Review Article

Old dogmas and new hearts: a role for adult stem cells in cardiac repair?

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Summary

The vast developmental repertoire of embryonic stem cells is well recognised. These primitive stem cells can differentiate *in vivo* and *in vitro* into cells of all three embryonic germ layers (endoderm, mesoderm, ectoderm), making them attractive potential agents to target for enhanced tissue repair and regeneration. Adult stem cells on the other hand are considered more restricted in their lineage differentiation capabilities. Recent research has challenged this dogma with the finding that bone marrow-derived stem cells can differentiate into a wide variety of cell types including muscle (skeletal and cardiac). Furthermore, although the myocardium has for decades been regarded as a post-mitotic organ, a series of studies has indicated that a population of stem cells exists which is capable of at least partial reconstitution of the myocardium following an ischaemic insult. It is therefore now accepted that adult stem cells could be used to enhance myocardial repair. This review discusses the current status of adult stem cell research in the light of its potential for improving myocardial repair.

Cardiovasc J South Afr 2004; **15**: 184–189. www.cvjsa.co.za

Adult stem cells are defined as cells capable of long-term self-renewal while also maintaining their ability to differentiate into mature cells with specific morphological and functional characteristics (see Table I for a list of stem cell definitions). Unlike embryonic stem cells, which are characterised by their place of origin (the inner cell mass of the blastocyst), adult stem cells have been isolated from different adult tissues (such as bone marrow, blood, brain, pancreas, fat and skin). As a result, the isolation of embryonic stem cells brings with it a significant amount of ethical baggage (as a developing embryo must be used as the source), something which adult stem cells are not lumbered with. While embryonic stem cells are arguably more versatile than adult stem cells, too much versatility may also have side-effects such as tumour formation. Therefore, we may find that in the future, adult stem cells become the preferred option for stem cell therapy.

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TABLE I. STEM CELL AND PROGENITOR CELL DEFINITIONS

Adult stem cell	An unspecialised cell, derived from a postnatal animal, capable of self-renewal **AND** generating specialised mature cells
Hematopoietic stem cell	A stem cell, which can proliferate and differentiate into mature blood cells.
Mesenchymal stem cell	A stem cell, which can proliferate and differentiate into mesenchymal tissues (such as bone, cartilage, muscle)
Mesodermal progenitor cell	An unspecialised cell capable of yielding mesodermal tissue (such as muscle). Progenitor cells are not capable of self-renewal.
Haemangioblast	Earliest mesodermal precursor of both blood and vascular endothelial cells.
Angioblast	An endothelial cell progenitor cell.
Multipotent adult progenitor cell	Cells isolated from postnatal bone marrow that can differentiate into mesodermal, neuroectodermal and endodermal cells *in vitro* and into all embryonic lineages *in vivo*.

Can adult stem cells transdifferentiate into cardiomyocytes?

The function of an adult stem cell has previously been assumed to be restricted to the tissue from which it is derived. However, new evidence suggests that this stem cell retains far more plasticity than was once thought. Of particular relevance to myocardial repair are the studies that demonstrate that multi-potent adult stem cells isolated from a variety of tissues are capable of differentiation into cardiomyocytes (Table II). These studies have increased the potential therapeutic value of adult stem cells in comparison to their previously favoured competitors, the embryonic stem cells. Furthermore, although long considered to lack any regenerative capability, the post-mitotic nature of the heart has been called into question.

Evidence supporting the proliferative ability of human adult cardiomyocytes was reported in 1998 by Kajstura *et al*.[1] Since then, the mitotic capability of the myocardium has been further supported by tracking expression of the nuclear protein Ki67 (present only in dividing cells) in the

TABLE II. ADULT STEM CELL DIFFERENTIATION INTO CARDIOMYOCYTES

Origin of stem cells	*Stem cell type*	*Species*	*Route of application*	*Reference*
Bone marrow	Mesenchymal	Rat	Transendocardial	4
Bone marrow	Mesenchymal	Mouse	Transendocardial	5
Bone marrow	Mesenchymal	Rat	Systemic	6
Bone marrow	Mesenchymal	Rat	Transendocardial	7
Fat	Mesenchymal	Rabbit	*In vitro*	8
Peripheral blood	CD34 positive	Human	Intravenous	48

human heart post-infarct or post-transplant. Mitotic indexes of between 0.03% (zone distant from infarct) and 0.08% (zone adjacent to infarct) were measured *post mortem* in patients who had died four to 12 days after myocardial infarction.[2] A mitotic index of 0.9% was also reported in the cardiomyocytes of female hearts transplanted into male recipients.[3] These studies therefore refute the dogma that the heart is completely post-mitotic, and provide further evidence for the existence of progenitor cells that are capable of differentiating into mature cardiomyocytes. However, the low observed mitotic indexes indicate that, to achieve clinical relevance within the setting of repair post-infarct, the process would clearly need to be manipulated and enhanced.

How do you heal the modern heart?

There are three basic steps of clinical importance when considering the use of adult stem cells to promote tissue repair: mobilisation, homing, and differentiation (Fig. 1). The environmental signals that stimulate each step are still relatively unclear.

Mobilisation

In response to physiological stress (such as that induced during tissue injury), chemokines and cytokines are secreted from the affected cells, from immune cells and also from the bone marrow. These promote the mobilisation of stem cells from the bone marrow, and possibly other stem cell niches, into the peripheral circulation. Recent evidence suggests that matrix metalloproteinase-9 (MMP-9), stem cell factor (SCF) and ckit may play important roles in promoting the mobilisation of adult stem cells with cardiomyogenic potential, making them particularly relevant to myocardial repair.[9]

There is therefore evidence that this mobilisation occurs following myocardial ischaemia, however the extent may be insufficient to substantially promote cardiomyogenesis and consequently, fibrosis dominates. Alternatively, the circulating stem cells may not be sufficiently attracted to the damaged myocardium.

Homing

In 1999 Bittner *et al.* demonstrated that circulating progenitor cells may home to the heart and act *in vivo* to replace necrotic myocardial tissue.[10] The appropriate homing of circulating adult stem cells to the myocardium may be regulated by a number of receptor–ligand interactions. SCF is secreted by macrophages in the damaged myocardium

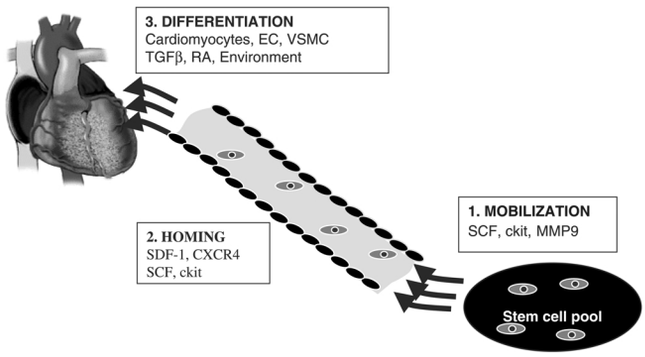

Fig. 1. Clinically important steps during adult stem cell transplantation and recruitment to the myocardium. EC: endothelial cell; VSMC: vascular smooth muscle cell; RA: retinoic acid; SCF: stem cell factor; SDF-1: stromal cell-derived factor 1; CXCR4: chemokine receptor 4.

and through interaction with its receptor ckit (commonly expressed on haematopoietic stem cells and now found to be expressed on putative cardiac stem cells), it could attract stem cells to the area of injury.[11]

Another recent study has found that stromal cell-derived factor 1 (SDF-1; a factor which promotes stem cell homing to bone marrow) is transiently expressed in the myocardium following infarction. The receptor for SDF-1, chemokine receptor 4 (CXCR4), is expressed on both haematopoietic stem cells and skeletal muscle satellite cells and its expression is important for the homing of circulating stem cells to SDF-positive niches such as the bone marrow and skeletal muscle respectively.[12-14] Therefore, it is conceivable that increased expression of SDF-1 in the damaged myocardium could also promote the trafficking of ckit-positive stem cells to the myocardial injury site.[14]

Differentiation

Appropriate environmental and physiological signals are required to promote optimal cardiomyocyte differentiation. A major concern in the use of stem cells for tissue repair is the risk that differentiation may not be 100%. Numerous cell models (e.g. embryonic stem cells, bone marrow cells, p19 embryonal carcinoma cells) have been used to further understand the factors regulating differentiation. Members of the transforming growth factor beta (TGFβ) superfamily of growth factors,[15-17] retinoic acid (RA),[18] ascorbic acid[19] and 5-azacytidine,[7,20-22] have all been shown to promote cardiomyogenesis *in vitro.* The use of 5-azacytidine on rat bone marrow mesenchymal stem cells has however subsequently been found to be ineffective unless these cells are immortalised, which calls into question the relevance of this stimulus (our unpublished data).[23] The cardiomyogenic stimulation of adult stem cell differentiation prior to transplantation may increase the percentage of terminal differentiation within the host tissue. However, the regulation of *in vitro* cardiomyocyte differentiation is still not understood well enough to apply this strategy as a routine therapy.

A landmark investigation by Orlic *et al.*[24] showed that, when injected into regions bordering the infarct zone, a subpopulation of bone marrow-derived adult stem cells

partially enhance the repair of the myocardium following infarct. Subsequently there has been a flurry of activity within the cardiac stem cell field.

Current research

Three main approaches have been taken in the quest to find the key to optimal myocardial repair (Fig. 2). The first relates to an improvement in the recruitment of endogenous stem cells, residing either in the bone marrow, the circulation, or in other stem cell niches such as the spleen, liver or fat. The second, and perhaps the currently favoured strategy, is cell implantation. Cell implantation involves the selection and isolation of autologous stem cells from an *in vivo* repository, the expansion of the cells *in vitro* and then the administration of the stem cells into the patient. The use of such autologous adult stem cell implantation would circumvent the problem of rejection. The third method revolves around the identification of resident cardiac stem cells and their proliferative stimulation, their migration towards, and differentiation within the injured area. Naturally for all three strategies the essential role of angiogenesis, to repair the damaged vascular supply, should not be overlooked, and indeed in many studies the transplanted stem cells have been shown to differentiate not only into cardiomyocytes, but also into vascular smooth muscle and endothelium.[1,2]

Recruitment

In the study by Bittner and colleagues (mentioned above), healthy bone marrow cells from a male murine donor were transplanted into muscular dystrophic female mice (mdx).[10] Y-chromosome-positive cardiomyocytes were subsequently found in the heart, indicating that endogenous bone marrow stem cells can migrate to the heart and differentiate into myocardial tissue. Further support that bone marrow-derived circulating stem cells can regenerate myocardial tissue was provided in a study using side population (SP) stem cells as donor cells.[25] The SP of bone marrow, isolated on the basis of their ability to extrude Hoechst 33342 (a nuclear dye), is a population highly enriched in haematopoietic stem cells, which have now been found to contribute to mesenchymal tissue repair.[25] In the Bittner study, the SP cells were transplanted to reconstitute the bone marrow of irradiated mice and three months later the myocardium was made temporarily ischaemic by left anterior descending artery occlusion. SP cells were subsequently found at the edge of the myocardial scar and also incorporated into newly formed blood vessels. A subsequent study later that year indicated that growth factor-stimulated mobilisation of the bone marrow could aid in myocardial repair.[26]

Mobilisation of lineage-negative (Lin^-), ckit-positive bone marrow stem cell using granulocyte colony-stimulating factor (G-CSF) and SCF increased the survival rate of infarcted mice from 17 to 73%.[26] Although infarct size in surviving mice was similar to that in the controls, a new band of myocardium was seen to occupy a large portion of the damaged area in the treated animals, and scar formation was minimal. No new myocardial tissue was observed in the damaged area of the control animals. However, a caveat

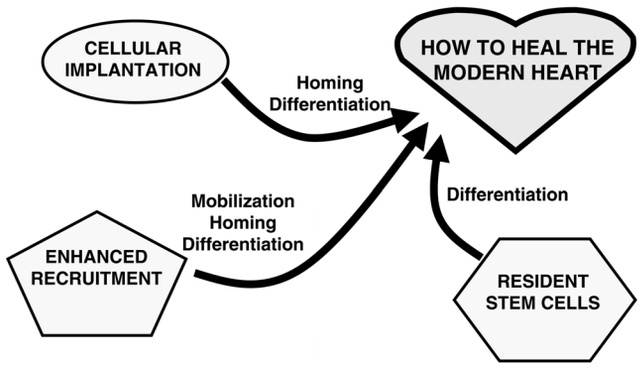

Fig. 2. Strategies for the use of adult stem cells to improve myocardial repair.

in the experimental protocol used is that the growth factors were administered prior to myocardial infarction, calling into question the direct therapeutic applicability of the findings. Furthermore, even though sufficient mobilisation of the stem cells should not be problematic (five million bone marrow-derived stem cells can be mobilised following several days of G-CSF administration), there is a concern that the accompanying increased level of leucocytes could potentially destabilise atherosclerotic plaques that probably already exist in an infarcted patient.

Cellular transplantation

Cellular cardiomyoplasty using adult stem cells holds enormous potential to repair the myocardium. Donor stem cells that have been most commonly used are derived from the bone marrow, but progenitor cells from skeletal muscle[27-30] and embryonic stem cells[31,32] have also been employed. A critical step in cellular transplantation is determining an efficient method of delivery. Intravenous delivery, although less invasive, may not be able to target sufficient stem cells to the damaged area. Intracoronary delivery has been used successfully in humans, however cells must be administered slowly to prevent cell clumping and embolism.[43,46] Transendocardial administration (into myocardial tissue bordering the infarct), although invasive, has also been shown to be successful in numerous animal models as well as in humans, and may be the way forward as this type of strategy delivers cells directly into the cardiomyogenic milieu (Table III).[44,45] However, direct injection into the avascular fibrotic area would in all likelihood not favour cardiomyogenesis and therefore this therapeutic approach may require pre-programmed differentiation of the stem cells down the cardiomyocyte lineage prior to implantation.

In 1999 Tomita and co-workers showed that mesenchymal bone marrow cells, which had been differentiated into myotubes using 5-azacytidine, stimulated angiogenesis when injected into the scar tissue of a cryo-injured rodent heart.[7] These injected differentiated cells also decreased left ventricular chamber hypertrophy and improved systolic and developed pressure, whereas undifferentiated bone marrow mesenchymal stem cells did not. This study indicated that, when appropriately administered, bone marrow stem cells could be incorporated into myocardial tissue and it further

TABLE III. FINDINGS FROM STUDIES USING TRANSENDOCARDIAL INJECTION OF BONE MARROW STEM CELLS TO IMPROVE MYOCARDIAL REPAIR

Population	*Species*	*Injection site*	*Delivery (post-infarct)*	*Cell number*	*Effects*	*Reference*
Adherent differentiated	Rat	Scar	21 days	10^6	Improved myocardial function	7
Adherent Lin^-	Mouse	Border	10 minutes	$5 \times 10^4 – 5 \times 10^5$	Reduced infarct size and fibrosis	5
Adherent differentiated	Rat	Scar	21 days	3×10^6	Myocytes in scar No functional studies	4
Mononuclear cells	Swine	Border and infarction	1 hour	10^8	Improved myocardial function	35
Lin^-, $ckit^+$	Mouse	Border	5 hours	$1.5 \times 10^4 – 1 \times 10^5$	Improved myocardial function	24
Unfractionated	Canine	Normal, border and infarction	30 days	2×10^7	No global improvement	34
Unfractionated	Sheep	Infarction	21 days	422×10^6	No engraftment No global improvement	33

suggested that differentiation into myogenic cells prior to transplantation may be advantageous to cardiac function post-repair. Further studies have found that injection of 5-azacytidine-treated bone marrow stem cells as late as three weeks post-damage increased the number of cardiomyocyte-like cells in the injured area.[4] However these cells did not form gap junctions with host tissue, suggesting that in the long-term, complete integration and synchronous contraction may not take place.

In 2001, Orlic *et al.* showed for the first time that lineage-negative (Lin^-), $ckit^+$ bone marrow cells, when injected into the myocardium bordering the infarct, can generate new myocardium consisting of myocytes, endothelial cells and smooth muscle cells. These new cardiomyocytes did integrate successfully within the myocardium (judged by expression of connexin 43) and the mice displayed an improvement in haemodynamic function.[24] A more recent study supported these findings by demonstrating that both cultured bone marrow stromal cells and Lin^- bone marrow cells can differentiate into cardiomyocytes and endothelial cells post-transplantation, and can reduce both infarct size and fibrosis.[5]

The timing and site of transplantation as well as the number and population of bone marrow cells is very important to ensure optimal engraftment and cardiac recovery (Table III). Two studies have shown that if injected (a) too late following myocardial damage and (b) into the scar rather than into the myocardial zone bordering the infarcted site, bone marrow stem cells will not facilitate significant improvements within the scar itself.[33,34] However, if injected within hours of the infarction into the ischaemic zone, an improvement in cardiac function was seen.[24,35]

Unlike cardiac muscle, it is well known and accepted that skeletal muscle contains a population of resident progenitor cells (satellite cells) which are normally quiescent but can be activated in response to conditions where either muscle repair or hypertrophy is required.[36] These represent a relatively accessible source of adult stem cells for repair and have therefore been analysed as a potential source of contractile tissue. However, although successfully transplanted and able to improve cardiac function in some studies,[27,37] these cells do not integrate into or electromechanically couple with the recipient myocardium[38] and do not trans-differentiate into cardiomyocytes in other studies.[39] Hence any potential use of satellite cells to repair infarcted myocardium is unclear.

Resident stem cells

Given the difficulties experienced in the studies reviewed above, the existence of putative cardiac stem cells in the myocardium itself has generated much excitement. The first indication that the myocardium may harbor progenitor cells arose from the studies of Quaini *et al.* and LaFlamme *et al*.[3,40] These groups studied human female-to-male transplants and used the presence of the Y-chromosome to indicate cells of extra-cardiac origin. Although there were discrepancies regarding the number of host-derived cardiomyocytes in the graft (0.04% vs 18% in the study by the LaFlamme and Quaini groups, respectively), these studies did indicate that some type of progenitor cells infiltrate the transplanted heart.

A subsequent study determined that a side population (SP) of cells exists within the adult heart, which may be a resident cardiac stem cell-like population.[41] More recently, clusters of Lin^-, $ckit^+$, stem cell antigen $(Sca\text{-}1)^+$ and $telomerase^+$ cells have been identified in the heart. The presence of these markers further supports the presence of resident cardiac stem cells.[42] In our own studies, we have also detected a small number of $ckit^+$ and $MDR1^+$ cells within the infarcted rat myocardium (unpublished data), which agrees with the recent report by Urbanek *et al*.[42] These stem cells could be capable of re-entering the cell cycle and contributing to an increase in cardiomyogenesis, if stimulated correctly.

Important insights from clinical trials

A number of small clinical trials are currently underway to test the efficacy and safety of using autologous bone marrow-derived or circulating stem cells to promote myocardial repair. Whereas the transplantation of skeletal myoblasts has resulted in ventricular arrhythmias, preliminary data from stem cell trials have not indicated arrhythmia to be a problem.[43]

The preliminary results of some of the trials (Table IV) have suggested a beneficial therapeutic effect of adult stem cell transplantation (bone marrow-derived or circulating)

with no apparent adverse side-effects. However it must be emphasised that these results have been generated from short-term, non-randomised trials with low patient numbers. Furthermore, in human studies, proof of transdifferentiation of injected stem cells is lacking. It is therefore too early to draw landmark conclusions.

Transdifferentiation versus facilitation

Although many animal studies have shown that adult stem cells do transdifferentiate into cardiomyocytes, whether they act entirely of their own accord to bring about myocardial improvement is unclear. In the majority of cases, researchers have reported a significant increase in angiogenesis in addition to the differentiation into cardiomyocytes.[7,10,24,25] Newly formed endothelial cells could play an important supporting function by acting as a paracrine signaling pathway to promote mitosis and differentiation of stem cells post-damage. Furthermore, new blood vessels could provide an additional supply of blood-borne stem cells. In human studies, investigators have also demonstrated an increase in angiogenesis.[34,47,49] In these studies, the injected stem cells were not tagged, making it difficult to establish firstly, that they indeed had differentiated and secondly, a cause–effect relationship between the stem cells and cardiac improvement.

Conclusion and cautionary remarks

The use of adult stem cells to promote repair following an ischaemic insult to the myocardium holds much promise. Animal studies have proven the validity of using adult stem cells to improve myocardial repair post-infarct. An obvious significant advantage of adult stem cells is the potential of autologous transplantation, thereby preventing host rejection. Studies using bone marrow stem cells have not reported any neoplasias, an adverse effect which may occur with other stem cell types. It should also be added that stem cell therapy is not limited to the treatment of ischaemic cardiac disease. Non-ischaemic cardiomyopathy may also be treated by stem cells. Cell transplantation experiments have been carried out in animal models of cardiac diseases (such as dilated cardiomyopathy) and have been shown to improve myocardial performance and to limit ventricular dilatation.[50] The number of experiments carried out in animals and humans with non-ischaemic cardiomyopathy is limited, but they do provide promising preliminary data.

From the successful small number of non-randomised clinical trials, it is reasonable to conclude that the use of bone marrow or circulating adult stem cells is safe, at least in the short-term. However, both experimental and clinical studies are still required to determine what the optimal cell source, the cell number, the delivery route, the timing of delivery and also the long-term safety implications are.

TABLE IV. CLINICAL TRIALS TO TEST SAFETY OF ADULT STEM CELL TRANSPLANTATION

Strategy	*Trial reference*	*Results*
Transendocardial	Perin *et al*. 2003[44]	14 patients: bone marrow Improved ventricular function (2 months) Improved ejection fraction (4 months)
	Tse *et al*. 2003[45]	8 patients: bone marrow Improved target wall thickening (3 months) ↓ anginal episodes (3 months)
Intracoronary	Strauer *et al*. 2002[46]	10 patients: bone marrow ↓ infarct size (3 months)
	Assmus *et al*. 2002[47] (TOPCARE-AMI)	9 patients: bone marrow 11 patients: blood derived Improved ejection fraction (4 months) ↓ end-systolic left ventricular volume (4 months)

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Comment

While debate about the use of embryonic stem cell therapy is waged in political, ethical and scientific circles, Niesler provides a highly topical, succinct review of the potential uses of the less emotive adult stem cells in promoting myocardial repair of ischaemic damage. Evidence is presented that challenges long-held views that adult stem cells are limited in their potential to differentiate into specialised cells, and specifically addresses the ability of selected cells to differentiate into cardiomyocytes. It also questions the tenet that the heart lacks a reservoir of cells capable of self-renewal and highlights the exciting prospect of the use of adult stem cells in autologous transplantation, thereby circumventing rejection.

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