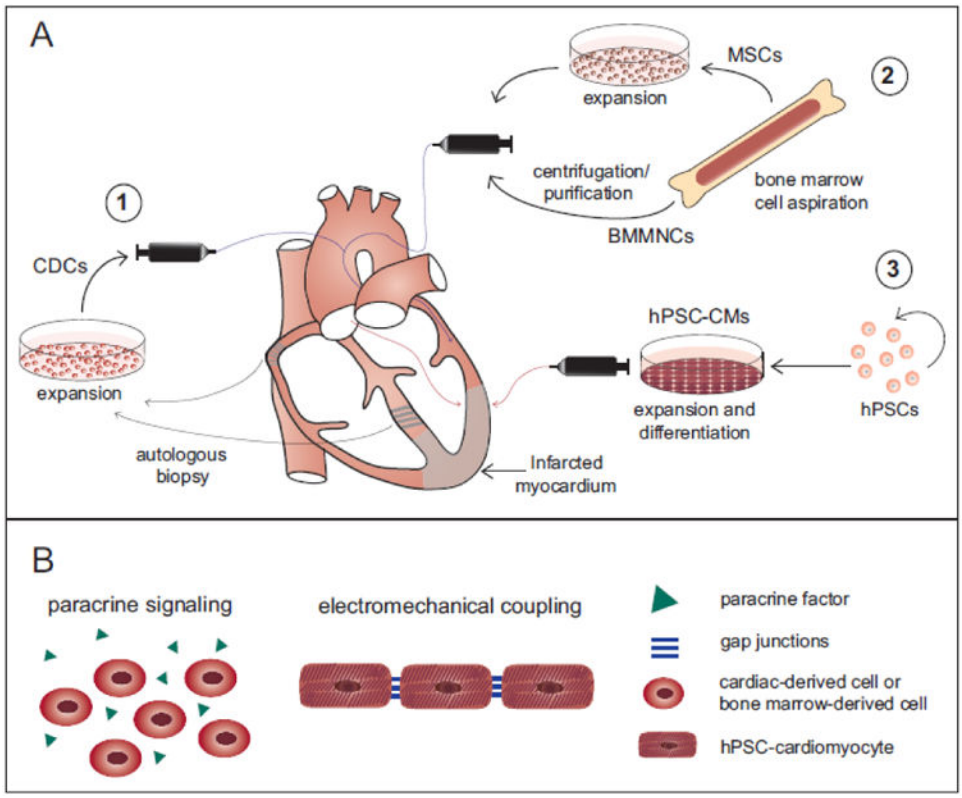

Figure 1.

Cell transplantation techniques and proposed mechanisms of cell therapy for heart regeneration. (A) Cell transplantation after myocardial infarction. (1) Cardiac-derived cells (CDCs) are isolated from either the atrial appendage or the septal wall, expanded *in vitro*, and transplanted via intracoronary catheter delivery. (2) Bone marrow mononuclear cells (BMMNCs) and mesenchymal stem cells (MSCs) are harvested from the bone marrow. BMMNCs may undergo purification steps followed by transplantation via intracoronary catheter delivery, while MSCs require *in vitro* expansion prior to transplantation. (3) Human cardiomyocytes are derived from human pluripotent stem cells (hPSCs) after *in vitro* expansion and directed cardiac differentiation. The proposed clinical delivery method for hPSC-cardiomyocytes (hPSC-CMs) is via transepicardial or transendocardial catheter-based injection. (B) Proposed mechanism of action after cell transplantation. Bone marrow-derived cells and cardiac-derived cells work primarily though paracrine signaling, in which transplanted cells secrete paracrine factors to the surrounding infarcted myocardium. HPSC-cardiomyocytes act primarily though the direct electromechanical integration with neighboring host cardiomyocytes. Paracrine factors may also be secreted by the hPSC-cardiomyocytes.

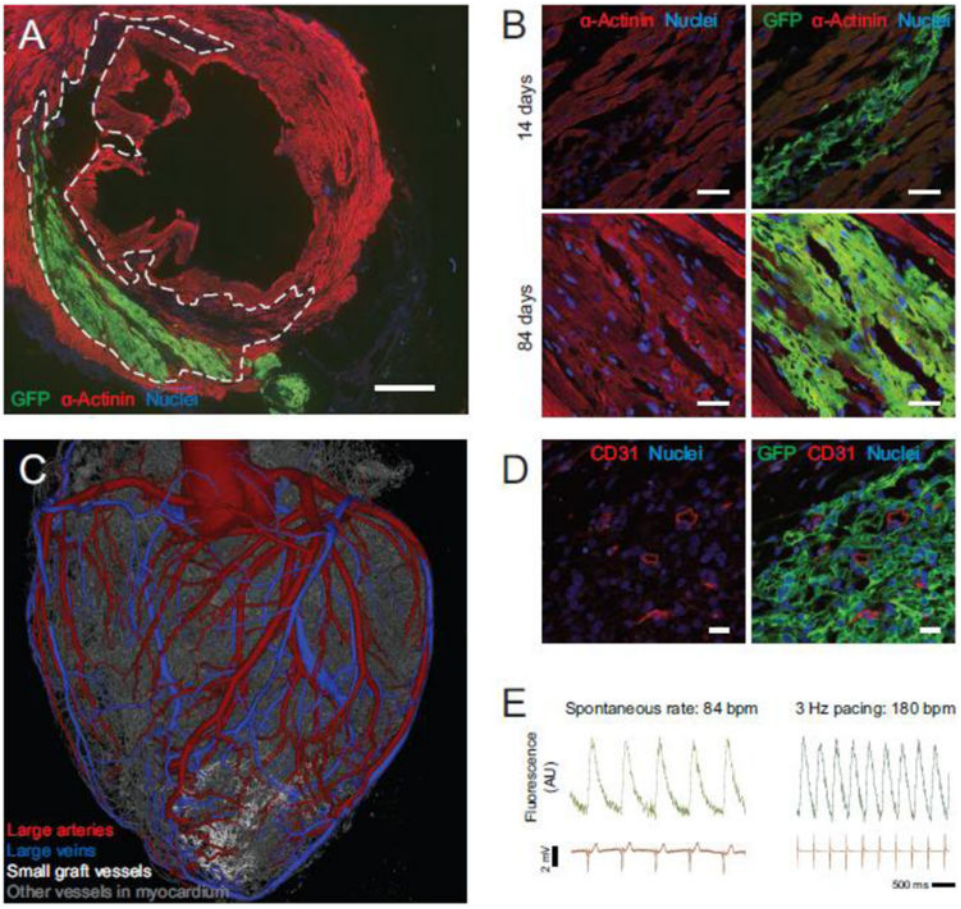

Figure 2.

Human pluripotent stem cell-derived cardiomyocytes remuscularize the infarcted macaque heart [63]. (A) HESC-cardiomyocytes robustly engraft in the infarcted myocardium, outlined by the dashed line, as indicated by confocal immunofluorescence at day 14 after transplantation. Engrafted cardiomyocytes express GFP (human, green) and both hESC-cardiomyocytes and host cardiomyocytes express the contractile protein alpha-actinin (human and monkey, red) with nuclear DAPI counterstain (blue). Scale bar = 2 mm. (B) The *in vivo* maturation of engrafted hESC-cardiomyocytes is evident from 14 days to 84 days post engraftment by co-staining for alpha-actinin (human and monkey, red) and GFP (human, green). At late timepoints, engrafted hESC-cardiomyocytes display a marked increase in cell size, sarcomere alignment, and myofibril content compared to early timepoints. Scale bar = 20 μm. (C) Host vasculature perfuses the hESC-cardiomyocyte graft at 84 days post engraftment, as visualized by three-dimensional rendered microcomputed tomography. Large host arteries and veins are shown in red and blue, respectively, and small vessels perfusing the graft are shown in white. Other vessels in the myocardium are shown in gray. (D) Host-derived blood vessels are found within the hESC-cardiomyocyte grafts at 84 days post engraftment. Host endothelial cells express CD31 (human and monkey, red) and infiltrate the GFP-positive graft area (human, green). Scale bar = 20 μm. (E) *Ex vivo* fluorescent GCaMP3 imaging indicates that engrafted human cardiomyocytes are electrically coupled to the infarcted macaque heart at 14 days post engraftment. GCaMP3

fluorescence intensity (green) and host ECG (red) are plotted versus time and demonstrate 1:1 coupling at spontaneos rate as well as during atrial pacing at 3 Hz.