Adult bone marrow-derived stem cells and the injured heart: just the beginning?

Ioannis Dimarakis\textsuperscript{a,}\textsuperscript{*}, Nagy A. Habib\textsuperscript{a}, Myrtle Y.A. Gordon\textsuperscript{b}

\textsuperscript{a}Department of Surgical Oncology & Technology, Imperial College Faculty of Medicine, Imperial College of Science, Technology and Medicine, Hammersmith Hospital Campus, Du Cane Road, London W12 ONN, UK

\textsuperscript{b}Department of Haematology, Imperial College of Science, Technology and Medicine, Hammersmith Hospital, London, UK

Received 21 March 2005; received in revised form 10 August 2005; accepted 11 August 2005; Available online 27 September 2005

Summary

Coronary artery disease leading to ischaemia of the human myocardium is one of the chief causes of morbidity and mortality in the western world. Cellular transplantation has recently been proposed as a novel alternative treatment modality. Adult bone marrow-derived autologous cells are one of the key cell types under investigation in both the experimental and clinical setting. A range of theories has been proposed with regard to the observed myocardial function improvement in both human and animal studies. A concerted interest in scientific questions needs to be constructed on the regenerative information made available throughout the last years. It is only now that we begin to fully understand this fast growing body of research and how it may have wide ranging implications for the treatment of ischemic heart disease.

\textcopyright{} 2005 Elsevier B.V. All rights reserved.

Keywords: Stem cells; Ischemic heart disease; Myocardial infarction; Myocardial injury; Cellular transplantation

1. Introduction

Congestive heart failure (CHF) is the common pathophysiologic endpoint of a number of diseases that affect the human myocardium. Degenerative changes can be identified at multiple levels including the nuclear (altered gene expression), the cellular (cardiomyocyte hypertrophy) and organ level (pump failure). Persistently high incidence and prevalence of heart failure in the western world can explain the growing interest of the scientific community in developing more effective treatment modalities\cite{1—3}. Although cardiac transplantation remains the sole radical treatment for end stage CHF, shortage of donors along with immunosuppression complications pose severe limitations to this approach. Techniques involving reshaping of the dilated left ventricle have been described and involve either endoventricular circular patch plasty (Dor procedure) or passive constraint and shape change\cite{4,5}. Left ventricular assist devices serving mainly as a bridge to cardiac transplantation cannot be considered a long-term solution.

Coronary artery disease with its clinical sequelae continues to be one of the leading causes of CHF. Following myocardial infarction, cardiomyocyte death and segmental scarring eventually contribute to the development of replacement fibrosis. The impact of myocardial fibrosis on the remaining healthy myocardium may range from sub-

\textsuperscript{*} Corresponding author. Tel.: +44 20 8383 2047; fax: +44 20 8383 3212. E-mail address: ioannis.dimarakis@imperial.ac.uk (I. Dimarakis).

Clinical to detrimental. The remodelling process initiated will eventually lead to impairment of left ventricular function\cite{6}. In an attempt to reverse the natural progress of the disease several alternative novel approaches are being considered. These include cellular transplantation in which de novo introduced cells are expected to repopulate areas of diseased myocardium.

2. Challenging old dogmas: regenerative potential of native human myocardium

Adult cardiomyocytes have traditionally been considered post-mitotic end-differentiated cells lacking the ability of division and proliferation following ischemic injury. Cardiomyocyte hypertrophy has been regarded the sole mechanism of compensation for loss of functional myocardium. Cellular proliferation has been theoretically restricted to endothelial and fibroblast cell populations leading to the development of collateral circulation and scar formation, respectively. A recent influx of experimental as well as clinical data is questioning the above postulates. Even though from a quantitative point of view these events may be of very limited magnitude they should be mentioned in brief as they shed light on myocardial cell biology.

The ability of terminally differentiated cells to re-enter the cell cycle has been already illustrated by Endo and Nada Ginard\cite{7}. Interestingly, when Kajstura et al. examined control human hearts by confocal microscopy about $14 \times 10^6$
myocytes were seen in mitosis. These figures were increased nearly tenfold in end stage ischaemic heart disease and idiopathic dilated cardiomyopathy [8]. It is apparent that a slow turnover of myocytes exists providing a possible explanation of why pathologies linked with myocyte death, such as diabetic cardiomyopathy do not lead to myocardial cellular ‘wipe-out’ [9]. In addition, Beltrami et al. by means of labelling for the nuclear antigen Ki-67 in post-mortem infarcted hearts demonstrated 4% of myocyte nuclei undergoing mitosis in the infarct border [10].

The origin of these cells undergoing cellular division remains to be ascertained. Whether arising from a resident cardiac stem cell population or having been recruited within the heart from the systemic circulation, ultimately being derived from the bone marrow awaits to be answered.

Resident cardiac stem cells capable of self-renewal and myocardial regeneration have been identified in rodent hearts by at least two groups. In one of these studies Lin⁺ c-kit⁺ cells were able to differentiate in vitro into all three main myocardial cell types (myocardial, endothelial and smooth muscle cells) as well as form de novo myocardium when injected into ischaemic rat myocardium [11]. Oh et al. published similar data by administering intravenously Sca-1⁺ cells isolated from mouse hearts into ischaemic mice [12]. Only recently, however, have Laugwitz et al. identified a resident population of cells within the murine, rat and human heart that is directly derived from a cardiac progenitor cell population [13]. These cells are characterised by the expression of the Islet1 gene and can be traced in the myocardium from the early embryonic stage. Islet1⁻ cells maintain the ability to self-renew and grow into mature cardiac myocytes under appropriate conditions.

Evidence is also accumulating in favour of cell recruitment from the systemic circulation within the myocardium. Bayes-Genis et al. reported the presence of cardiac chimerism in recipients of peripheral blood and bone marrow stem cells with the use of a sensitive polymerase chain reaction assay for donor and recipient genotyping [14]. Furthermore, homing of recipient progenitor cells in the myocardium has been shown in sex-mismatched cardiac transplantations [15,16]. Based on their observations, Quaini et al. hypothesise that the donor heart harbours a population of resident primitive cells as well as attracting a second population of progenitor cells from the recipient.

More importantly Kucia et al. have demonstrated that the postnatal BM harbours a non-haematopoietic population of cells that express markers for cardiac differentiation. They hypothesise that it may be these cells that are responsible for any observed myocardial regeneration attributed to bone marrow-derived stem cells [17].

3. Overview of stem cells in myocardial regenerative studies

3.1. Adult bone marrow-derived stem cells

The attractive feature of adult stem cells for researchers in the field of myocardial regeneration is their innate plasticity. Minimal criteria for stem cell plasticity as outlined by Verfaillie include capability for self-renewal, ability of a single cell to differentiate into cells of the tissue of origin and at least one different cell type, and functional differentiation in vivo into cells of the tissue of origin and at least one cell type other than the tissue of origin [18]. At least two distinct populations of stem cells reside within the adult bone marrow: haematopoietic stem cells (HSCs) and mesenchymal stem cells (MSCs). The former are the precursors of all blood lineages and the latter give rise to stromal cells of the bone marrow including osteogenic, chondrogenic and adipogenic lineages. It has recently been suggested that the bone marrow harbours additionally non-haematopoietic tissue-committed stem cells; these cells appear to be highly mobile and express mRNA/proteins for various markers of early tissue-committed stem cells [19].

The sole assay available at present for identification of HSCs remains the reconstitution of the haematopoietic system of a myeloablated host. Surface markers such as CD34 or the more primitive CD133 (AC133) help in distinguishing subpopulations enriched in HSCs in humans; these include the CD34⁺ CD38⁻ cell population as well as the side population. The latter cell type may reside in various organs besides the bone marrow and there is conflicting data as to the origin of these cells with some evidence supporting the fact of them actually being bone marrow-derived HSCs rather than tissue-specific [20,21]. As murine progenitor cells do not express the same surface markers, lineage depletion (Lin⁻) is the major criterion to identify enriched populations [22]. Endothelial progenitor cells or angioblasts and HSCs are thought to share a common precursor, the haemangioblast [23]. Studies have shown the connection between angioblasts residing within the bone marrow and neovascularization [24,25]. MSCs (also known as marrow stromal cells) have two main characteristics: the ability to adhere to culture dishes as well as to differentiate into a variety if tissues under the appropriate conditioning. In addition, they appear uniformly negative for typical ‘haematopoietic’ surface markers.

A very appealing adult bone marrow-derived population of high plasticity was reported by the Minnesota group [26]. These cells which are known as multipotent adult progenitor cells (MAPCs) were isolated from mesenchymal stem cell cultures and maintain the ability to differentiate in vitro in cells of the three germ layers. Only recently has another group reported the identification of a similar subpopulation from adult human bone marrow [27]. Intramyocardial transplantation of these cells in a rodent myocardial infarction model lead to in vivo differentiation into multiple lineages including cardiac, endothelial and smooth muscle cell phenotypes.

Besides, the bone marrow several sources of adult stem cells are known. Zuk et al. have demonstrated that adipose tissue contains both HSCs and mesenchymal stem cell populations [28]. Peripheral blood is also a source of HSCs and endothelial progenitor cells. The latter may be isolated from blood and used for cellular transplantation.

3.2. Other stem cell types

Even though this review focuses on adult bone marrow-derived stem cells it should be mentioned that the armamentarium of potential donor cells is not strictly confined to this group. Ethical considerations continue to
be the chief argument against clinical or even laboratory research involving embryonic stem cells. These totipotent/pluripotent cells may be derived from embryos at the 100-cell-stage blastocyst and are capable of differentiation into all somatic cell types in vitro, including cardiomyocytes. Other issues complicating clinical adaptation include insufficient availability, associated disadvantages of allograft transplantation, unpredictable electrical behaviour as well as the risk of tumour formation.

Umbilical cord blood stem cells comprise another promising group of cells. Compounding a relatively immature nature with potential for autologous cell transplantation they may pose in the future as a patient-specific donor cell type immediately available from cryopreservation tissue banks.

An autologous population of established phenotype that overcomes most of the above restrictions is skeletal myoblasts. These precursor cells residing within skeletal muscle can be easily obtained by muscle biopsy. They may be expanded in culture, are ischaemia resistant and provide fatigue resistant, slow-twitch fibres. When compared to CD133+ bone marrow-derived haematopoietic progenitor transplantation in an animal model similar results of functional improvement were recorded [29]. Encouraging phase I clinical trial data also exists incorporating both transepicardial and trans-coronary-venous approaches [30,31]. The main concern of introducing skeletal myoblasts within myocardial tissue is the lack of electrical communication with native myocytes. There has been no demonstration of gap junction formation post-transplantation, which may potentially contribute to the generation of arrhythmias seen in patients receiving these cells. Recently, genetic modification of myoblasts to express the gap junction protein connexin43 has been proposed as an antiarrhythmic measure [32]. Once again it may be questioned why anyone attempt ‘connecting’ to the native electrical circuit cell groups with an unpredictable electrical behaviour.

4. Homing of stem cells to injured myocardium

Little is known with regard to the regulatory mechanisms that control the homing and holding of haematopoietic progenitor cells within the bone marrow. Of significant clinical importance and relevance is the CXCR4/SDF-1 chemotactic axis. Stromal cell-derived factor-1 (SDF-1/CXCL12) is a marrow stroma and bone-derived chemokine chemotactic axis. Stromal cell-derived factor-1 (SDF-1/CXCL12) is a marrow stroma and bone-derived chemokine thought to form a decreasing gradient from the extravascular toward the intravascular compartment within the bone marrow. Implicated in bringing about the relocation of fetal progenitor cells within the bone marrow. Of significant that control the homing and holding of haematopoietic progenitor cells within the bone marrow. Of significant clinical importance and relevance is the CXCR4/SDF-1 chemotactic axis.

Bone marrow-derived progenitor cells are well known to migrate to localised areas of injury following ischaemic insults. Two factors that seem to be associated with this process are SDF-1/CXCL12 and vascular endothelial growth factor (VEGF) [37,38]. It has been demonstrated by Ceradini et al. that SDF-1 gene expression is partially controlled by the transcription factor hypoxia-inducible factor-1 (HIF-1) transcribing thus to increased levels in the presence of tissue hypoxia [39]. HIF-1 is also an early transcription factor for VEGF, which is also up-regulated in myocardial infarction [40,41]. This has been confirmed in human ventricular biopsies taken during surgical revascularization procedures following myocardial infarction [42]. Locally delivered SDF-1 may enhance vasculogenesis and subsequently contribute to ischaemic neovascularization as shown in an ischaemic hind limb model [43]. Finally, by blocking the binding of SDF-1 to receptor CXCR4 in a rodent myocardial infarction model inhibition of stem cell homing has been seen [44].

It has been suggested that the establishment of a positive concentration gradient between ischaemic tissue and bone marrow promotes mobilization and subsequent homing to sites of injury [45]. In theory, this would be in agreement with the observation that granulocyte colony-stimulating factor (G-CSF) achieves bone marrow mobilization by decreasing the bone marrow levels of SDF-1 [46]. Kucia et al. have only recently suggested that the bone marrow actually may act as a ‘hideout’ for non-haematopoietic CXCR4+ tissue-committed stem cells opening new areas of research [19].

It is apparent that mobilization and subsequent homing of stem cells is a dynamic process involving various chemokines, cellular surface receptors, metalloproteinases, and adhesion molecules in addition to remodelling of the extracellular matrix. Besides, the aforementioned factors a variety of factors and mediators are produced as a result of ischaemic injury [47]. Even if not directly involved in generating homing signals their significance may lie in creating the appropriate microenvironment required for integration of the homed cells.

5. Representative small animal studies

The contribution of small animal myocardial infarction models has proven invaluable in assessing various cell populations for potential myocardial regenerative capability. It was not than from a small number of studies that the initial encouraging data of myocardial regeneration following cellular transplantation of bone marrow-derived stem cells emerged. In one of the earlier reports, the transplantation of autologous bone marrow cells (BMCs) was documented to stimulate angiogenesis in the recipient ischaemic myocardium [48]. Having been labelled previously with bromodeoxyuridine (BrDU) cells were implanted directly into a myocardial scar created by cryoinjury three weeks earlier. When explanted hearts were examined eight weeks post transplantation, increased capillary density was seen in comparison to the control group, with BrDU positive cells present within the wall of the newly formed vessels. Functional improvement was observed only in the recipients of the mesenchymal stem cells that had been treated with 5-azacytidine.

Undisputedly, the most important paper of this era was published two years later by Orlic and co-workers in 2001 [49]. In a mouse ligation-induced myocardial infarction model, the transplantation of autologous lineage negative (Lin−) bone marrow cells in the acute ischaemia phase was assessed. Cells were isolated from male transgenic mice expressing enhanced green fluorescent protein and the
receptor for stem cell factor (c-kit+) and injected directly into the myocardium of female mice 3–5 h after coronary ligation. When hearts were removed 9 days post transplantation, proliferating myocytes and vascular structures occupied 68% of the infarcted segment of the left ventricle. The authors concluded that Lin– c-kit+ bone marrow cells have the capability of regenerating acutely significant amounts of contracting myocardium.

An interesting protocol to investigate the regenerative capacity of the side population (highly enriched haematopoietic stem cell fraction residing within the bone marrow) was developed by Jackson and colleagues [50]. Lethally irradiated mice transplanted with side population cells were subsequently rendered ischaemic by transient coronary occlusion and reperfusion. Mice were sacrificed at two to four weeks after injury. Examination of the hearts once more suggested that the bone marrow cells were able to transdifferentiate and engraft into myocardium and endothelial cells. In another model of myocardial ischaemia, systemic infusion of human bone marrow-derived endothelial cell precursors was able to interrupt the remodelling process of the left ventricle [51]. The observed neovascularization prevented apoptosis of hypertrophied myocytes reducing collagen deposition and subsequent scar formation. Post-transplantation ventricular function improved as well.

Taking their previous work a step further Orlic et al. hypothesised that cytokine mobilization of the animals own bone marrow should increase the trafficking of desirable cells to the site of myocardial injury [52]. Not surprisingly for them, a 68% decrease in mortality with significant haemodynamic improvement was documented when cytokine (stem cell factor and G-CSF) mobilization was undertaken in lethally irradiated mice transplanted with side population cells were subsequently rendered ischaemic by transient coronary occlusion and reperfusion. Mice were sacrificed at two to four weeks after injury. Examination of the hearts once more suggested that the bone marrow cells were able to transdifferentiate and engraft into myocardium and endothelial cells. In another model of myocardial ischaemia, systemic infusion of human bone marrow-derived endothelial cell precursors was able to interrupt the remodelling process of the left ventricle [51]. The observed neovascularization prevented apoptosis of hypertrophied myocytes reducing collagen deposition and subsequent scar formation. Post-transplantation ventricular function improved as well.

In all the above studies, cell administration was performed by direct intramycocardial injection. Owing to technical difficulties, intracoronary infusion in a rodent model cannot be performed in the same way as in large animal models or humans. In an attempt to reproduce intracoronary delivery, two weeks after coronary artery ligation Wang et al. infused, under direct vision, mesenchymal stem cell suspensions in briefly occluded ascending aortas [53]. Injected cells could be identified after three weeks as cardiomycocytes or fibroblasts. Once more it was concluded that cells migrated through the coronary circulation to sites of injury and under the effect of the local microenvironment transdifferentiated into required phenotypes.

The above studies constitute a mere representative sample of small animal studies involving adult bone marrow-derived stem cell populations. A vast number of projects are continuing to be published since these reports investigating a variety of cell types in conjunction with various cytokines or even gene therapy techniques. Whole bone marrow [54,55], mononuclear cell fractions [56], MSCs [57–59], angioblasts [60,61] and even novel subpopulations [62] are being scrutinised.

6. Representative large animal studies

Even though small animal models can offer great insight in cellular transplantation it is large animal constructs that resemble humans the closest. Unquestionably it is non-human primate models that are most similar to humans. A remarkable similarity exists between the porcine and human coronary anatomy making this model favourable to scientists. Finally, the canine and ovine models should not be overlooked as they may provide useful alternatives. Apart from anatomic and physiologic proximity these models provide the ability to perform minimally invasive procedures (angiography, coil embolization, etc.) with instrumentation developed and used in human subjects. In addition, periprocedural assessment may be performed with conventional imaging techniques thus avoiding the need for developing or even adapting equipment to the anatomic and physiologic standards of small animals.

Autologous bone marrow-derived cells were injected by Fuchs et al. via the transendocardial route guided by ventricular electromechanical mapping in pigs previously rendered ischaemic [63]. Regional wall thickening was increased in the order of 50% as measured by echocardiography in the cell transplanted group while no significant change was seen in the control group. Of interest is the fact that the only observed improvement in myocardial perfusion was localised entirely in injected ischaemic areas. In neither group was a positive effect in regional myocardial blood flow demonstrated. Histopathological examination revealed a 100% increase in total endothelial cell area in the ischaemic collateral-dependent zone compared with the non-ischaemic territory of the cell treated group.

Four weeks after occlusion of the left anterior descending artery with coils and Gelfoam sponge Tomita et al. injected directly into porcine ischaemic myocardium BrdU labelled marrow stromal cells [64]. Animals were sacrificed 4 weeks after cellular transplantation. Tissue slices from transplanted areas contained a number of BrdU labelled cells that stained positive for troponin I; some endothelial cells from the same areas also stained positively for BrdU. Although the authors were unable to identify any junctions between these cells and host cardiomycocytes, once more a significant improvement both in myocardial blood flow and left ventricular performance was shown by scintigraphy in the cell transplant group compared to the control animals. Shake et al. also demonstrated engraftment, using double staining for membrane and muscle-specific proteins, along with functional improvement [65]. This was once again performed with transplantation of mesenchymal stromal cells in pigs with left anterior descending coronary artery ligature-induced ischaemia.

The toxicity and therapeutic potency of autologous bone marrow cell transplantation has been also assessed in a canine chronic coronary occlusion model by Hamano et al. [66]. Thirty days after left anterior descending coronary artery ligation autologous BMCs were introduced directly into infarcted, marginal as well as healthy myocardium. The control group received injections of phosphate-buffered saline in respective sites. Cardiac function along with local wall motion was evaluated by echocardiography thirty days after implantation. Angiogenesis was measured by microvessel counting under light microscopy. A significant increase in local wall thickening and density of microvessels was documented only in the marginal area of the BMC treated group. No further differences were seen between the two
groups. Once again no systemic or local toxicity was observed.

Investigating whether increased cardiac nerve density can contribute to improved cardiac function Pak et al. observed overexpression of cardiac tenascin, increased cardiac nerve sprouting and amplified magnitude of atrial sympathetic hyperinnervation two months post transplantation of mesenchymal stem cells [67].

In a non-human primate model, male and female baboons were treated with haematopoietic growth factors following ligation of the lateral branches of the circumflex coronary artery [68]. Norol et al. showed regeneration of vascular structures in treated animals, but no evidence of cardiac cell differentiation. Although in a few animals regional myocardial blood flow for the infracted area increased, no overall improvement was seen in myocardial function between the two groups.

A controversial study in a canine model carried out by Vulliet et al. reported microinfarctions in subjects following intracoronary infusion of mesenchymal stromal cells [69]. Early passage cells were infused into the circumflex coronary artery of healthy dogs. Hearts were examined after 7 days and the presence of microinfarctions in areas supplied by the circumflex artery was documented.

A great interest has also been shown in similar models to develop reliable techniques that will facilitate implantation and continuous tracking of stem cells into myocardial tissue [70–72]. A percutaneous transvenous approach through the coronary venous system has also been described and demonstrated in a porcine model [73].

7. Human trial data

The abovementioned pre-clinical data have encouraged clinicians to initiate clinical studies/trials of adult stem cell transplantation in myocardial ischaemia (Table 1). All research protocols must be in accordance with the Declaration of Helsinki for medical research involving human participants [74]. Crucial aspects of every study design include the selection of the cell type to be delivered, timing of delivery with regard to the natural progress of the disease as well as the optimal route of delivery. Factors guiding researchers are the phase of disease (acute myocardial infarction or well-established myocardial ischaemia) and patient’s clinical status (end-stage heart failure patients cannot be enrolled in a trial were cell delivery is combined with open-chest surgery).

7.1. Transepicardial delivery

First clinical confirmation of the safety of adult bone marrow stem cell transplantation was demonstrated by Hamano et al. [75]. The five patients enrolled in this pilot study underwent local implantation of the mononuclear fraction of autologous bone marrow in conjunction with standard coronary artery bypass graft (CABG) surgery. Bone marrow harvested and processed during the CABG procedure was injected directly into ischaemic myocardial areas not amenable to grafting. The control group consisted of thirty patients having standard CABG during the same period. All participants made an uncomplicated recovery with no new-onset arrhythmia or significant bleeding seen. Significant improvement was documented in 60% of patients at one-year follow-up as assessed with myocardial scintigraphy. An observation worth mentioning is that computed tomography one year after treatment did not reveal the presence of calcification or mass in the injected myocardium.

Clinical safety and enhancement of cardiac function has been reported from one more group following a very similar protocol [76]. Before sternal split, 5–7 ml of bone marrow was aspirated from the sternum of 14 patients. CABG was carried out once again while the bone marrow was prepared by an aseptic technique. All scarred areas in the left ventricle besides the ones residing within the intraventricular septum were injected with an unmanipulated cell preparation. Functional improvement was only seen in areas were auto transplantation was combined with bypass grafting. Once again no complications were documented and benefit was maintained at ten months of follow-up.

In mid 2001 the Rostock group recruited patients for the first phase-1 study of autologous BMC intramyocardial implantation [77,78]. Having aspirated autologous bone marrow the day before surgery, selection of CD133+ cells took place. From the previously isolated mononuclear fraction CD133+ cells were selected with the use of specific monoclonal antibodies conjugated to superparamagnetic particles and passage through a CliniMACS Magnetic Cell Separation device (Miltenyi Biotech, Germany). The final cell suspension was injected the following day in the border zone of an infarct that was not accessible to routine revascularization. All participants made an uncomplicated recovery with no procedure-related complications recorded up to 14 months after transplantation, including new arrhythmias or neoplastic changes. A significant improvement in both left ventricular function and myocardial perfusion was reported by echocardiography and thallium scans.

In an attempt to reduce manual cell handling under GMP compliant conditions, Ghodsizad and coworkers published a refined method that enables intraoperative isolation and processing of BMCs in patients simultaneously undergoing coronary surgery [79]. Following standard CABG, LASER channels were shot in predefined areas within the hibernating myocardium. CD133+ cell separation and processing was concurrently performed in an area connected to the operating room where the patients underwent surgery. The purified autologous fraction was subsequently injected around the laser channels. Cellular transplantation in the emergency setting is, therefore, a theoretical possibility as suggested by the authors.

The feasibility of combining off-pump coronary artery and autologous bone marrow-derived CD34+ transplantation was shown by Nagamine et al. [80]. Using the Isolox 300i magnetic cell selection system, CD34+ were selected and injected directly into ischaemic myocardium. Improved perfusion of ungraftable ischaemic areas was reported.

The method of cell collection in the above studies has been solely bone marrow harvest, mainly from the iliac
Table 1  
Adult stem cell therapy studies/trials

<table>
<thead>
<tr>
<th>Study</th>
<th>Delivery route</th>
<th>Study groups</th>
<th>n</th>
<th>Cell type</th>
<th>Cell dose</th>
<th>Evaluation modes</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hamano et al. [75]</td>
<td>Transepicardial</td>
<td>BMCs</td>
<td>5</td>
<td>Mononuclear cells</td>
<td>0.3—2.2×10⁹</td>
<td>Echocardiography</td>
<td>Safety confirmation</td>
</tr>
<tr>
<td></td>
<td>Control</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Galinanes et al. [76]</td>
<td>Transepicardial</td>
<td>BMCs</td>
<td>14</td>
<td>Unfractionated BM</td>
<td>From 5 to 7 ml BM</td>
<td>Echocardiography</td>
<td>Segmental wall motion score</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stamm et al. [77,78]</td>
<td>Transepicardial</td>
<td>BMCs</td>
<td>12</td>
<td>AC133⁺</td>
<td>1.02—2.8×10⁶</td>
<td>Echocardiography</td>
<td>Perfusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ghodzizad et al. [79]</td>
<td>Transepicardial</td>
<td>BMCs</td>
<td>5</td>
<td>CD133⁺</td>
<td>1.9—9.7×10⁶</td>
<td>Echocardiography</td>
<td>EF</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nagamine et al. [80]</td>
<td>Transepicardial</td>
<td>BMCs</td>
<td>1</td>
<td>CD34⁺</td>
<td>2.1×10⁷</td>
<td>Echocardiography</td>
<td>End-systolic volume</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pompilio et al. [81]</td>
<td>Transepicardial</td>
<td>Molilised BMCs</td>
<td>4</td>
<td>CD133⁺</td>
<td>0.13—2×10⁹</td>
<td>Echocardiography</td>
<td>Restoration of viability</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ozbaran M et al. [82]</td>
<td>Transepicardial/Intracoronary</td>
<td>Molilised BMCs</td>
<td>6</td>
<td>Mononuclear cells</td>
<td>1.35—8×10⁷</td>
<td>Echocardiography</td>
<td>Quality of life improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Strauer BE et al. [83]</td>
<td>Intracoronary</td>
<td>BMCs</td>
<td>10</td>
<td>Mononuclear cells</td>
<td>2.8±2.2×10⁷</td>
<td>Echocardiography</td>
<td>NYHA score improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BOOST [84]</td>
<td>Intracoronary</td>
<td>BMCs</td>
<td>30</td>
<td>Unfractionated BM</td>
<td>2.4±0.9×10⁹</td>
<td>Echocardiography</td>
<td>Hypokinetic area</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>30</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TOPCARE AMI [85]</td>
<td>Intracoronary</td>
<td>BMCs</td>
<td>29</td>
<td>CD34⁻CD45⁺</td>
<td>2.1±0.7×10⁷</td>
<td>Echocardiography</td>
<td>Contractility infarct region</td>
</tr>
<tr>
<td></td>
<td></td>
<td>CPCs</td>
<td>30</td>
<td>Endothelial progenitors</td>
<td>1.6±1.2×10⁷</td>
<td>Echocardiography</td>
<td>End-systolic volume</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chen S et al. [88]</td>
<td>Intracoronary</td>
<td>BMCs</td>
<td>34</td>
<td>BM MSCs</td>
<td>4.8—6×10¹⁰</td>
<td>Echocardiography</td>
<td>Cardiac MRI, PET</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>35</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aviles et al. [90]</td>
<td>Intracoronary</td>
<td>BMCs</td>
<td>20</td>
<td>Mononuclear cells</td>
<td>7.8±4.1×10⁷</td>
<td>Echocardiography</td>
<td>LVEF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>13</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kuehne S et al. [91]</td>
<td>Intracoronary</td>
<td>BMCs</td>
<td>5</td>
<td>Mononuclear cells</td>
<td>3.9±2.3×10⁷</td>
<td>Echocardiography</td>
<td>End-systolic volume</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kang HJ et al. [92]</td>
<td>Intracoronary</td>
<td>BMCs (G-CSF mobilized)</td>
<td>10(7)</td>
<td>Leucocytes (first 3 patients)</td>
<td>1×10⁹</td>
<td>Echocardiography</td>
<td>LVEF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>G-CSF alone</td>
<td>10(3)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>7(1)</td>
<td>Mononuclear cells</td>
<td>1×10⁹</td>
<td>Echocardiography</td>
<td>Perfusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fuchs S et al. [93]</td>
<td>Transendocardial</td>
<td>BMCs</td>
<td>10</td>
<td>Unfractionated BM</td>
<td>7.8±6.6×10⁷</td>
<td>Echocardiography</td>
<td>Restenosis rate ~70%</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>7(1)</td>
<td>Mononuclear cells</td>
<td>1×10⁹</td>
<td>Coronary angiography</td>
<td>CCS score improvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Perin et al. [94]</td>
<td>Transendocardial</td>
<td>BMCs</td>
<td>14</td>
<td>Mononuclear cells</td>
<td>2.5±0.6×10⁷</td>
<td>Echocardiography</td>
<td>Perfusion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Control</td>
<td>7</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tse HF et al. [97]</td>
<td>Transendocardial</td>
<td>BMCs</td>
<td>8</td>
<td>Mononuclear cells</td>
<td>From 40 ml BM</td>
<td>Echocardiography</td>
<td>Target wall thickening/motion</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Silva GV et al. [98]</td>
<td>Transendocardial</td>
<td>BMCs</td>
<td>5</td>
<td>Mononuclear cells</td>
<td>From 50 ml BM</td>
<td>Echocardiography</td>
<td>Myocardial volume oxygen consumption</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

AMI indicates acute myocardial infarction; BMCs, bone marrow cells; CPCs, circulating progenitor cells; LVEF, left ventricular ejection fraction; CCS, Canadian Cardiovascular Society; NYHA, New York Heart Association; MRI, magnetic resonance imaging; PET, positron emission tomography.
crests. In a very recent publication, Pomplio et al. have performed intramyocardial delivery of bone marrow mobilised stem cells obtained via subcutaneous administration of G-CSF [81]. Once mobilised, patients were subjected to apheresis with subsequent CD133+ cell fraction isolation by means of a CliniMACS device (Miltenyi Biotech, Germany). Out of the four patients included in this study three had accompanying off-pump coronary artery bypass grafting performed. In the fourth patient, cells were delivered via a minimally invasive transfemoral approach without any additional treatment. Patients tolerated both procedures well and made uncomplicated postoperative recoveries. Promising preliminary follow-up data has been reported once more.

Mobilised peripheral mononuclear cells were also transplanted in six patients with end-stage congestive heart failure [82]. Participants shared a reduced left ventricular ejection fraction (LVEF ≤25%) and were not suitable for standard CABG. Interestingly, the cell suspension collected by apheresis was not only delivered to the injured non-fibrotic myocardial tissue directly, but was also infused via the coronary arteriotomies while performing the bypass graft surgery. Significant improvement was noted in patients’ symptoms during follow-up with some benefit documented also on echocardiography, thallium scintigraphy, and positron emission tomography.

7.2. Intracoronary delivery

Adult stem cell therapy has also taken its place in the centre of clinical research in the field of interventional cardiology. Trying to cash in from the possible regenerative processes at the myocardial level a vast number of studies incorporating intracoronary infusion have been employed. The preponderance of these entails delivery within the first days after an acute coronary episode thus attempting to interrupt the natural progress of the infarction.

Strauer et al. infused autologous mononuclear BMCs in ten patients after they received standard therapy for acute myocardial infarction [83]. Bone marrow was harvested under local anaesthesia the day before transplantation and mononuclear cells isolated. The cell suspension was infused at high pressure under stop-flow conditions into the infarct-related artery above the infarct border zone. Significant improvement was seen in left ventricular function at three months follow-up in comparison to the control group. A considerable decrease in the perfusion defect was recorded as well on scintigraphy.

Six-month follow-up data from the BOOST trial was published by the Hanover group in mid2004 [84]. Having received standard treatment following acute ST-segment elevation myocardial infarction sixty patients were randomly assigned to a whole BMC infusion or control group, respectively. After routine bone marrow harvesting and 4.8 days on average post myocardial infarction cells were infused via the central lumen of an over-the-wire balloon catheter in the infarct-related artery. Mean global left ventricular ejection fraction had increased by 6.7% in the cell—infusion group compared to 0.7% in the control group. Left-ventricular function was notably enhanced in myocardial segments adjacent to the infarcted areas.

During the same period the Frankfurt group reported the one year follow-up results of the TOPCARE AMI trial [85] of which preliminary data had already been announced to the medical community [86,87]. The 69 patients were allocated to two groups depending on the type of progenitor cell infused, being either bone marrow-derived or circulating progenitor cells. A significant improvement in left ventricular function with reduction of end-systolic volumes was documented. No early or late procedure-related complications were seen.

In another study from China 69 patients having been treated for acute myocardial infarction were once again randomised to receive either autologous bone marrow MSCs or saline as the control arm [88]. A considerable improvement was seen at three-month follow-up both in left ventricular haemodynamics as well as in myocardial perfusion.

Encouraging results from twenty patients transplanted with bone marrow-derived mononuclear cells was also reported by Aviles et al. [89,90]. In contrast to the previous reports, Kuethe et al. did not reveal any significant difference regarding left ventricular function or coronary flow reserve at three and twelve-month follow-up [91]. This study involved five patients with large anterior myocardial infarctions receiving mononuclear cell suspensions. One last study worth mentioning comes from a Korean group [92]. Investigators examined the efficacy of intracoronary delivery of cytokine mobilised peripheral stem cells. Despite some encouraging data an increased rate of in-stent restenosis was the cause of an early termination of this trial.

7.3. Transendocardial delivery

Advances in cardiac electrophysiology have allowed cardiologists to deliver cell suspensions in desired areas as seen on left ventricular electro-mechanical mapping. This theoretically could prove a valuable approach especially in the subgroup of patients with no other standard revascularization options. The first study in this set provided evidence of feasibility with demonstration of potential efficacy [93]. Perin et al. treated 14 patients with autologous bone marrow-derived cell suspension [94–96]. All had severe ischaemic heart failure with no alternative treatment options available. Patients were followed up at 2, 6 and 12 months post implantation. There was significant improvement in exercise capacity and myocardial perfusion as compared with controls. Tse et al. recorded similar findings at three-month follow-up of eight patients of the same status treated with autologous mononuclear cell injections [97]. An increase in exercise capacity and myocardial volume oxygen consumption was also documented in five patients with end-stage heart failure awaiting transplantation [98].

It should be emphasised that all above studies have included limited numbers of subjects and with the exception of the BOOST trial lack randomisation, placebo controls and double blind assessment. All these parameters should be taken into consideration whilst undertaking interpretation of outcomes. The aftermath of this ‘rush’ to enter the clinical trial setting may backlash on the integrity of the whole study when critically analysed.
8. Discussion

Even if all the theoretical pathways of cell trafficking and homing to ischaemia generated signals were to be elucidated and fully understood, what takes place once the ‘homed’ cells reach or are directly introduced to their new microenvironment is a great mystery and topic of dispute. Functional improvement observed in animal and human studies may constitute manifestations of either direct mechanical reinforcement of the left ventricular wall, incorporation and integration of implanted stem cells into native myocardial tissue, improvement in neovascularization, or even be a result of paracrine factor production and delivery exerted by the implanted cells. Responsible mechanisms remain to be unravelled.

The main attraction of bone marrow-derived stem cell transplantation for scientists remains their innate plasticity. Proof of principal has been shown by trans-differentiating marrow stromal cells into cardiomyocytes in vitro. This has mainly been accomplished by treating cells with chemicals promoting DNA demethylation, such as 5-aza-2‘deoxycytidine [99–101]. All cells were seen to express cardiomyocyte specific genes and could beat on the petri dishes. More surprisingly cardiomyocyte-like cells have been reported to be obtained from adipose tissue-derived stem cells with and without pre-treatment [102,103]. In experimental studies ultimate evidence of successful engraftment and incorporation of pre-labelled cells is the identification of cells that stain for marker dyes as well as cardiac proteins. The perceived plasticity of transplanted cells has been attributed mainly to the mechanisms of milieu dependent differentiation. The double staining for marker dyes and cardiac proteins post transplantation can also be explained by cell–cell fusion. Recent work has shown that this phenomenon may actually have greater dimensions than acknowledged [104]. Cell–cell fusion has been documented for both stem cells and cardiomyocytes [105,106]. Finally, Oh et al. have shown the two phenomena to happen to a similar extent for murine cardiac stem cells transplanted into injured left ventricle [12].

At this point it should be mentioned that a few investigators have published work questioning the trans-differentiation of haematopoietic cells transplanted into the injured myocardium [107–109]. When unfractonated bone marrow labelled with a fluorescent dye was transplanted in infarcted areas of myocardium in an ovine model no engraftment or transdifferentiation was seen [110]. As noted by the authors the discrepancy between results may be ascribed to differences in protocol design.

The ultimate aim of cellular transplantation remains the regeneration of lost heart muscle along with reversal of the remodelling process. Functional myocardium is made up primarily of three cell types: cardiomyocytes, endothelial and smooth muscle cells. Despite the accumulating data of the existence of cardiac stem cells and tissue specific stem cell subpopulations little information is usually provided in the human trials about the evidence of the multipotential phenotype of the injected cells. Expression of certain transcripts in these cells in conjunction with alterations in baseline levels of message as well as protein once tissue specific differentiation in vitro is demonstrated should probably be established as part of the pre-clinical bench work.

In addition to failing to identify the actual cell type responsible for the observed improvement, other cell-type specific complications will not be recognised and attributed. By transplanting crude bone marrow cell suspensions or the whole aphaeresis leukocyte yield adverse effects may take place as cells that do not take part in the regeneration process are introduced directly into the intracoronary circulation or myocardium [92].

Local inflammation or failure to ‘home’ to the site of injury may affect post-transplantation survival rates. Mode of delivery and phase of injury are crucial determinants in this process. It is therefore only reasonable to accommodate for these losses when calculating absolute numbers of delivered cells in any study design. If the desired effect is attributed to a certain subpopulation (CD133+, MAPCs, etc.) expansion ex vivo may be required to achieve adequate number of progenitors and avoid delivering suboptimal cell counts. In this context, studies with studies where a very small amount of whole bone marrow is delivered and no control groups exist should be perceived with caution, as the final effective population of cells delivered may be negligible.

Local injury has been shown to be a pre-requirement for engraftment in animal studies. Cells delivered in control animals with no injury did not show any sign of engraftment [111]. Intracoronary infusion of cell suspension in healthy canines lead to micro-infarctions, as no local cues were available for cell homing and attraction, and there probably was no distinguishing factor between these cell boluses and systemic emboli [92]. The time of delivery should be at the cross over point in time where the local microenvironment is ready to receive cells at the optimum time of their development for that particular phase of the disease. Lu and coworkers have shown that following myocardial infarction in a rat model the local cues for homing of circulating cells at the site of injury are restricted to days 3 and 7 post infarction [112]. They concluded that the potential for bone marrow-derived progenitor cell regeneration resides within the two first weeks. The degree of differentiation required should be established before transplantation. An immature, more plastic cell may be more effective than an ex vivo pre-differentiated, committed cell and vice versa. Bittira et al. have actually shown that in the acute setting unmodified marrow stromal cells facilitate myocardial angiogenesis and myogenesis, whereas converting scar into myogenic tissue may be augmented by cell preprogramming before implantation [113].

Ischaemic myocardium constitutes a hostile environment for transplanted cell populations. In an effort to overcome this and improve cell survival during this stage many strategies are being developed. These include angiogenic pretreatment with injection of an adenovirus encoding VEGF [114], prevascularization with gelatin microspheres containing various growth factors [115] and finally, ex vivo retroviral transduction of cells to overexpress prosurvival genes [59].

From the animal studies Kocher carried out to the Rostock experience, endothelial progenitor cells have been shown to provide functional improvement of the injured left ventricle.
It remains unclear if the observed neovascularization is the direct result of trans-differentiation/incorporation of injected cells into newly formed vessels or the indirect effect of paracrine factor delivery. With the use of high-power laser scanning confocal microscopy two groups have demonstrated that double staining for marker dyes and endothelial proteins post-transplantation with conventional light or fluorescence microscopy may be dubious [116,117]. Transplanted cells were not actually in the vessel wall but the perivascular space.

A topic of concern associated with the observed angiogenesis following progenitor cell transplantation is the possible accelerated development of atheromatous plaques. Re-endothelialization and inhibition of neointimal hyperplasia are considered key functions of endothelial progenitor cell populations [118,119]. On the other hand promotion of neointimal hyperplasia has mainly been attributed to smooth muscle progenitor cells. Progenitor cell transplantation has been implicated in the promotion of neointimal hyperplasia either via direct differentiation of progenitor population into vascular smooth muscle cells or attraction of these cells via paracrine effects [120].

Lastly, the argument of an early paracrine effect being responsible for the observed myocardial functional improvement has been strengthened by the work of Dai et al. [121]. Following allogenic mesenchymal stem cell transplantation in postinfarcted rat myocardium surviving mesenchymal stem cells were seen up to 6 months expressing muscle and endothelial phenotypes. The documented functional improvement was transient though being limited to the first four weeks.

9. Closing comments

It was more than thirty years ago when Sen described his ‘snake-heart operation’, consisting of multiple full-thickness acupunctures of the ventricular wall to improve myocardial blood supply [122]. Today not only have researchers surpassed the concept of offering patients ‘reptile’ hearts, but are allowed to aim for de novo reconstitution of injured human myocardium. Identification of cell subpopulations responsible for the observed myocardial regeneration, improvement in cell expansion techniques along with development of in vivo dynamic cell tracking methods are areas of future research. Large, prospective, double blind randomised controlled trials are also warranted. Results from the laboratory and clinical front will decide if autologous cellular transplantation (alone or in conjunction with established therapies) will be offered routinely to patients suffering from ischaemic heart disease.

References


[106] Innaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow-derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res 2004;94(5):678–85.


