Stem cell therapy for cardiac repair: benefits and barriers

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Cardiovascular disease remains the leading cause of death worldwide. Acute ischaemic injury and chronic cardiomyopathies lead to permanent loss of cardiac tissue and ultimately heart failure. Current therapies aim largely to attenuate the pathological remodelling that occurs after injury and to reduce risk factors for cardiovascular disease. Studies in animal models indicate that transplantation of mesenchymal stem cells, bone-marrow-derived haematopoietic stem cells, skeletal myoblasts, or embryonic stem cells has the potential to improve the function of ventricular muscle after ischaemic injury. Clinical trials using primarily bone-marrow-derived cells and skeletal myoblasts have also produced some encouraging results. However, the current experimental evidence suggests that the benefits of cell therapy are modest, the generation of new cardiac tissue is low, and the predominant mechanisms of action of transplanted stem cells involve favourable paracrine effects on injured myocardium. Recent studies show that the adult heart possesses various pools of putative resident stem cells, raising the hope that these cells can be isolated for therapy or manipulated in vivo to improve the healing of cardiac muscle after injury. This article reviews the properties and potential of the various stem cell populations for cardiac repair and regeneration as well as the barriers that might lie ahead.

Cardiovascular disease is the leading cause of death worldwide. Of the almost 17 million people who die each year from cardiovascular causes, over 11 million die as a result of cardiac disease and 5.5 million deaths are related to stroke. Myocardial infarction carries a short-term mortality rate of about 7% (with aggressive therapy), and congestive heart failure an even more distressing 20% one-year mortality (Ref. 1). Despite significant strides in therapy, thanks to newer treatment modalities and risk-reduction strategies, the global burden remains substantial (Refs 2, 3, 4, 5). This continued health problem has prompted research into new therapeutic approaches.

Stem cell therapy is a relatively new frontier in the battle against cardiovascular disease that has sparked intense research and criticism. With the

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discovery of various stem cell populations possessing cardiogenic potential, and the subsequent ability to isolate and expand these cells, the notion of a restorative therapy has begun to take shape. Although much knowledge has been gained through more than a decade of research, numerous barriers to true cardiac regeneration remain. In the pursuit of this endeavour, it has become apparent that we need to better understand the processes that lead to both damage and repair if we are to realise the true potential of stem cell therapy.

Ischaemia and infarct

Myocardial ischaemia, whether acute or chronic, begets a cascade of events leading to cellular injury or death with resultant scar formation and ultimately mechanical dysfunction, electrical uncoupling and loss of structural integrity (Ref. 6). Other than early restoration of blood flow, which engenders its own complications, the process is largely irreversible (Refs 7, 8). True regeneration is extremely limited.

Within seconds of an ischaemic insult, aerobic glycolysis ceases, leading to marked ATP depletion and lactic acid accumulation (Refs 9, 10). Early in the process, clinical reduction in myocardial contractility occurs secondary to the build up of various tissue metabolites that reduce the Ca^{2+} sensitivity of contractile myofilaments (Ref. 11). Continued oxygen deprivation leads to failure of the Na^+/K^+-ATPase pump, an increase in intracellular solute, and subsequent swelling (Refs 7, 12). Accumulation of lactic acid reduces the cellular pH, limiting the activity of essential enzymes and increasing the release of lysosomal products that lead to cellular breakdown. In addition, the failure of the Ca^{2+} pump leads to Ca^{2+} influx, with damaging effects on numerous intracellular components including ribosomal dissociation and mitochondrial membrane potential reduction, ultimately ending in apoptosis (Ref. 13). Cellular death signals macrophage and neutrophil infiltration, originally to the periphery and later to the centre of the infarct. As phagocytosis ensues, the necrotic tissue is removed and replaced with fibrovascular granulation tissue, leading to a decrease in the thickness of the muscle wall. As the process continues, neutrophils are replaced with myofibroblasts and subsequent

deposition of collagen (predominantly type I and III). Finally, the cellularity is reduced, leaving only a dense collagenous scar (Ref. 6).

Scar formation is an essential aspect of rapid wound healing, especially in the injured myocardium, which is under constant wall stress. Without rapid wound healing, the ischaemic region would be subject to rupture, which is generally incompatible with life. Scar formation therefore offers protection from immediate danger by providing a rapid mechanical barrier (Ref. 14). However, scar tissue is largely acellular and lacks the normal biochemical properties of the host cells. This leads to electrical uncoupling, mechanical dysfunction, and loss of structural integrity, ultimately resulting in a dilated cardiomyopathy (Refs 15, 16). Limiting scar formation or even reversing the process could thus prove beneficial in maintaining the overall function of the organ.

To date, the mainstays in treatment of heart disease focus on reducing myocardial oxygen demand, increasing its supply and limiting the ischaemic burden in an effort to prevent scar formation and enhance myocardial function. However, once scar formation has occurred, a vicious cycle ensues, first with localised dysfunction and later with remodelling and dilation of the surrounding myocardium, leading to heart failure.

It is well known that following injury many species of amphibians and fish undergo complete regeneration (Refs 17, 18). Moreover, embryos respond differently than adults to tissue injury, with rapid, almost complete, regeneration and little scar formation (Ref. 19). This is believed to be a result of both the intrinsic function of embryonic fibroblasts as well as the external milieu surrounding the embryonic cells (Ref. 17). A better understanding of these intrinsic regenerative mechanisms may lead to novel potent therapies in the future.

The discovery of the proliferative capacity and plasticity of various stem cell populations has sparked much interest and debate regarding their use as a potential therapy. Over the past decade, several different stem cell types have been studied in an effort to find the best source for cardiac regeneration. Each stem cell population has its own advantages and complications (Table 1). Here, we examine the

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Table 1. Characteristics of stem cell populations used for cardiac repair

Stem cell	Derived from	Advantages	Disadvantages	Clinical trials
Embryonic stem cells	Inner cell mass of the pre-implantation blastocyst	Theoretically unlimited self-renewal capacity; pluripotent	Ethical considerations; teratoma formation; graft-versus-host disease	None
Bone marrow mononuclear cells, haematopoietic stem cells, circulating progenitor cells	Bone marrow, peripheral blood (also umbilical cord, placenta)	Easy to isolate; proven safe and feasible to implant	Controversy whether true cardiovascular differentiation takes place	Large-scale trials: modest and transient benefits; significant reduction in subsequent cardiovascular events (Refs 50, 51, 52, 53, 54, 55, 56, 57)
Mesenchymal stem cells	Bone marrow (adherent cells), adipose tissue	Easy to isolate and expand in culture; less immunogenic than other lines; multipotent	Large heterogeneity; heterotopic differentiation (e.g. ossification)	Safety and feasibility, and small-scale studies (Ref. 74)
Endothelial progenitor cells	Bone marrow, peripheral blood	Mobilised from bone marrow or present in peripheral blood; important in neovasculogenesis	Heterogeneity; small populations; reduced in individuals with cardiovascular comorbidities	Safety and feasibility, and small-scale studies (Refs 96, 97, 98, 99, 100, 101)
Skeletal myoblasts	Mature muscle (between the sarcolemma and basement membrane)	Extensive scalability; resistance to ischaemia; multipotent; no teratoma formation	Potential for arrhythmias; lack of cardiomyocyte differentiation (dysynchronous beating)	Large-scale clinical trials: no benefit (Ref. 120)
Cardiac stem cells	Special niches in the myocardium (in deep tissue at the atria and apex)	Resident cells; robust cardiovascular differentiation potential; reduced tumour formation	Stem cell pool appears to undergo senescence; scalability largely unknown	None

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various stem cells utilised in animal models and clinical trials thus far, discussing briefly their benefits, disadvantages and evidence supporting their use.

Embryonic stem cells

Mouse and human embryonic stem cells (ESCs) can be removed from the inner mass of the blastocyst and expanded practically indefinitely in vitro (Refs 20, 21, 22). ESCs remain pluripotent in an undifferentiated state in culture; when allowed to differentiate, usually as embryoid bodies, ESCs are able to give rise to most somatic cell lineages (Refs 23, 24, 25). In this regard, their regenerative capacity is theoretically limitless. Furthermore, by culturing the embryoid bodies in various growth media, one can drive differentiation towards a desired cell type such as the cardiomyocyte (Refs 23, 24). These cells can then be implanted into the corresponding organ. This approach to repair cardiac tissue after injury has been tested in preclinical studies with encouraging results (Refs 26, 27, 28, 29). In fact, of the various stem cell populations studied so far, perhaps the greatest capacity for cardiac cell differentiation and long-term cell survival has been seen in studies using ESCs (Ref. 30).

To date, no human trials have been attempted using ESCs for cardiac repair. There have been three main concerns regarding their use as a treatment modality. First, differentiating embryoid bodies contain cells from all three germ layers of ectoderm, mesoderm and endoderm, and therefore possess the capacity to differentiate along any or all of these lineages. This increases the likelihood of teratoma formation at the implantation site. Although these teratomas are believed to be largely benign in vivo, reasonable concerns have been raised because some cells have been found to express markers similar to those found in malignant tumors (Ref. 31). There is some evidence that the host tissues secrete factors that help drive the stem cells along a particular differentiation pattern (Refs 29, 32). Subsequently, there has been increased interest in culturing the embryoid bodies in specific media to promote differentiation to specific cell types (Ref. 33). These partially, or in some cases fully, differentiated cells can then be implanted, alleviating some of the risk of teratoma formation. Studies with these differentiated cells have shown increased engraftment and functional improvement (Refs 28, 30). While no long-term studies have been carried out to assess the real risk of teratoma formation, the theoretical concern remains an important obstacle.

The second issue regarding the use of ESCs pertains to immunity. Once thought to be uniquely immunoprivileged, increasing evidence has demonstrated that ESCs express specific human leukocyte antigen (HLA) subclasses (Ref. 34). This raises the worry of graft rejection and might necessitate immunosuppression. Steroid use without concomitant stem cell implantation has been known for some time to be harmful to ischaemic myocardium (Ref. 35). Not only does immunosuppression complicate the treatment with stem cells, but it may in fact undo any benefit derived from the addition of the stem cells to the ischaemic milieu. There is currently ongoing research to help limit the immunogenicity of the cells for allogeneic transplantation.

Finally, the origin of ESCs has raised considerable ethical concerns and led to heated debates among scientists and the wider public. The recent discovery that it is possible to generate ESC-like cells, called inducible pluripotent stem (iPS) cells, by reprogramming adult somatic cells with genes regulating ESC pluripotency may resolve the ethical and immunogenic issues associated with the use of ESCs (Refs 36, 37, 38).

Bone-marrow-derived stem cells
Bone marrow haematopoietic progenitor/ stem cells

Bone marrow haematopoietic stem cells, or circulating peripheral blood progenitor cells, were shown to differentiate into cardiomyocytes in culture, making them of particular interest in the treatment of cardiac disease because they represent a well-characterised and ample source of progenitor cells (Refs 39, 40, 41, 42). A number of landmark studies showed significant improvement in cardiac function when bone-marrow-derived cells were implanted directly or mobilised from endogenous reservoirs. Some analyses not only showed improved ventricular function, but actually demonstrated

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regeneration of contracting cardiomyocytes and vascular beds (Refs 43, 44, 45, 46). However, other investigations found limited or no differentiation of bone marrow cells to cardiovascular cell types (Refs 47, 48), suggesting a beneficial effect independent of tissue regeneration (Ref. 49). Nevertheless, the improvements seen in ventricular function prompted a number of clinical trials using autologous bone marrow cells to treat heart failure patients or patients who had suffered a myocardial infarction. The clinical studies used circulating haematopoietic progenitor cells, or bone marrow mononuclear cells (MNCs), which also contain the small population of haematopoietic stem cells.

Early smaller studies were encouraging. However, larger, randomised, placebo-controlled and blinded studies have shown some mixed results (Refs 50, 51, 52, 53). The REPAIR-AMI trial (the largest of the randomised, placebo-controlled trials) was positive in that it not only demonstrated improved left ventricular function, but also showed a reduction in the combined clinical endpoint of death, myocardial infarction or revascularisation at one year (Ref. 54). The BOOST trial also showed improved left ventricular function early on compared with control patients, but by 18 months that difference had disappeared as control patients caught up with those who received cell therapy (Refs 55, 56). In contrast to the improved left ventricular function results of the REPAIR-AMI and BOOST trials, a double-blind, randomised controlled study, using autologous bone marrow MNCs in patients with myocardial infarction 24 h after successful percutaneous coronary intervention, showed no benefit in left ventricular ejection fraction, but a significant reduction in infarct size and improved regional left ventricular function (Ref. 53).

A recent meta-analysis of 18 randomised and nonrandomised trials involving 999 patients with acute myocardial infarction or chronic ischaemic cardiomyopathy found that transplantation of adult bone marrow cells improved left ventricular ejection fraction by 5.40%, decreased infarct scar size by 5.49% and lowered left ventricular end-systolic volume by 4.80 ml (Ref. 57).

It is possible that the apparently conflicting results among different trials are secondary to the cell preparation or the timing of the cell administration. There is clearly a need for further large-scale trials to assess the role of infused bone marrow cells in cardiac repair in order to improve their therapeutic efficacy.

Mesenchymal stem cells

Mesenchymal stem cells (MSCs) are a subset of stem cells that inhabit the stroma of bone marrow and can differentiate into osteoblasts, chondrocytes and adipocytes (Refs 58, 59). They can be separated from haematopoietic cells by their ability to adhere to the culture dish (Ref. 60). MSCs can also be induced to differentiate in vitro into cardiomyocytes, which has stimulated a large number of animal and clinical studies to evaluate the efficacy of MSCs for cardiac repair and regeneration (Refs 61, 62, 63). MSCs are potentially advantageous as they are thought to be less immunogenic than other lines (Refs 64, 65). This alleviates the need for immunosuppression or autologous therapy.

Preclinical studies using transplantation of MSCs in post-infarct mice demonstrated improved left ventricular function and reduction in infarct size (Refs 62, 64, 65, 66, 67, 68, 69), and a decrease in mortality (Ref. 70). These improvements were seen despite small numbers of cells undergoing differentiation to cardiomyocytes (Refs 68, 71, 72, 73). A clinical study of MSCs in 69 post-infarct patients also demonstrated improved left ventricular function (Ref. 74).

Difficulties may arise, however, because of the broad differentiation capacity of MSCs. There remains significant heterogeneity among MSC populations and thus they are less predictable when implanted. Most notably, some studies found that implanted MSCs had differentiated into osteoblasts inside ventricular tissue (Refs 75, 76). This is an obvious cause for concern and needs to be addressed prior to full-scale therapy.

Endothelial progenitor cells

Another bone marrow cell type, the endothelial progenitor cell (EPC), has shown great promise as a potential therapy. Angiogenesis was once thought to occur solely though the proliferation of mature endothelial cells at sites of injury. This was challenged with the discovery that bone-marrow-derived EPCs home to sites of injury and incorporate into the microvasculature (Refs 77, 78). This revolutionised our understanding of vascular

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growth and repair and became an intriguing concept for therapeutic manipulation.

Although there is some controversy regarding their true definition, EPCs can be identified by their ability to acquire endothelial cell characteristics in culture and in vivo. They express cell-surface makers such as cluster of differentiation molecule 133 (CD133), the vascular endothelial growth factor receptor 2 kinase (VEGFR-2; also known as KDR), CD34 and vascular endothelial cadherin (VE-cadherin). Of these, CD34$^+$ and CD133$^+$ cells are the most widely recognised and utilised, although these markers are also shared by haematopoietic stem cells (Ref. 79). EPCs are mobilised from bone marrow in such injurious states as burns, myocardial infarction and cancer (Refs 80, 81, 82, 83). Furthermore, they have been shown to contribute anywhere from 5% to 25% of neovessel formation (Refs 84, 85). Not only do EPCs aid in vasculogenesis, but there is also evidence that they can differentiate to cardiomyocytes (Ref. 86).

Subsequently, the search began to find ways to enhance their mobilisation or to directly incorporate them into the vasculature of injured tissues. Both VEGF and granulocyte colony-stimulating factor (G-CSF) have been shown to increase EPC mobilisation from bone marrow (Refs 87, 88, 89). It should also be noted that statins (3-hydroxy-3-methylglutaryl coenzyme-A reductase inhibitors) have been shown to stimulate the mobilisation of EPCs from the bone marrow as well, pointing to yet another aspect of the ever-evolving understanding of the many therapeutic benefits of the drug (Refs 90, 91).

The first preclinical studies with implanted EPCs were hind-limb ischaemia experiments, which demonstrated significant improvement in blood flow recovery and limb salvage (Refs 77, 92, 93). Furthermore, injection of EPCs into infarcted myocardium improved left ventricular function and inhibited fibrosis (Refs 79, 94, 95). These results led to clinical experiments to assess safety and feasibility (Refs 96, 97, 98). The results of several small trials have shown trends toward improvement in left ventricular function with both acute and chronic ischaemia, without adverse effects (Refs 97, 99, 100, 101).

EPCs have already found a niche in the field of interventional cardiology. The earliest stents used were bare metal stents without drug coating. Although beneficial, these stents have an increased tendency to restenose (narrowing of the vessel via a localised inflammatory response) over time. Drug-eluting stents (impregnated with various chemicals that inhibit neointimal thickening) reduce the restenosis rate, but increase rates of in-stent thrombosis, a potentially fatal event. A newer technology for stents may be on the horizon. GENOUS stents are coated with anti-CD34 antibodies, which serve to trap circulating EPCs and augment the endothelialisation process in an effort to prevent restenosis (Ref. 102). They have already proven safe for implantation and ongoing studies will assess whether we are able to reduce the restenosis rate without the concern for in-stent thrombosis.

There are, however, barriers to the use of EPCs as therapeutic agents. First, is the heterogeneity of this cell population. EPCs circulating in the peripheral blood span the full range of differentiation from angioblasts to mature endothelial cells. This in part could explain differences in results from various studies. Second, the stem cell pool of EPCs is quite limited and only through ex vivo expansion can one attain appreciable numbers to surmount any significant injury or ischaemic event (Ref. 79). Last, the circulating pool of EPCs is reduced in patients with cardiac ischaemic disease comorbidities such as diabetes mellitus, hypertension and hypercholesterolaemia (Refs 103, 104). This is problematic as this cohort is essentially the very one that would need to be treated with EPCs – namely patients with coronary artery disease and other ischaemic risk factors. These challenges require further research to enhance the therapeutic efficiency of EPCs in ischaemic tissue.

Skeletal myoblasts

Skeletal myoblasts have been seen as an attractive source of stem cells and were among the earliest cell types considered for cardiac repair. Often called satellite cells, they are found beneath the basal membrane of muscle tissue where they lie dormant until stimulated to proliferate by muscle injury or disease (Ref. 105). These cells are further differentiated than ESCs and are thus less prone to teratoma formation. Furthermore, they can be harvested from the host, expanded in vitro, and autologously reimplanted, thus avoiding the need for immunosuppression (Ref. 106). Skeletal

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myoblasts are especially apt for cardiac repair as they are resistant to ischaemia, an inherent obstacle to the function of stem cells in injured myocardium (Ref. 107). Finally, skeletal myoblasts have the capacity to differentiate in vitro into nonmuscle cell types (Refs 108, 109). These properties prompted their consideration in cardiac repair.

Animal transplantation experiments in cardiac disease models were subsequently performed with encouraging results. Most of these studies showed improved left ventricular function and decreased remodelling possibly because the implanted cells form myotubules that are able to contract (an event possibly mediated by stretch receptors; Refs 106, 107, 110, 111, 112). Furthermore, the cells have been shown to decrease matrix breakdown both in the peri-infarct area as well as remote myocardium, which likely contributes to reduced remodelling (Ref. 113).

However, skeletal myoblasts do not fully differentiate into cardiomyocytes in vivo after intramyocardial transplantation and the contracting myotubules do not operate in synchrony with the surrounding myocardium (Refs 112, 114). This is due at least in part to a lack of connexin activity and electrical coupling with the surrounding myocardial cells. However, regardless of the processes involved, the improvement in left ventricular function in animal models prompted a series of clinical investigations.

Early clinical studies were aimed at assessing the feasibility and safety of implantation (Refs 107, 115, 116, 117, 118, 119). These studies proved the therapy possible and showed that skeletal myoblasts survive in the human heart, although only marginal benefit was seen. Larger-scale clinical trials were then undertaken to assess the benefit of myoblast therapy. The most notable to date was the MAGIC trial, which randomised patients to receive either stem cell injection or culture medium. Results from this trial have been disappointing in that no significant benefit was seen with stem cell implantation (Ref. 120). Further clinical studies are ongoing and may reveal differing results.

Several barriers still remain in the use of skeletal myoblasts. First, there has been considerable concern regarding the potential for arrhythmias (Refs 114, 121, 122). Early studies did report rare cases in human patients (Refs 117, 123). However, since then, there have been conflicting results and the data from more-recent large clinical trials did not record increased arrhythmic events in vivo after intracardiac injection of skeletal myoblasts (Refs 120, 124). Animal experiments also showed that the electrical coupling of skeletal myoblasts to resident cardiomyocytes is increased when the skeletal cells are induced to overexpress connexin 43, indicating that there might be ways to overcome the arrhythmogenic obstacle (Refs 125, 126).

Another limitation is the relative paucity of engraftment of the injected cell population to the surrounding tissue. Cellular lethality of the order of 90% within the first few days has been demonstrated in mice (Ref. 127). Some studies in humans have shown similar cell death tolls (Ref. 107). The cells that survive are scarce. In addition, the engrafted cells differentiate into myotubules and not cardiomyocytes and therefore do not demonstrate a true regenerative therapy.

Finally, there is much variability and complexity involved in the use of skeletal myoblast populations. For example, female myoblasts demonstrate a higher proliferation potential than do male lines (Ref. 128). Moreover, although myoblasts are easy to harvest and expand in culture, the process is labour intensive and takes considerable time. This largely precludes autologous use in acute ischaemic events such as myocardial infarction.

Cardiac stem cells

The modest functional effects of transplanted progenitor cells from bone marrow and skeletal muscle in human studies stimulated further research into the natural regenerative mechanisms of the cardiac tissue. The heart has traditionally been viewed as a postmitotic organ because mature cardiomyocytes withdraw from the cell cycle and cease to proliferate. Interestingly, contradictory data began to accumulate as cardiomyocyte proliferation and cycling were found under certain pathological conditions – namely ischaemia and hypertension (Refs 129, 130, 131). This idea was further advanced with the discovery of male cardiomyocytes and endothelial cells in donor female cardiac tissue transplanted into a male

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recipient (Refs 132, 133). These findings raise the possibility that Y-chromosome positive, male cells migrated either from the recipient atrial stump or the bone marrow into the cardiac tissue and differentiated into functional cardiomyocytes. Moreover, estimates of the death rate levels of adult cardiomyocytes also led to the consideration of a pool of cardiac progenitor cells (Ref. 134). This evidence prompted a search to locate such resident cardiac cells. Subsequently, several different cell types were discovered in the adult heart with stem cell characteristics.

For example, a typical property of some stem cell populations is the cytoplasmic exclusion of vital dyes such as Hoechst 33342 and Rhodamine 123. The dye-negative cells have been called the side population (SP) cells. SP cells have been identified in various organs including bone marrow, skeletal muscle and adipose tissue (Ref. 135). Staining of dissociated cardiac tissue revealed that the heart also has a resident pool of SP cells (Refs 136, 137). Interestingly, isolated cardiac SP cells can differentiate to cardiomyocytes, suggesting that they represent cardiac progenitor cells (Refs 138, 139). SP cells are mobilised after cardiac injury (Ref. 140) but their regenerative potential is still unclear. One study documented differentiation of transplanted SP cells to cardiomyocytes, endothelial cells and smooth muscle cells (Ref. 139).

A second putative resident progenitor population comprises cells expressing the stem cell factor receptor c-Kit (also known as CD117), which are located in small clusters within the adult heart (Ref. 141). c-Kit$^+$ cells have regenerative potential after transplantation, giving rise to cardiomyocytes, endothelial cells and smooth muscle cells. c-Kit$^+$ cell transplantation after ischaemic injury leads to significant improvement in ventricular function (Refs 141, 142, 143, 144).

A third cell type in the heart with stem cell features consists of cells expressing the stem cell antigen 1 (Sca-1$^+$) (Ref. 145). Sca-1$^+$ cells home to infarcted myocardium and differentiate to cardiomyocytes around the injury area (Ref. 145). The Sca-1$^+$ cell subpopulation, which does not express CD31, was shown to differentiate into both cardiomyocytes and endothelial cells in culture (Ref. 146). Transplantation of Sca-1$^+$CD31$^-$ cells in mice after myocardial infarction improved cardiac function and promoted new blood vessel formation (Ref. 146).

Finally, cardiac progenitors from mouse hearts were isolated by enzymatic digestion to obtain round cells that form so-called cardiospheres in suspension (Ref. 147). Cardiosphere-derived cells can differentiate to cardiomyocytes, endothelial cells and smooth muscle cells. An equivalent human cardiac stem cell population can be obtained via endomyocardial biopsy and subsequently grown in suspension as cardiospheres that exhibit remarkable proliferation and differentiation capacity (Refs 147, 148, 149). Once isolated, this cell population can be induced to differentiate into spontaneously beating aggregates of cardiomyocytes, which can then be implanted into injured myocardium at a later time (Refs 149, 150). The injection of cardiosphere-derived cells has shown some benefit in preclinical studies (Refs 148, 149, 150, 151). In much the same manner as the previous progenitor cell populations, the benefit appears to be largely by way of improved left ventricular function. There has been some regeneration seen in small numbers, but not enough to explain the functional improvement.

Cardiac stem cells (as well as stem cells from other tissues) appear to reside in specialised niches, which support the growth and maintenance of the stem cell pool (Refs 152, 153). Putative niches have been localised throughout the myocardium, concentrated in deep tissue at the atria and apex (Refs 141, 154). Recent evidence has also shown that there is a marked increase in the number and migration of such cells to the injury areas following an ischaemic insult (Ref. 145). Although the different cardiac stem cell pools are small relative to the mature resident cardiomyocytes, they are believed to be the source of new cells in normal organ homeostasis as well as in stressed myocardium (Ref. 155). At present, it is unclear if the various cardiac stem cells are distinct types or whether they represent different stages of a single cell lineage.

One seemingly contradictory aspect of endogenous cardiac stem cells is the apparent lack of regeneration seen in the chronic damage that occurs in ischaemic cardiomyopathy. It is puzzling why these pools of stem cells, which are induced to differentiate and migrate to sites of injury, are not able to reverse tissue losses. It is possible that the resident stem cell populations do not survive in the hypoxic

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environment after an ischaemic insult and they undergo apoptosis along with mature myocardium. Furthermore, it appears that the cardiac stem cell pool diminishes with ageing, possibly contributing to the lack of efficacy of regeneration in elderly individuals (Ref. 155). Since it is largely the elderly who experience increased mortality from cardiomyopathies, it raises the need to enhance or rejuvenate this senescent stem cell population.

Favourable paracrine effects of stem cells

As experimental evidence about the outcomes of stem cell therapy accumulated, a peculiar pattern began to emerge. Although many studies involving different stem cell populations and various administration modalities show significant benefit (often in the form of improved left ventricular function), there seems to be little differentiation of the infused stem cells into mature cardiovascular cell types. Moreover, few of the implanted cells persist for any appreciable length of time (Refs 127, 156). Also, the cardioprotective effects of stem cells are already evident 24 h after transplantation, a time frame that is too short for true regeneration (Ref. 157). These results have been recapitulated in many studies whereupon following a brief inhabitance in the ischaemic milieu the infused cells can no longer be found despite the persistent functional improvement of the myocardium (Ref. 158). Another peculiarity is a similar benefit has been derived using a wide range of stem cell populations. Finally, those studies that do demonstrate engraftment have shown numbers so small that it is hard to attribute the haemodynamic improvements to the incorporated cells.

The benefits witnessed therefore require further elucidation. If the implanted cells do not remain in the tissue and differentiate into functional cardiomyocytes in appreciable numbers, then how is this benefit derived? The hypothesis began to emerge that the stem cell populations exert a favourable paracrine effect on the injured myocardium, perhaps preventing apoptosis and promoting healing (Refs 156, 159).
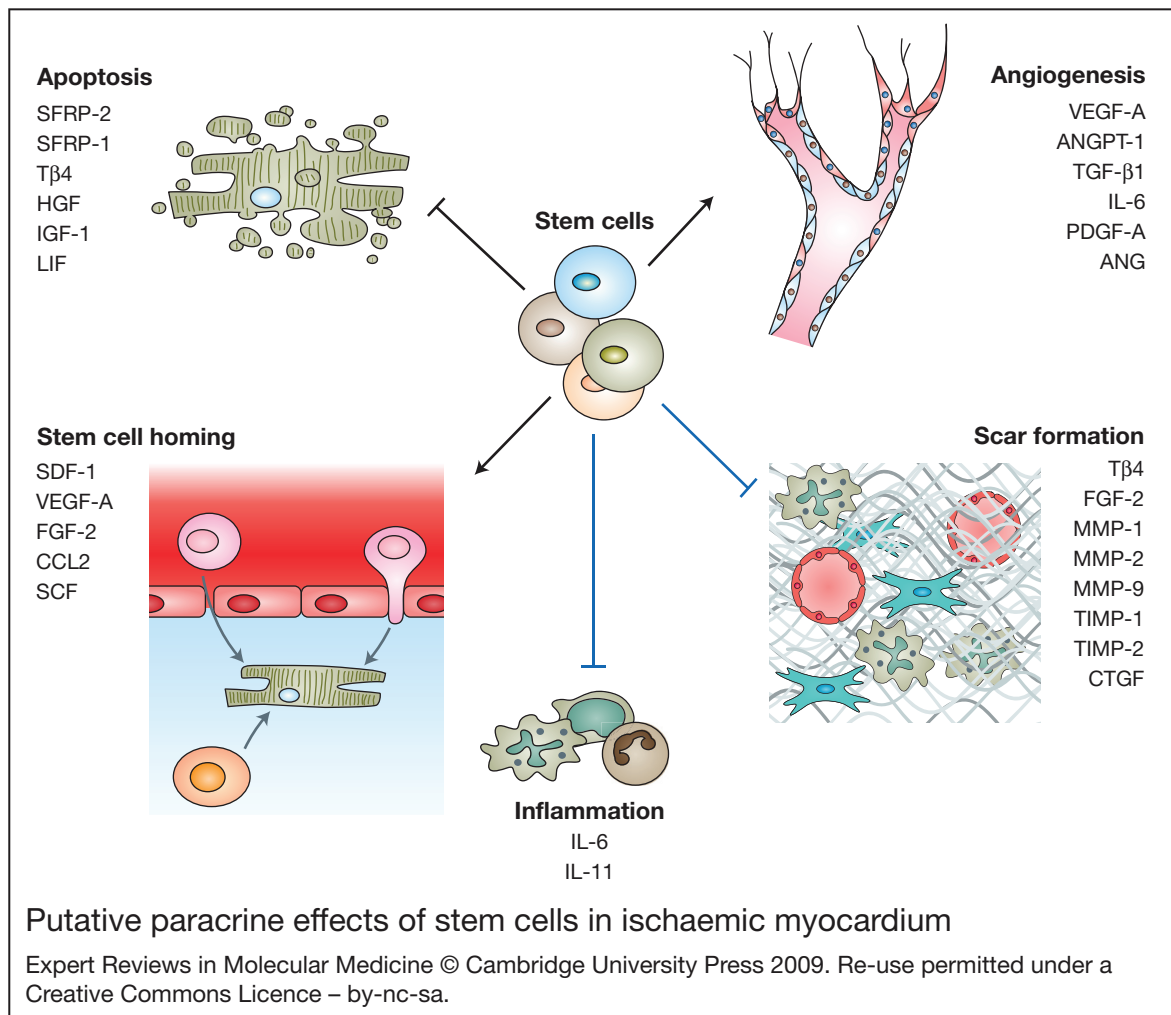
Indeed, various studies showed that progenitor cells secret survival factors, which stimulate tissue recovery after ischaemic injury and minimise the infarct size (Refs 156, 157, 160, 161, 162). The beneficial effects have been thus far attributed to specific products of transplanted progenitor cells such as thymosin β4, which promotes wound healing, or the Wnt antagonist SFRP-2 (secreted frizzled-related protein 2), which protects cardiomyocytes from hypoxia-induced apoptosis (Refs 163, 164, 165). In addition, based on the gene expression profiles of various stem cell types (Refs 156, 166), it is likely that stem-cell-secreted factors attenuate inflammation, decrease apoptosis, induce angiogenesis, recruit other stem cells and reduce the extent of fibrosis (Refs 156, 162, 167) (Fig. 1).

Taken together, the experimental evidence suggests that current benefits derived from stem cell therapy are at least in part secondary to a favourable paracrine effect of the stem cells acting on the host tissue. Whether or not the administration of isolated stem cell products or the physical presence of stem cells in the injury site is a more ideal form of therapy remains to be seen. However, it is apparent that in response to ischaemia many factors, acting in concert, work to limit damage and enhance repair. It is therefore possible that by providing the injured tissue with a functioning stem cell population, which can react to the internal milieu and respond with sustainable, targeted production of cardioprotective peptides, greater damage attenuation can be achieved than by simply infusing fixed quantities of specific agents. Perhaps the dynamic presence of a tissue repair biocatalyst is the most beneficial effect of stem cell implantation, which better equips injured tissue with the tools and blueprints to aid recovery and regeneration.

Future directions

Since the discovery of various resident stem cell populations and the subsequent ability to extract and culture them for therapeutic use, there has been a wealth of research into the potential of regenerating injured tissue. The current evidence suggests that stem cell therapy has great promise for attenuating remodelling and transforming inert scar into biochemically functional myocardium. However, the past decade has shown that translating the potential of stem cell therapy into actual practice is not easy, and many barriers would need to be overcome before this therapy attains its full potential.

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Apoptosis

SFRP-2
SFRP-1
Tβ4
HGF
IGF-1
LIF

Angiogenesis

VEGF-A
ANGPT-1
TGF-β1
IL-6
PDGF-A
ANG

Stem cells

Stem cell homing

SDF-1
VEGF-A
FGF-2
CCL2
SCF

Scar formation

Tβ4
FGF-2
MMP-1
MMP-2
MMP-9
TIMP-1
TIMP-2
CTGF

Inflammation

IL-6
IL-11

Putative paracrine effects of stem cells in ischaemic myocardium

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Figure 1. Putative paracrine effects of stem cells in ischaemic myocardium. Stem cells secrete factors that: promote survival of ischaemic cardiomyocytes and reduce apoptosis; induce angiogenesis, improving perfusion around the ischaemic area; modulate protease activity and scar formation; and produce factors that recruit circulating (pink) or resident (orange) progenitor cells. The improved disease environment attenuates inflammation and fibrosis, curtailing subsequent cardiac tissue remodelling (based on Refs 156, 162). On the figure, inflammation is depicted by a monocyte, macrophage and neutrophil; scar (or granulation) tissue is represented by myofibroblasts, macrophages and capillaries in a collagen matrix. Abbreviations: ANG, angiogenin; ANGPT, angiopoietin; CTGF, connective tissue growth factor; FGF, fibroblast growth factor; HGF, hepatocyte growth factor; IGF, insulin-like growth factor; IL, interleukin; LIF, leukaemia inhibitory factor; CCL2, chemokine (C-C motif) ligand 2 (also known as monocyte chemoattractant protein 1; MCP-1); MMP, matrix metalloproteinase; PDGF, platelet-derived growth factor; SCF, stem cell factor (c-Kit ligand); SDF, stromal-cell-derived factor; SFRP, secreted frizzled-related protein; Tβ4, thymosin β4; TGF, transforming growth factor; TIMP, tissue inhibitor of metalloproteinases; VEGF, vascular endothelial growth factor.

Despite these obstacles, the observed functional improvement with or without long-term engraftment of the stem cells has spurred continued animal and clinical studies along several different directions. First, there is ongoing research into ways to better enhance the recruitment, survival and long-term engraftment of implanted stem cells (Refs 168, 169). If true regeneration is to take place, then a sizeable percentage of the stem cells need to remain viable, differentiate into fully functional cardiomyocytes and incorporate into the resident tissue. Second, further analyses of stem cells that exhibit robust cardiac potential (i.e.

human ESCs and autologous iPS cells) are also needed to generate pure cell populations of cardiomyocytes with appropriate functional characteristics. Third, the interesting notion that stem cells exert their influence largely through paracrine activity has sparked research into how this effect is brought about. By gaining more understanding of the molecular interactions between donor stem cells and host tissue, we could discover ways to harness this effect. Finally, the discovery of various cardiac stem cell populations has renewed interest in the innate regenerative capacity of the human heart to enhance endogenous repair or mimic it with exogenous stem cell therapy. Although much more work needs to be done, stem cell therapies in conjunction with current treatment modalities may help to further reduce the mortality and improve the quality of life in cardiovascular disease patients.

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Further reading, resources and contacts

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Website

The public homepage of the Cardiovascular Cell Therapy Research Network provides background and information for several ongoing multicentre clinical trials in the USA using stem cells for cardiac therapy:

http://ccct.sph.uth.tmc.edu/cctrn/Public/PublicHome.aspx

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Features associated with this article

Figure
Figure 1. Putative paracrine effects of stem cells in ischaemic myocardium.

Table
Table 1. Characteristics of stem cell populations used for cardiac repair.

Citation details for this article