Review

Is the clinical use of adult stem cells a realistic possibility for myocardial regeneration?

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Cardiac disease is the leading cause of death in the Western World and as such presents a large incentive for finding a cure. There is much current interest in exploiting adult stem cells in cardiac therapies, with both heart tissue and bone marrow being considered as potential sources or stem cells. Some promising results for future therapies are emerging, but it remains unclear how long it will take for the research to translate into clinical use. Further basic research into stem cell biology needs to be conducted before such therapies can be used with confidence.

Key words: adult stem cells, myocardial regeneration, cardiac stem cells.

It is widely hoped that stem cells (SCs) will offer a route to regenerative medicine, i.e. to develop ways of re-growing damaged organs and tissues. This review focuses specifically on one proposed use of SCs, namely the regeneration of cardiac tissue. Cardiovascular disease is the leading cause of death of all non-communicable diseases world wide and as such, is an important target for the development of new treatments. Chronic heart disease and heart failure represent a significant load on the Western World’s health care systems. As such, development of cell-based therapies aimed at ameliorating survival rates from cardiovascular disease offers an attractive research incentive. It is the ultimate goal of regenerative medicine to provide cures for diseases that can at present only be treated by delaying end-stage progression.

Prior to discussion of the main research published in this field, however, it is useful to first define what an SC is and specifically, for the context of this article, what characterizes an adult stem cell (ASC). Generally speaking, SCs are thought to exhibit the following characteristics. SCs are specialized cells capable of generating progenitor daughter cells that can differentiate (i.e. can turn into other specialized cell types), have self-renewal capacity or be clonogenic (Fig. 1). These cells in turn can develop into specific lineages depending on the type of SC from which they originated, via asymmetric divisions (an SC is also produced every time a progenitor is created). Therefore upon activation, SCs should give rise to progenitor cells that, in the natural setting, can be guided down a specific differentiation pathway (or pathways), producing precursor cells that eventually terminally differentiate. Progressive differentiation leads to a loss of SC characteristics such as self renewal and clonogenicity.

SCs can be divided into different types; embryonic stem cells (ESCs), foetal stem cells (not discussed here) and ASCs. ESCs are described as pluripotent, meaning that they have the potential, if given the correct stimuli, to generate any tissue in the body, bar the placenta i.e. all the cells of the blastocyst (reviewed by Lovell and Mathur1). Although this is true for murine ESCs, human ESCs can also produce cells of placental origin (trophoeendoderm). ASCs are more restricted in their differentiation potential than ESCs and are said to be multipotent; they can develop into a subset of lineage potentials of the approximate 200 cell types of the body, usually into the cell types of a specific organ. As more is understood about ASCs, however, increasing reports are emerging5 of ASCs developing into cell types divergent from their initial classification either directly (transdifferentiation), or by a process of dedifferentiation and redifferentiation6 (Fig. 1).

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When SCs are stimulated to grow (e.g. in normal homeostatic response to minor tissue damage) mitosis occurs to replace lost cells. Ki67 is a protein expressed only during mitosis and its presence is exploited to identify activated SCs in a snapshot of time when the histological section is taken. Labelling with a fluorescent antibody allows this protein to be visualized by immunohistochemistry, and Ki67 labelling has contributed to the discovery of CSCs.

The existence of CSCs represents a paradigm shift to occur in cardiac biology. Previously the heart was thought to be a post-mitotic organ, i.e. comprised entirely of terminally differentiated cells. However, hard evidence is being accrued for the genuine existence of CSCs, including one group providing convincing evidence that Ki67-labelled cells had not simply undergone fusion with endogenous differentiated cells.

CSCs are a subset of ASCs that reside within the heart tissue itself, and are now thought to contribute to natural replenishment of heart cell tissue in periods of minimal damage or general wear and tear. As with most ASCs, these cells are believed to exist within a specific physical location, termed the stem cell niche, within the human heart. Similar niches have also been located in the murine heart. Cardiac cells were thought to exit the cell cycle after birth, and thus disease was thought to occur by a progressive loss of myocardial cells. Through adult life compensatory hypertrophy of the remaining terminally differentiated cells occurred to maintain cardiac output, until disease developed when these cells failed to meet the demands of the organism. Studies now support the view that these cells could be involved in regeneration of the myocardium post-infarction. One study found myocyte hyperplasia (division) accompanied by concomitant activation of resident CSCs in the setting of human cardiac hypertrophy. After aortic stenosis, cardiac mass regeneration occurred via hypertrophy of existing cells and division of others originating from CSCs, thus supporting the hearts status as a regenerative organ. Some doubt exists regarding the capacity of CSCs to regenerate large sections of the whole organ. Yet, evidence is mounting to suggest that the heart could be persuaded to regenerate itself, at least in part, if given the correct stimuli (such as growth factors).

Human CSCs have been positively identified in myocardial tissue, located in clustered niches in the atria and the apex of the heart. A true CSC expressed no cardiac-specific transcription factors or cytoplasmic proteins themselves, indicating that they remain in an undifferentiated state. The CSCs were surrounded by slightly differentiated progenitor cells, expressing the transcription factor GATA-4. As GATA-4 is the cardiac-specific member of this transcription factor family, the presence of this protein is a strong evidence of their cardiac lineage. Rapidly dividing new myocyte cells were also found forming over the damaged area to replace the myocardium. These findings support the idea of CSCs

Two principal sources of ASCs for cardiac therapy are being considered; those found within the heart itself, i.e. cardiac stem cells (CSCs) and a variety of populations within the bone marrow; bone marrow stem cells (BMSCs).

SCs are relatively rare within adult tissues, and it has been necessary to develop a number of molecular techniques to distinguish ASCs from the more populous ‘normal’ cells in the surrounding tissue. One important technique exploits the relatively slow cycling of SCs. BromodeoxyUridine (BrdU) is a nucleoside that can be incorporated into replicating DNA in place of thymidine. Antibodies specifically recognizing BrdU can be used to monitor its presence within DNA. If a pulse of BrdU is provided, successive rounds of DNA replication will effectively dilute the concentration of BrdU within any given cell and hence the amount of antibody binding is diminished until it is effectively undetectable.

Since SCs are slow cycling under non-activated conditions, they tend to be ‘label retaining’ and can be identified under histological section. Confocal microscopy is usually used to visualize these cells and is a powerful technique to assess SC labelling.

![Diagram](https://academic.oup.com/biohorizons/article-abstract/1/1/67/233178/68)
being involved in cardiomyocyte homeostasis and that CSCs could be responsible for the regenerative response to cardiac damage after injury.

There are two postulated mechanisms by which CSCs could repair damaged myocardial tissue. If damage occurs close to the niche in vivo it seems likely that these cells could reconstitute the damaged myocardium. Alternatively, it is also thought that transmigration of CSCs from the niches could happen. This would allow potential regeneration of all parts of the heart. Transmigration could occur through the fibroblast extracellular matrix or via coronary arteries resulting in attachment to the site of injury any where in the myocardial tissue. The latter idea would be of most benefit to potential therapy as it could be employed to regenerate areas of the myocardium spatially separated from the niche. This latter theory however has not yet been demonstrated conclusively in vivo.

An interesting study showed human CSCs are mobilized to sites of damage in the heart in response to acute myocardial infarction (MI) and, to a lesser extent, in hearts from patients with chronic ischemic cardiomyopathy. The presence and activation of a discrete CSC population in the hearts was demonstrated when the level of CSC growth and senescence was measured. The hearts were harvested from patients who had endured a fatal acute MI or from patients who had received a heart transplant after suffering with end-stage postinfarction cardiomyopathy. All the tests were compared with control hearts. The authors found the resident CSC pools in the studied hearts to be free of the haematopoietic blood cell markers, but possessing sca-1-like epitopes, c-kit and MDR-1 (molecular surface proteins/receptors used as markers), suggesting the presence of SCs free of haemopoietic stem cell lineage. The sca-1 tagged cells were also reported to be free of endothelial markers, such as Von Willebrand factor and vascular endothelial-Cadherin, thus suggesting that the endothelial SCs could not have contributed to the detected CSC pool. The isolated CSCs were devoid of cardiac cytoskeletal proteins or transcription factors thus demonstrating their multipotency, and taken together, these factors support the basis for the identified cells being of CSC origin. The study ultimately found that in the control and acutely infarcted heart, CSC activation did occur due to ischemic injury. CSCs were found to be more senescent in chronically injured hearts than in the acutely injured heart (determined from the level of p53 and p16\(^{INK4a}\), of which high levels show a blocking of the cell cycle at G_0. Associated telomerase dysfunction was observed by the lower levels of full length PARP (Poly ADP Ribose Polymerase) in chronically infarcted hearts compared with control or acute hearts. PARP is a molecule that serves as an associated protein for working telomerase, which protects the ends of DNA from degradation during nuclear replication. This increased senescence may be due to the death of CSC reserves in the chronic heart.

Proliferative ability of the CSCs was assessed by the amount of Ki67. Proliferation was increased 19-fold in acutely infarcted hearts, while the chronic hearts were only found to have a 9-fold increase over control hearts when looking at the amount of Ki-67 labelled CSCs. This was concurrent with the activity of telomerase in the proliferating CSCs, as DNA division is only maintained when telomerase activity is present over successive divisions. Overall, this study showed that CSCs can and do respond to ischemic injury in humans and highlighted an interesting point that CSC proliferation decreases in the chronically infarcted heart. Compared to acute hearts, it was found that there was a 73% decrease in the amount of fully competent CSCs in the chronic heart; this may ultimately prove a hurdle for SC intervention in chronic heart dysfunction.

It seems, therefore, that the CSC research community is largely in agreement that true CSCs exist and are located in the ventricular and atrial regions of the heart. Progenitors of these cells reside in the niche with the CSCs, and have a slightly differentiated phenotype as shown by the presence of cardiogenic specific transcription factors, while still containing the same cell surface markers present on CSCs. The progeny of these cells (precursors) subsequently become more differentiated and thus lose their cell surface CSC epitopes, with the concomitant up-regulation in expression of transcription factors and cytoskeletal proteins of either of the three main cell types of the heart; myocytes, smooth muscle cells and endothelial cells. Differentiation into these three cell types has been shown in vitro, but the molecular signalling pathways governing cell fate are yet to be elucidated. Although there is a body of evidence supporting the existence of CSCs with the ability to become up-regulated in response to MI injury, the infancy of the research field means that there are no current clinical studies utilizing CSCs. Progress in this area is presently constrained by both the limits of scientific understanding and problems with sourcing and collecting the cells. CSC collection would require a highly invasive procedure and the numbers of cells available for collection would require extensive ex vivo expansion to form an effective therapy. Indeed, due to the minute sample sizes, it has been noted that CSCs would require far more expansion than bone marrow SCs or endothelial progenitor cells intended for cardiac use. However, a study isolating cells that generated cardiospheres from the murine and human heart biopsies could possibly address the issue of supply by their extensive ability to proliferated ex vivo while still maintaining their SC potential. It could therefore be possible to isolate cells from heart biopsies of patients who were already having an invasive procedure and expand them relatively quickly under in vitro conditions. These cells could therefore be used in an autologous manner provided the wait would not endanger the patient.

It has been hypothesized that injection of an, as yet unknown, CSC-specific growth factor would be the ultimate aim of therapeutic strategies in this field. This would bypass
invasive procedures to collect cells for expansion ready for re-injection and allow for a direct injection in the setting of acute MI allowing CSC pools to be stimulated immediately after insult. This miraculous therapy would boost the heart’s innate ability to heal itself and perhaps lessen the extent of fibrosis that occurs post MI, with the concomitant bypassing of immune rejection or systemic mobilization of SCs or progenitor cells. Unfortunately, due to the current status of research into CSCs, this eventual aim is far from being realized. Yet even with these drawbacks, the National Heart Lung and Blood Institute (NHLBI) in the USA have agreed to fund a CSC clinical trial in the near future.2

Due to certain research findings it is unclear at present whether it will be possible to stimulate resident CSCs in patients with heart failure, perhaps limiting its clinical application.13 In heart failure it is thought that the normal homeostasis capabilities of the myocardium from regenerative CSC niches have been compromised.8 This may be due to CSC pools being destroyed as they were in the vicinity of an infarct or due to a gradual increase in senescence of these cells as the heart ages.16

The potential therapeutic efficiency of using CSCs to treat damaged cardiac tissue would undoubtedly revolutionize the treatment of many cardiovascular disorders, for example recovery from MI and reversal of heart failure. The tantalizingly thought of a ‘clean’ regenerative therapy using CSCs seems, therefore, to remain a long way off.

Bone marrow, consisting of a heterogeneous collection of lineage-determined cells and sub-populations of unspecialized stem cells, may represent an alternative source. To date, bone marrow is the best understood source of ASCs since extensive research has been conducted into the regulation of haematopoietic signalling pathways and differentiation capabilities.17 Bone marrow comprises cells from both mesenchymal (stromal) and haematopoietic lineages which, respectively, form the support cells and precursor cells for the blood cells of the body. SC types that are undergoing research for use in cardiac regeneration are mesenchymal stem cells (MSCs) and haematopoietic stem cells (HSCs). Other specific side population cells originating from the bone marrow, and found by fluorescence-activate cell sorting (FACS) analysis have also been studied.18 A seemingly large degree of plasticity has been documented within the pool of BMSCs. Recent research has shown that BMSCs can not only develop into tissues from mesenchymal and blood cell origins, but have been reported to cross the presumed lineage boundaries of cell types in foetal development and transdifferentiate into cell types of other embryonic lineages (ectoderm and endoderm tissues).19, 20 For example, cardiac precursor cells and cells capable of transdifferentiation into cardiac lineages have been isolated from both HSCs and MSCs, and thus bone marrow holds a large potential for cardiac regenerative therapy. BMSC collection is a well practised method (by aspiration) and sidesteps the need for collection of organ-specific ASCs that require invasive procedures (e.g. opening the chest cavity to obtain CSCs), making autologous therapy feasible.21 Certain bone marrow side population cells display markers of cardiac origin,19 and therefore the cells would require less transdifferentiation to cardiac phenotypes in vivo. Several research groups have isolated and tested different lineages of BMSCs in the treatment of MI, with some promising results in animal and early clinical trials.22 24 Therefore, at least in the immediate term, these cells show more potential impact on the application to clinical medicine than CSCs.

In a paper published in 2001, convincing evidence was presented showing that HSCs could improve functioning of the heart in experimentally infarcted mice.22 In this experiment, Lin–, c-kit+, bone marrow cells transgenically expressing Extra Green Fluorescent Protein (EGFP) from male donor mice were isolated and injected into the area of infarct post-injury in female mouse hearts (Fig. 2). Nine days after injection, the authors reported that 68% of the infarct was reconstituted by the injected cells, with a success rate of inducing repair in 40% of the subject animals. The source of the cells covering the infarcted area were concluded to be the injected cells, since the cells expressed EGFP fluorescence and were positively stained for the Y chromosome. These stained cells were not present in the viable myocardium, suggesting that the injected cells selectively populated the damaged area and did not randomly diffuse through the heart tissue. The group also presented data that showed the expression of endothelial, myocyte and smooth muscle proteins in 58% ± 8% of the EGFP/Y chromosome labelled cells overall, signifying these cells were differentiating into cardiac lineages within the damaged area. Coronary arteries were observed that formed from the injected cells, showing the acquisition of functionality in these cell types. Myocyte functionality was assessed by staining for the foetal transcription factors Csx/Nkx 2.5, which are involved in myocyte differentiation, and with MEF2 and GATA-4, which are also cardiac-specific transcription factors. MEF2 and GATA-4 are involved in transcribing cardiac genes needed for the translation of the contractile machinery, and these were found to be expressed in all of the cells in the regenerative band. Csx/Nkx 2.5 was found in 40% of the EGFP cells, therefore suggesting induction of the foetal cardiac gene programme and thus differentiation of the injected cells. Connexin 43 expression also suggests functional acquisition of the cells as this molecule allows coupling between myocyte cells needed for efficient contraction. Additionally, Ki67 and BrdU were used to label the cells to see how many were actively dividing and therefore producing more cells to repopulate the myocardium. 40–50% of the cells with loosely organized myofibrils were also positive.
for Ki67 and BrdU, with the concomitant expression of EGFP. When compared with a control group of mice, the left ventricular end diastolic pressure was 36% lower in the experimental group. Unfortunately, delivery of the cells required thoracic surgery, and this therefore presents significant restraint on the therapeutic potential of such a procedure.

A subsequent study by the same team tested the hypothesis that an injection of stem cell factor (SCF) and granulocyte-colony stimulating factor (GSF) would mobilize bone marrow SCs in mice, and that these would subsequently home to damaged myocardium and repopulate it post MI. The study found significant amounts of tissue regeneration 27 days post insult, with the additional improvement in cardiac output of these mice. Mortality was decreased by 68%, with a 40% decrease in the size of the infarcted area. Substantial improvement was seen in the ventricular function of all the animal subjects. Unlike the protocol in the earlier work, this method of administration is simple and non-invasive and, as such, holds great promise for therapeutic use. Indeed, treatment of MI in human subjects by the administration of GSF has proved successful. However, both these cytokine studies include drawbacks regarding their interpretation, and thus more conclusive studies should be conducted to understand the molecular pathways involved.

Several early clinical trials used BMSCs of various types to experimentally repair damaged myocardium either in the setting of MI or chronic heart disease. Of these trials, one notable study, conducted by Chen et al., used bone marrow MSCs. The cells were transplanted 3 weeks after percutaneous coronary intervention (PCI), which itself occurred 8 days after MI. Autologous bone marrow aspiration harvests were used, with extensive in vitro expansion before injection to the damaged area. Patients were monitored 3 and 6 months after the injection and were found to have improved left ventricular function after the cell implant. Results were attributed to the MSC injection and not the PCI due to the time lapse between the procedure and the cell injection. MSCs harvested for in vitro expansion were found to be capable of achieving cell populations of up to $10^3$ cells if cultured for 14–21 days, depending on the starting density. Therefore, in this experiment, at least,
there was a realistic time window for ex vivo expansion in the clinical treatment of MI.

Interestingly, the study by Perin et al.,\textsuperscript{25} showed that endomyocardial injection of Autologous Bone Marrow MonoNuclear stem cells (ABMMNCs), improved heart function in chronically infarcted hearts. Most research has been focused on the effect of SC therapy on MI, and so this study is important due to its wider implications on cardiac therapy. The study found that there was an improvement in left ventricular function resulting from injection into the endothelial membrane that lines the interior of the heart, a method previously untested in human candidates. This injection site was thought to be beneficial as the function of ischaemic areas could be boosted directly by increased angiogenesis and perfusion in the damaged area thus assisting chronic infarct. The implications of such a study are great, since chronic heart failure is not presently considered treatable with the stimulation of resident CSCs mentioned above.

One drawback however is that the BMSC studies involved cells originating from heterogeneous populations of BMSCs, and as such their identity of each type is not easily distinguished. A recent study, however, has used molecular techniques to isolate a specific subset of BMSCs that were shown to improve cardiac function. The study showed that clonally purified non-haematopoietic MSCs positively contributed to functioning of the heart post MI when compared against other BMSC types.\textsuperscript{19} This study explored the effects of human MSCs, bone marrow mononuclear cells (BMMNCs), peripheral blood derived mononuclear cells (PBMMNCs) and un-purified mesenchymal stem cells (uMSCs) on recovering of heart function after MI in a rat model. It is not clear if the BMMNCs in this study are the same type studied by Perrin et al.\textsuperscript{25} Purification of MSCs was achieved by depleting BMMNCs of cells containing CD45\textsuperscript{+}/Gly\textsuperscript{+} cells by micro-magnetic beads. The remaining cells were expanded in culture; clones were selected and diluted and then collected when the clones reached 80% confluence. These cells were then incubated with magnetic activated cell sorting (MACS) colloidal super-paramagnetic microbeads that had an antibody conjugated to it selective for the cell surface marker CD34 (a haematopoietic progenitor cell marker). The mixture was passed down a column that depleted the magnetic bead-attached cells, and the resulting cells were checked for purity by FACS analysis. The cells were administered to the culture; clones were selected and diluted and then collected.

Even with this success in early animal models and clinical trials, there are still reservations from some scientists as to the safety of implementing a therapy that clearly works, but is poorly understood at the molecular level. Additionally, failure by other laboratories to replicate early findings by Orlic et al. has fuelled arguments over the validity of the results. The study by Murry et al.\textsuperscript{28} had isolated HSCs that possessed the same cell surface markers as the HSCs used in Orlic et al.'s\textsuperscript{25} study, but they completely failed to transdifferentiate into cardiac cells, the observation reported in the primary study. Compared with Orlic, Murry did use slightly different techniques to visualize their Sca\textsuperscript{1+}, c-kit\textsuperscript{+} cells. Orlic’s group primarily used immunofluorescence detection, and Murry’s group used transgenic markers of lineage and phenotype (being MHC-nLAC and β-Act-EGFP), but it is unlikely that these technological disparities should have caused a difference in the ability of the HSC to adopt cardiac functionality. There is therefore a need for more extensive animal studies to clarify the molecular basis of such a conversion.

Due to differing data and the easier expansion of MSCs in vitro, MSCs at present are thought to hold more potential for cardiac therapy.

The use of growth factors has also raised questions regarding safety. In Orlic et al.'s\textsuperscript{23} study, the authors were unable to show definitively that GSF was only stimulating bone marrow cells, thus allowing space for this treatment to be non-specific and possibly propagate malignant growth. Further study is warranted regarding other cell populations that could be mobilized by GSF, especially if resident CSCs could be stimulated to proliferate under such circumstances. Understanding of this type must be established before moving into properly controlled clinical trials. Kueht,\textsuperscript{24} who used GSF to mobilize BMSCs in human subjects, has also questioned what other effects may occur by GSF injection. The modern practise of medicine requires a mechanism of action to be understood before therapies can be added into clinical practise. The effect of whole body exposure to growth factors, even in a transient state has not been studied, and therefore worries over malignant growth need to be studied.

The conflicts in this field result from a relative lack of understanding of the molecular markers and, interestingly, there is currently no universally accepted description of markers for MSCs.\textsuperscript{30} To date, clinical trials have been small. Randomized, placebo-controlled double-blind studies need to be conducted once molecular characterization has been understood.\textsuperscript{31} Even with these shortcomings, it is important not to forget that BMSCs have a positive effect on patients with MI in some instances, and further research will allow development of these promising and complex cells.
In summary, SC research aimed at improving cardiac function, either using cells derived from the bone marrow or intrinsic CSC, is an emerging field in regenerative medicine. These two areas aim to provide eventual cures for cardiac diseases that at present contribute greatly to mortality levels world wide. Studies into BMSCs have demonstrated that this cell type can exhibit plasticity, but understanding why studies work in mice and not in humans remains to be answered. Clearly there is greater complexity in the issue than we presently understand and thus further molecular research is warranted.

Studies using CSCs have only just reached the animal stage of research, but this preliminary work shows great promise for future therapies. This method holds the advantage of being able to be an autologous therapy, perhaps with just an injection of growth factor to stimulate the body to heal itself. However more basic biochemical and molecular bench work research on CSCs is needed before they can move towards the clinic. Animal studies do not always mean efficacy in human equivalents and thus it still remains to be seen how well CSCs will contribute to the future of medicine.

Evidence derived from the research reviewed here seems to endorse the potential of future treatments using ASCs, but also implies that it may take many years to convert the potential into successful and reliable therapies. ASCs are a realistic scientific source as well as being a more ethical source than presented by ESCs, yet both fields are still in the early stages of research and development. Scientists are now faced with the mammoth task of sifting through this data and early clinical or animal findings to understand the cells being pioneered for therapies. The new ASC research facility being set up in Cambridge could help us understand the basis of ASCs behaviour in general, but again this is yet to be seen. Speculation over a probable positive outcome is all we have to go on when we try to decide how influential ASCs will be, let alone in the field of cardiac regenerative therapy. The evidence collected so far is highly promising; the future is bright, but therapeutic use of SC therapies in cardiac regeneration remains to be fully established.

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