

Chapter 2

Treating Cardiac Disorders with Stem Cells

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Abstract Heart failure is one of the leading causes of death in the western world and its incidence is increasing in the east. One of its causes is myocardial infarction which results in loss of muscle mass through death of cardiomyocytes. Replacing these by transplanting stem cells or encouraging cells in the heart itself to multiply are among the ways being investigated to prevent heart failure developing. The only stem cells which can form cardiomyocytes though are pluripotent stem cells, until recently only available from human embryos. Other types of (adult) stem are not able to form cardiomyocytes but, if transplanted, may help the heart recover and be of short term benefit through other mechanisms. One area using human embryonic stem cells is controversial because of its ethics, the other because of its sometimes disputed clinical outcome. Here, a critical overview of the issues is presented.

Keywords Cell therapy • Stem cells • Cardiomyocytes • Heart infarct • Clinical trials

2.1 Introduction

Among the ailments discussed in the context of cell transplantation therapy, those of the heart feature most prominently because of the large numbers of patients in the prime of life with cardiac disease. Cardiac failure almost inevitably follows myocardial infarction because the remaining healthy heart tissue attempts to compensate for the loss of heart cells by working harder. This leads to hypertrophy (swelling) followed by thinning of the heart wall and eventually its collapse. Cardiac failure and hypertrophy may also result from other (life-style) conditions independent of myocardial infarction, such as high blood pressure, obesity, or diabetes. The clinical

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cardiologist's interest in using cell therapy is based on the assumption that dysfunction of the left ventricular chamber of the heart, which pumps oxygenated blood around the body, is largely due to the loss of a critical number of cardiomyocytes. Implanting new contractile heart cells into the regions of scarring or wall thinning could inhibit or even reverse the process. Scientists have been searching for the best cellular sources on which to base therapies and have included cells derived both from embryonic stem or progenitor cells and adult tissues. Some patients have already been treated with the safest option: cells from their own bodies. These are not rejected by the immune system as being "foreign." These adult stem cells are most commonly derived from the bone marrow and other tissues, such as fat. Benefits so far seem modest and temporary and the mechanisms are poorly understood. Contrary to initial studies suggesting that bone marrow cells might be "plastic" and turn into heart cells once injected into the heart, this turns out not to be the case. The present assumption is that any benefit may derive from limiting ischemic damage after myocardial infarction or creating new blood vessels that improve blood supply to the heart muscle. On the other hand, evidence is unequivocal that human embryonic stem cells (hESC) can become cardiomyocytes. These could perhaps be used to repair heart tissue by contributing contractile force, but they have other associated risks specifically in humans, namely the ability to beat spontaneously in the absence of any pacemaker cells that could cause lethal arrhythmias. Unlike bone marrow cells, they would not be autologous and so would be rejected by the body unless immunosuppressive drugs were used, and could form tumors (teratomas) if rogue undifferentiated cells remained present in the transplanted cell preparations. The issue of rejection could possibly be addressed by using induced pluripotency stem cells (iPSCs) derived from the patient's skin as a source of cardiomyocytes; this approach would, however, likely be too expensive for individualized therapy. These issues aside, the greatest obstacle is still likely to be proper and stabilized integration of the grafted cells from whatever (pluripotent or progenitor) cellular source into the host tissue. Will it be possible to align new heart cells so that there is more contractile force? Will there be sufficient blood supply to the graft to support survival? Will grafts and any associated scar tissue interrupt the electrical circuitry of the heart and interfere with sequential contraction of the chambers? How will we repair the tiny muscles that open and close the valves if these have also been damaged by ischemia? These are all questions that need addressing as basic research advances towards clinical practice. It is, however, clear that restoring function of failing hearts by replacing damaged cardiomyocytes is straightforward in principle but probably among the most challenging paradigms of regenerative medicine.

2.2 Adult Stem Cells

Bone marrow cells (BMCs) were among the first non-cardiomyocyte sources of regenerative cells described for the heart. Genetically tagged BMCs expressing green fluorescent protein (GFP) as a visible marker were transplanted to the

experimentally infarcted hearts of normal mice [1]. The general approach in pre-clinical studies for determining the effects of stem cell transplantation on heart function after myocardial infarction are shown in Fig. 2.1. Initially, transdifferentiation of the transplanted BMCs to cardiomyocyte was thought to take place since GFP coincided with cardiomyocytes marker expression, explaining functional improvement to the heart compared to controls. This later turned out to be autofluorescence from scar tissue or dead cells with fusion between injected and host cells possibly contributing to co-expression of markers. After 4–6 weeks, no injected cells were ever recovered [2–5]. Nevertheless, these early results evoked an unprecedented progression to completion of the first randomized clinical trials within 5 years. Early reports of non-controlled pilot studies were unanimously positive but in later randomized (placebo-) controlled trials, effects were more modest, especially after longer follow-up times. Most researchers now agree that if BMCs improve cardiac function after myocardial infarction, it is more likely to result from

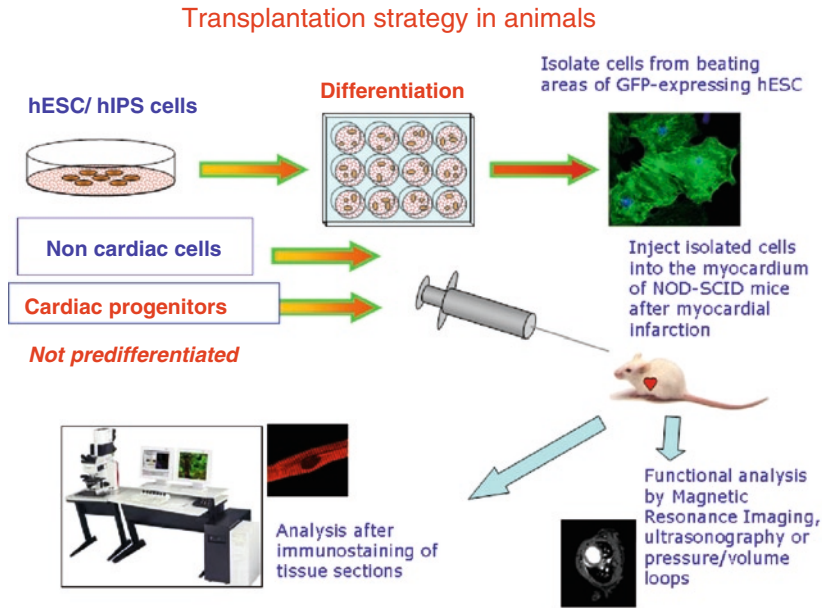


Fig. 2.1 Transplantation of stem cells or their derivatives to a mouse heart after myocardial infarction. The schematic diagram shows how these experiments are generally carried out. The cell of interest is usually labeled in some way (for example, genetically, fluorescently, or using iron particles) and injected into the (anaesthetized) mouse either through the tail vein or directly into the heart muscle. The mouse is usually immunodeficient to prevent cell rejection and a suture used to tie off one of the blood vessels of the heart. The cells are usually introduced at the same time as the surgery for myocardial infarction. Variable numbers of cells may be used. One group of mice usually functions as a sham control. Heart function in the mouse is determined over time after infarction and cell delivery. Non-invasive magnetic resonance imaging, echography, or invasive “pressure-volume loop” are the most common ways of determining cardiac function. The fate of the transplanted cells over time is monitored by immunohistochemistry or the like

early salvage of ischemic myocardium by some kind of paracrine action from the transplanted cells (reviewed in [6]). This does not mean the concept of cardiac regeneration (intrinsic repair) or repair (building tissue from an external source) should be abandoned. For instance, the underlying causes of cardiovascular disease affect BMC function; it may be of value to select subgroups of patients without BMC dysfunction for inclusion in trials. Parameters such as infarct remodeling or exercise capacity did appear to improve after BMC treatment, at least for up to 4–6 months, patients with the largest infarcts generally benefiting most [7]. Moreover, a small group of patients with angina pectoris (chronic chest pain) caused by underlying cardiac hypertrophy rather than acute myocardial infarction, have reported significant quality of life and modest functional improvements when bone marrow cells were injected into the heart [8]. Further basic research on the mechanism underlying any benefit will be required before the best cell type, intrinsic or extrinsic, can be mobilized or isolated for transplantation based therapy.

2.3 Pluripotent (Embryonic) Stem Cells and Cardiac Progenitors: Sources of Cardiomyocytes

This group of cells will be considered together because all have the capacity to generate the most important cellular components of the heart (reviewed in [9, 10]). Human embryonic stem cells (hESCs) are pluripotent cells derived from blastocyst-stage embryos. They proliferate indefinitely in vitro in an undifferentiated state and have the potential to differentiate into derivatives of all three primary germ layers (ectoderm, endoderm and mesoderm) and thus all 220 cell types of the adult individual. Mesoderm is the embryonic origin of the four major cell types of the heart: cardiomyocytes, vascular smooth muscle cells, endothelial cells and cardiac fibroblasts. HESCs are therefore a potential cell source for tissue regeneration including that necessary in the heart following myocardial infarction (MI). Likewise, human induced pluripotent stem cells (hiPSCs), derived by reactivating a set of pluripotency genes in somatic cells, can also self-renew indefinitely and differentiate to the same spectrum of cell types as hESC. Finally, cardiac progenitors endogenous to the heart could be a source of different cardiac cell types. These have been isolated from human fetal and adult hearts, shown to divide in culture to some extent but not indefinitely and to differentiate to one or more of the cell types in the heart. Their identity, however, is still a matter of discussion [11].

Multiple methods have been described to induce differentiation of stem and progenitor cells to cardiovascular derivatives. These range from methods that attempt to recapitulate normal development, such as growth as aggregates in suspension called “embryoid bodies,” and/or addition of specific growth factors, to more artificial methods, such as addition of demethylating agents that alter gene expression through epigenetic mechanisms. The cardiomyocytes obtained beat spontaneously and, in contrast to adult cardiomyocytes, express sarcomeric proteins,

cardiac transcription factors and multiple cardiac ion channel genes. They may have ventricular, atrial or pacemaker action potentials, respond as expected to positive and negative chronotropic agents, and form gap-junctions. Differentiated derivatives of stem and progenitor cells have an immature or fetal phenotype. Differentiated cell populations from pluripotent stem cells rarely exhibit a single phenotype and selection is necessary to obtain more pure populations. Much research today is focused on how best to do this; genetic marking, cell surface antibodies and physical methods based on differences in cell size or elasticity are all methods being tested. Independent of whether drug discovery and toxicity studies or regenerative medicine are the goals, obtaining well-defined homogeneous cell populations under defined conditions will be essential.

The first transplantation of hESC-derived cardiomyocytes demonstrated their potential to act as biological pacemakers in electrically silenced pig hearts, where the intrinsic pacemaker activity had been blocked by destroying the small region of pacemaker cells. At the same time, these studies demonstrated the risk of injecting immature (beating) cardiomyocytes into the heart – their potential to induce local arrhythmias. Transplantation studies were rapidly extended to regenerating the working myocardium. After transplantation into the healthy myocardium of immunodeficient rats or mice [12–14], hESC-derived cardiomyocytes were described as surviving and maturing for between 4 and 12 weeks [13, 14]. Although mixed cell populations were injected, preferential survival of cardiomyocytes was reported, with non-cardiac elements lost over time. Disconcertingly, however, grafted human cells formed a syncytium with each other but were largely separated from the rodent myocardium by a layer of fibrotic tissue. When transplanted into infarcted hearts in rodents, cardiomyocytes formed considerable grafts, with one study showing that addition of a pro-survival cocktail increased graft size. Cardiac function in animals receiving cardiomyocyte-containing populations was better during the first few weeks following transplantation than when non-cardiomyocyte derivatives were injected, and this was in turn better than when vehicle alone was injected, that is it seems any cell type is better than nothing, at least in mice and rats, cardiomyocytes work best. Hence, there were cardiomyocyte-specific benefits; these were quantitatively correlated in one study with the degree of neovasculture derived from the host in the border zone of the infarct. Only one study so far has, however, extended functional follow-up to 12 weeks after transplantation. At this point in time, the advantage of cardiomyocytes over non-cardiomyocyte-containing populations was no longer present, even when the number of cells transplanted or the number of injections was increased. “Priming” hESC with a growth factor called Bone Morphogenetic Protein (BMP) has been suggested as an alternative therapeutic option [15]. Primary fetal human cardiac progenitors, although difficult to obtain routinely, have also been described to differentiate in the rodent heart after MI and improve cardiac function, perhaps for even longer periods than hESC-CM [16]. Overall, though, functional enhancement by stem- or progenitor-derived cardiomyocytes even when neovasculture also forms, appears limited to mid-term at most. The therapeutic benefit of the cell therapy may be prolonged when using a pro-survival cocktail or different timing of injection, for example after the initial inflammatory phase when the environment may be hostile to the donor cells, but this remains to be

proven. A fibrotic layer can develop between injected and host cells that may or may not impede transduction of electrophysiological signals. The question arises of whether rodents are the most useful model animals to address this potential safety issue in humans. Rodent hearts beat at 400–600 times per minute, those of humans at 60–100. Injection of human cardiomyocytes with intrinsic different electrical properties into rodent hearts is therefore unlikely to contribute to cardiac function. The more slowly beating human cardiomyocytes would likely die from tachycardia if they really coupled to rodent host cardiomyocytes. Transplantation in the rodent myocardium is less likely to create arrhythmic substrates than the same procedure in humans. Transplantation of new cardiomyocytes from any stem or progenitor cells source into the human heart is thus likely to be fraught with safety and efficacy issues at the outset.

2.4 Summary and Conclusions

Almost any cell will cause functional improvement in the heart of a mouse with a myocardial infarction, independent of whether it is transplanted as or can become a cardiomyocyte or not. The only published exception so far appears to be skin fibroblasts (reference in [9]) which have no effect. Short term effective cell therapy in animals or humans does not appear to require that the cells survive permanently or even long-term in the heart. Current evidence suggests that any (transient) benefit to heart function and/or quality of life derives from new vessel formation or an as-yet undefined paracrine mechanism. Although ethically acceptable, likely safe and even commercially available as an unproven “treatment,” the long-term benefit of adult non-cardiac stem cells from any source, however, remains an open question. “Stem Cell Tourism,” a serious concern of the International Society of Stem Cell Research (www.isscr.org), which recently issued a recommendation on this subject, reflects the ethical and health concerns on excessively positive information on the benefits of stem cell treatment that are not provided as evidence-based therapies. Co-funding of basic and preclinical research through private and public partnerships may, however, accelerate clinical introduction of validated therapies. Animal studies and models could certainly be improved. Here exists the greatest disparity between basic scientists and cardiologists. Basic scientists make very clean “wounds” in the hearts of otherwise healthy mice; a normal heart patient would be stented (a small tube inserted) very quickly after myocardial infarction so the vessels would be reopened. In mice, the vessel is usually tied off permanently and cells transplanted immediately. In the case of heart failure in patients, the disparity with mouse models is even greater: large areas of hypertrophy interspersed with areas of necrosis and scar tissue and brittle vessels, small but crucial muscles operating valves inside the heart chambers damaged and dysfunctional. A large animal, such as a pig or sheep, could model aspects of human disease more effectively because of their greater physiological similarity to humans than rodents. For example, vessels could be tied off temporarily, then released and stents inserted as is done in patients. The timing of cell transplantation after infarction could be

varied, and different numbers and types of cells could be compared directly and simultaneously. The best delivery method could be identified. Should cells be injected deeply, or more towards the outer surface? Should a liquid matrix or gel be used, and would the presence of additional growth or survival factors be beneficial?

So far, these experiments are only being done on a limited scale, in part because they are very expensive. A pig can cost 10,000–20,000 euros compared to, say, 50 euros or less for a mouse. Housing of pigs during follow-up is significantly more costly than for mice. In Europe, preclinical studies of this kind in non-human primates, arguably the best animal model, are largely precluded by ethical considerations although a few large centers in the USA and Russia do carry out preclinical studies in these animals. Tissue engineering may also represent a way forward, particularly when the goal is to use contracting (immature) cardiomyocytes for transplantation, since these would allow prealignment of cardiomyocytes into myocardial “patches” for repair. If biodegradable scaffolds were used, then over time only the transplanted cardiac and vascular cells would remain.

In summary, there is clearly much basic preclinical research to be done. If cell therapy for the heart had already been proven, then the justification for using embryonic stem cells as a potential cell source for repair may be less controversial on ethical grounds. However, to close this avenue of research prematurely may deprive patients of potential therapies in the future, given the experimental options that remain to be explored. At present, it remains essential to carry out these studies in parallel to identify those with the most robust clinical potential in the shortest time frame. On the other hand, properly controlled clinical trials are imperative to combat the epidemic of “stem cell tourism” [17, 18].

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