Review: Current Perspective

Stem Cell Repair of Infarcted Myocardium
An Overview for Clinicians

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Under appropriate cell culture conditions, stem cells are capable of differentiating into cardiac myocytes and endothelial cells (Table 1). If these results can be reproduced in vivo, it may be possible to use stem cells to impede progressive deterioration into heart failure or even to restore cardiac function in patients with acutely or chronically damaged myocardium. In this article, we will provide for the clinical cardiologist a brief overview of the conceptual basis for stem cell therapy and review the current laboratory evidence supporting its potential value. We will describe the clinical options in stem cell treatment of damaged myocardium and the potential limitations that need to be assessed in randomized clinical trials.

The Conceptual Basis of Stem Cell Therapy

Stem cells share the following two defining characteristics: the capacity to differentiate into a spectrum of different cell types and the capacity to renew themselves.1 The biological principle that underlies stem cell therapy is tissue-directed differentiation. For example, adult stem cells isolated from liver tissue and reinjected into liver become hepatocytes, whereas the same cells injected into myocardium become myocytes.2 Stem cells have been engrafted into a broad spectrum of tissues, including regenerating bone, neural tissue, dystrophic skeletal muscle, and injured skeletal muscle.3

The classification of stem cells, based on a large number of cell markers, is still in evolution, and interested readers are referred to excellent reviews in the basic science literature.4,5 The primary distinction is between embryonic and adult stem cells. Most cardiovascular research has used adult stem cells derived from the bone marrow. Within the bone marrow, a simplified distinction is between CD34+ hematopoietic stem cells, which are precursors of blood and endothelial cells, and CD34− mesenchymal stem cells, which are precursors of stromal cells, including fibroblasts and osteocytes. An array of additional markers is then used to further identify and classify the cells. A recently identified CD34− subgroup has been classified as a multipotent stem cell on the basis of its capacity to differentiate into both lineages.

The Clinically Relevant Steps in Stem Cell Engraftment

Stem cell repair of cardiac and vascular tissue is a naturally occurring process after injury.6,7 Circulating CD34+ mononuclear cell counts and plasma levels of endothelial growth factor are significantly increased in patients with acute myocardial infarction, peaking on day 7 after onset.8

The three clinically important steps in this natural process are mobilization of stem cells from the bone marrow, homing of these cells to the site of injury, and differentiation of the stem cell into a functional cell of the injured tissue. These three steps drive the strategies of therapeutic myocardial engraftment, given that it is possible to intervene at each step.

Initial Studies of Myocardial Restoration

Attempts to replace infarcted myocardium began with direct injection of fetal and skeletal myoblasts, which can engraft into normal and infarcted myocardium. With follow-up periods as long as 2 months, cardiac function is reported to be better after myocardial infarction compared with controls.9–13 Engraftment with viability, however, does not establish functional integration. The principal concern with myoblasts is that they do not transdifferentiate into myocytes, they contract and relax like myocytes only when externally stimulated, and they do not develop meaningful gap junctions.14–16 This concern has now emerged as a central issue in initial clinical trials (see below).

Evidence That Transplanted Stem Cells Become Phenotypic Myocytes

In cell culture studies, stem cells can differentiate into cardiomyocytes with cardiac-specific genes that express structural proteins and ion channels. The proteins are organized in a developmentally regulated manner that recapitulates the sarcomeric organization of the embryonic heart.17 Yoon et al18 cloned multipotent bone marrow stem cells from a single cell. Depending on culture conditions, these cloned cells differentiated into cells that expressed epitopes specific for endo-, meso- and neuroectodermal lineages. For instance, cells cocultured with rat neonatal cardiomyocytes expressed cardiac troponin and other cardiac-specific proteins. When
the rat cardiomyocytes were removed and the stem cells were exposed to the cultured media alone, differentiation did not occur. The authors speculate that the tissue type of the differentiated stem cell is regulated, at least in part, by cell-to-cell signals. On the other hand, some authors have found no cardiac transcription factor expression in bone marrow cell (BMC) culture and have suggested that the putative transdifferentiation of BMCs observed during transplantation must be due to the in vivo milieu.19

**Direct Engraftment: Intramyocardial Stem Cell Injection**

The most direct way to deliver stem cells is by injection into the damaged myocardium, thereby bypassing the need for mobilization and homing (Figure 1). A number of laboratories, including our own, have reported that stem cells can transform into cardiomyocytes in vivo based on developmental pattern, structural characteristics, and expression of cardiac-specific proteins such as actin and troponin. These reports commonly include histological analyses and functional assessment of myocardial perfusion and perfusion. For example, Fuchs et al20 found that intramyocardial injection of autologous bone marrow promotes collateral circulation in ischemic porcine myocardium. Over a period of 4 weeks before injection, expression of vascular endothelial growth factor and macrophage chemoattractant protein-1 by the cultured BMCs increased progressively. The cells then were injected into ischemic myocardium created by an aoroid constriction of the left circumflex coronary artery. Collateral flow and regional myocardial wall thickening during adenosine stress increased by ≈50% in the treated group compared with controls.

A number of studies have confirmed this effect of stem cell transplantation on cardiac function after acute myocardial infarction.21–24 Orlic et al21 injected stem cells into the peri-infarct myocardium of mice 5 hours after left coronary artery occlusion. Nine days after infarction, left ventricular developed pressure had increased by 32%, and left ventricular end diastolic pressure had decreased by 36%. Nevertheless, not all injected cells become myocytes or endothelial cells.

Some of the stem cells injected into infarcted myocardium also express a fibroblastic phenotype and are incorporated into the scar tissue.22 Consistent with this histological finding, others have shown in rats that stem cell injection into the peri-infarct region reduces left ventricular dilatation and remodeling.22,23 Thus, as with skeletal myoblasts, the conclusion that stem cells transform into new, functioning cardiac muscle and thereby improve cardiac performance is inferential. It also possible that the better cardiac function compared with controls could reflect more fibrous tissue formation with limitation of remodeling. Figure 2 shows the potential beneficial mechanisms of stem cell therapy after myocardial infarction.

**Homing: Eliminating Direct Myocardial Injection**

In bone marrow transplantation therapy, homing refers to the phenomenon by which intravenous stem cells specifically engraft in the bone marrow and not in other organs. Homing of bone marrow stem cells to injured myocardium is now also thought to occur after myocardial infarction. Not yet well defined, homing is believed to be a complex multistep process.25 An early step in the process appears to be stem cell mobilization. The stimulus may be stem cell factor and/or granulocyte colony-stimulating factor (G-CSF) and other cytokines.26 Extracellular matrix proteins and proteolytic enzymes facilitate cell mobilization. After the cells enter the circulating blood, adhesion molecules expressed at the injury site mediate attachment to endothelial cells. Transendothelial migration is then in part driven by chemokines.27,28
tissue-directed differentiation within the injured organ is probably influenced both by cell-to-cell contact and by growth factors.

The natural homing phenomenon suggests that stem cells could be delivered by intravenous injection. Kocher et al.29 collected heterologous human bone marrow-derived angioblasts mobilized by administration of G-CSF, before intravenous injection in the rat infarct model. Stem cell infiltration was observed 2 days after injection in the injured myocardium but not in unaffected myocardium or in sham-operated rats. At 15 weeks, new blood vessel formation in the infarct bed (vasculogenesis) and proliferation of preexisting vasculature (angiogenesis) was observed. Capillary formation was 3-fold greater in the treated group and the number of apoptotic cells was reduced 6-fold. Compared with controls, the percentage of myocardium infarcted was reduced from 36% to 12%, with a corresponding hemodynamic benefit in cardiac output, which fell by 48% in controls versus 26% in the treated group.

To enhance adhesion and proliferation before injection, the genetic makeup of injected stem cells can be modified in vitro before injection. Iwaguro et al.30 transduced heterologous endothelial progenitor cells with the VEGF gene before injection into athymic mice with hind limb ischemia. Neovascularization and blood flow improved, accompanied by a 64% reduction in limb necrosis compared with control animals. Nonetheless, the theoretical malignancy potential of altering progenitor cells with growth factors will need to be investigated before any clinical application.

**Mobilization: Marrow Stimulation of Stem Cell Release After Myocardial Infarction**

Naturally occurring BMC mobilization in response to injury suggests that the cytokines might be effective as therapy. In mice, Orlic et al.30 administered human G-CSF and rat stem cell factor for 5 days before infarction and 3 days afterward, aiming to stimulate translocation of the splenectomized recipient’s own BMCs to the infarcted myocardium. At 27 days, in the cytokine-treated animals’ mortality decreased by 68%, infarct size by 40%, and left ventricular end-diastolic volume by 26%. Ejection fraction improved progressively by 48%, 62%, and 114% versus controls at 9, 16, and 26 days, respectively. The authors estimated that there was the formation of $15 \times 10^6$ new myocytes, which were connected with the circulation of the unaffected ventricles by arterioles and capillaries. The obvious limitation of this study from a clinical standpoint is that the agents were delivered before infarction. Subsequent unpublished studies, however, suggest that G-CSF need not be administered before infarction to be effective.

Several groups, however, have reported that labeled hematopoietic stem cells do not differentiate into muscle, brain, kidney, or liver cells, even though the donor cells are identifiable in the host tissue.31 Consequently, as with cell culture studies, the issue of stem cell differentiation into functioning myocytes in the intact heart also is still a source of debate.

**Cell Fusion as a Potential Confounding Factor**

A potential explanation for these divergent results is that what was thought to be stem cell transdifferentiation actually represents cell fusion. Several authors have cocultured labeled embryonal cells with adult BMCs or neural stem cells. After two to four weeks the labeled cells were found to express protein markers consistent with transdifferentiation. Further examination, however, revealed cells with 4 sex chromosomes (XXXY) and twice the usual amount of DNA, strongly suggesting cell fusion.32,33 Advocates of transdifferentiation, however, point out that cell fusion appears to be a very low-frequency event, occurring perhaps once in 10 000 to 100 000 cells, whereas transdifferentiation has been reported to occur in as much as 55% in coculture assays, and regeneration of liver mass and development of substantial capillary networks have been followed in vivo injection of adult bone marrow stem cells.34–37 In addition, some authors have found that transdifferentiated stem cells contained a normal number of chromosomes. Nonetheless, if even some injected stem cells retain twice the normal number of chromosomes, ie, are genetically abnormal, this might preclude human use.

**Extension to Human Application: A Number of Potential Limitations**

Beyond the fundamental issue of whether stem cells actually differentiate into functioning myocytes and blood vessels, practical questions remain unanswered even as investigators begin clinical trials (Table 2). First, the size of the murine heart and its infarct is tiny compared with its human counterpart. Thus, it is possible that the number of stem cells required for remodeling a larger mass of myocardium might exceed practical limits. A second practical limitation of small animal studies is the frequent disabling of the recipient immune system, eg, by radiation, thymectomy or splenectomy. Because these procedures will not be used in human applications, the type of stem cells, timing of injection, and the mode of delivery need to be more clearly defined.
Our laboratory and others used the porcine model of myocardial infarction and injected allogeneic mesenchymal stem cells.\textsuperscript{38,39} No immunosuppression by surgery, radiation, or drugs was used. We also tested the possibility of injecting stem cells in the subacute phase of recovery from myocardial infarction. In one study, for instance, $2 \times 10^6$ stem cells were injected into the peri-infarct zone at 1 month after infarction. One month later, the labeled stem cells had developed the phenotypic characteristics of cardiac myocytes, endothelial cells, smooth muscle cells, and fibroblasts. Compared with untreated controls, cardiac function stabilized. In the treated group, ejection fraction at 2 and 3 months after infarction stabilized at 52%, whereas in the untreated group ejection fraction progressively deteriorated to 45%. Because ejection fraction stabilized but did not increase, it is possible that injection at 1 month represents interruption of remodeling rather than restoration of cardiac muscle function. Because the porcine and human hearts are similar in size, we may speculate that delivery of stem cells by intramyocardial injection may provide a sufficient number of cells to preserve but not necessarily restore myocardial function.

Most studies have used hematopoietic stem cells because mesenchymal stem cells were thought to lack the capacity to differentiate into both endothelial cells and myocytes. Recently, however, a population of postnatal human bone marrow CD34\textsuperscript+ stem cells that copurify with mesenchymal stem cells, yet differentiate into visceral mesoderm, neuroectoderm, and endoderm in vitro, has been identified.\textsuperscript{40,41} Several laboratories have used cells that are defined as mesenchymal stem cells by traditional criteria (CD34\textsuperscript+ cells that adhere to the walls of culture plates) to induce both new myocyte and new vessel formation by direct myocardial injection. These multipotent progenitor cells have now been isolated not only from the bone marrow but also from brain, muscle, and adipose tissue. Whether these cells are originally derived from circulating BMCs or are intrinsic to each organ is not yet established.\textsuperscript{41}

These new findings raise the issue of autologous versus allogeneic cells. If allogeneic hematopoietic stem cells are used, there is the potential for graft-versus-host reaction, necessitating use of the patient's own bone marrow. The marrow can be filtered and injected without expansion in culture. This approach, however, would deliver far fewer stem cells than the number used in animal studies. Alternatively, autologous stem cells can be isolated and expanded, but this approach will require a second visit to the catheterization laboratory for cell injection unless the cells are delivered intravenously. The third alternative is to use allogeneic mesenchymal stem cells. Allogeneic mesenchymal stem cells can home to injured tissue, and transplantation might be accomplished without the need for significant host immunosuppression.\textsuperscript{42,43} If studies prove they are as effective as hematopoietic stem cells in preserving myocardial function and perfusion, they have substantial potential clinical importance because they are commercially available.

Stem cell mobilization with G-CSF is appealing as a potential therapy because it offers both ease of treatment and universality of application. In mice, restoration of myocardial function appears to be of similar magnitude independent of whether the cells are injected directly, intravenously, or mobilized with cytokines. Mobilization of a sufficient number cells in the infarct patient should not be a problem, given that $5 \times 10^6$ circulating stem cells can be removed from the peripheral blood by apheresis of normal human donor blood after several days of G-CSF stimulation.\textsuperscript{44} When the mobilization strategy is tested in humans, however, there are also potential limitations. There is no animal model in which the coronary arteries have the multiple unstable coronary plaques that probably exist in patients with acute myocardial infarction.\textsuperscript{45,46} Theoretically, these plaques might be destabilized by an increased number of circulating leukocytes after G-CSF administration. An elevated white cell count does predict an adverse prognosis in acute myocardial infarction.\textsuperscript{47} On the other hand, this correlation may reflect the magnitude of myocardial injury rather than an adverse effect of leukocytes.\textsuperscript{48} Indeed, acute myocardial infarction patients with circulating immature white cells immediately after infarction have a higher ejection fraction at 6 months than patients without circulating immature cells.\textsuperscript{49} Short-term injection of G-CSF in humans does not increase the level of circulating inflammatory cytokines, and in clinical hematological practice, this procedure does not seem to have been associated with an increased risk of acute or late coronary events.\textsuperscript{50,51} The most common significant side effect of G-CSF administration is bone pain, which could be a substantial hindrance to the performance of randomized clinical trials.

Independent of the issues surrounding stem cell delivery, it is possible that stem cells may have longer-term adverse effects. We have found sympathetic nerve sprouts in atrial tissue distant from the infarct area after intramyocardial injection of mesenchymal stem cells. These nerve sprouts could cause cardiac sympathetic hyperinnervation and an increased risk of cardiac arrhythmia. Stem cell–derived cardiomyocytes studied in vitro using whole-cell patch clamps show spontaneous activity, low dV/dT, prolonged action potential duration, and easily inducible triggered arrhythmias.\textsuperscript{52} Thus, in theory, transplanted cells could be arrhythmogenic by reentry, automaticity, or triggered

### TABLE 2. Advantages and Disadvantages of Potential Clinical Strategies for Preservation or Restoration of Myocardial Function

<table>
<thead>
<tr>
<th>Strategy</th>
<th>Advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>Intramyocardial skeletal myoblasts$^{34}$</td>
<td>Abundant cell source; clinical trials ongoing</td>
<td>Engraft but do not transform; ventricular arrhythmias</td>
</tr>
<tr>
<td>Transendocardial stem cells$^{59}$</td>
<td>Short-term safety very likely; clinical trials ongoing</td>
<td>Imaging technology and catheterization expertise required</td>
</tr>
<tr>
<td>Intracoronary stem cells$^{14}$</td>
<td>Possible wide use in catheterization laboratory</td>
<td>Preparation of cells complex</td>
</tr>
<tr>
<td>Intravenous stem cells$^{29}$</td>
<td>No intramyocardial injection; may be equally efficacious</td>
<td>Autologous cells impractical; No. of cells may be inadequate</td>
</tr>
<tr>
<td>Cytokine mobilization$^{31}$</td>
<td>Widespread practicality</td>
<td>Uncertainty regarding risk and timing</td>
</tr>
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Cytokine mobilization$^{31}$

Intracoronary stem cells 58 Possible wide use in catheterization laboratory Preparation of cells complex

Intravenous stem cells 29 No intramyocardial injection; may be equally efficacious Autologous cells impractical; No. of cells may be inadequate

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Preliminary Insights From Clinical Trials
A randomized clinical trial of stem cell therapy in cardiovascular disease has been conducted in patients with peripheral vascular ischemia. Twenty-two patients received either bone marrow mononuclear cells or peripheral blood mononuclear cells. The patients treated with stem cells had major and statistically significant improvement in ankle-brachial index and transcutaneous oxygen pressure. Two patients had myocardial infarctions within 2 years of the procedure, which were considered unrelated to the stem cell therapy. The authors proposed that the ability of marrow isolates to supply endothelial progenitor cells and to secrete various angiogenic factors or cytokines suggested that the approach "could be safe and effective for achievement of therapeutic angiogenesis." Nonetheless, enthusiasm for this approach must be tempered by concern over low numbers of identifiable endothelial progenitor cells in such bone marrow specimens.

Clinical investigation in patients with both acute myocardial infarction and angina pectoris is currently in the early stage of nonrandomized testing of feasibility and safety. Transplantation of autologous skeletal myoblasts has been conducted in a phase I safety and feasibility study in patients with left ventricular ejection fraction <35% after myocardial infarction. An average of 874 million cells were injected in a mean of 37 akinetic sites at the time of bypass surgery. At a mean of 10.9 months follow-up, 60% of the akinetic tissue segments had regained some contractility. Mean New York Heart Association class increased from 2.7 to 1.6, with a parallel increase ejection fraction from 24% to 32%. Four of the patients, however, had episodes of sustained ventricular tachycardia requiring implantation of an automatic cardioverter-defibrillator. In the first phase I trial of transcatheter endocardial injection of skeletal myoblasts in patients with prior old infarction and reduced ejection fraction, however, again a high frequency of both nonsustained and sustained ventricular tachycardia was encountered, leading to the need for implantable cardiac defibrillators. The trial is now restricted to patients who have had an implantable cardioverter-defibrillator implanted for at least 3 months. Thus, these early results suggest that the development of symptomatic cardiac arrhythmias within weeks of cell transfer is a serious problem with myoblast transplantation. Investigators have hypothesized that because the transplanted skeletal myoblasts remain committed to their lineage, they may retain an action potential duration different from that of adjacent myocardium, and that this electrical inhomogeneity predisposes to ventricular arrhythmia.

In contrast, the preliminary safety and feasibility trials of stem cell therapy have not yet reported arrhythmias to be a problem. No authors have reported a significant short-term safety problem, but as yet the efficacy data are insufficient to support any conclusion. Because none of the preliminary trials are randomized, each study design has a major limitation. For instance, we do not know the magnitude of improved function or perfusion that might have been observed with conventional therapy. Stamm et al injected up to 1.5×10⁶ autologous AC133⁺ mononuclear bone-marrow cells (an isolate that includes both hematopoietic and multipotential stem cells) into the peri-infarct zones of six post-infarct patients during coronary artery bypass surgery. The authors reported that at 9 to 16 months after surgery, none of the patients had experienced ventricular arrhythmia. All six patients reported a notable improvement in exercise capacity to New York Heart Association functional class I, with a change in mean ejection fraction from 37% to 48%. Myocardial perfusion scans were reported to have improved strikingly by qualitative analysis in five of six patients.

The TOPCARE-AMI study is evaluating the delivery of circulating endothelial progenitor cells (EPCs) or BMCs directly into coronary arteries in patients with reperfused acute myocardial infarction. Although this study has a control group, it is not randomized. Treated patients were compared with noncontemporary historical controls. In the first 20 patients, 11 received EPCs and 9 received BMCs. At 4 months, left ventricular ejection fraction had improved by 8.5% in both EPC and BMC patients, compared with rates of 2.5% seen in the reference group. Echocardiography revealed substantial enhancement of regional wall motion. Coronary flow reserve measured by stress echo was improved in both the target vessels and reference vessels. Quantitative F-18-fluorodeoxyglucose–positron emission tomography revealed a statistically significant increase in myocardial viability in the infarct zone. There were no adverse events of treatment in any of the patients and no cases of arrhythmia, or increase in creatine kinase and troponin.

Strauer et al used contemporary controls, but the patients were not randomized, creating a potential issue of selection bias. Ten treated patients who received stem cell therapy were compared with 10 patients who refused. Intracoronary autologous BMCs were injected in 10 patients 5 to 9 days after acute myocardial infarction. The delay in part reflects the time required for aspiration, separation, harvest, and cultivation of autologous BMCs. The cells were delivered by high-pressure infusions of 2 to 3 mL of cell suspension directly at the site of the former infarct occlusion during 2- to 3-minute coronary occlusion by a balloon. At 3 months, the control patients exhibited no statistically significant change in cardiac function or perfusion. The treated group had a reduction in infarct size as a segmental wall motion defect from 30% to 12% and a reduction in infarct region as a 201-thallium perfusion defect from 174 to 128 cm². Although stroke volume increased significantly, ejection fraction remained unchanged. The authors reported, "No side effects were observed at any point in time." In the absence of randomized controls, critics have questioned whether the number of injected vascular or myocardial progenitor cells, which we might estimate to be in the range of 10⁶ cells, would be sufficient to explain the apparent improvement in cardiac function and perfusion.

Finally, a number of trials of transendocardial injection of commercially produced mesenchymal stem cells or freshly aspirated BMCs are now underway. Injection is controlled by the same left ventricular electromechanical mapping technology now widely used in electrophysiology catheterization laboratories during ablation procedures. Although early
experience suggests that this approach is technically feasible and can be performed safely, as with other clinical trials data are as yet insufficient to assess efficacy. Thus, the final arbiter of the effectiveness of stem cell therapy in the management of acute and chronic myocardial infarction must be randomized clinical trials that assess the balance between likely benefit and theoretical risk.

References


Key Words: myocardial infarction ■ heart failure ■ remodeling ■ stem cells