Stem Cell Therapy in Perspective
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The concept of regenerative medicine using the body’s own stem cells and growth factors to repair tissues may become a reality as new basic science works and initial clinical experiences have “teamed-up” in an effort to develop alternative therapeutic strategies to treat the diseased myocardium. In particular, revealing the signals that mediate cellular growth and differentiation may provide novel tools designed for myocardial regeneration in patients sustaining ischemic cardiomyopathy syndromes. We attempt herein to provide a critical overview of recent developments of myocardial cell transplantation strategies.

Stem Cells
Stem cells are a population of immature tissue precursor cells capable of self-renewal and provision of de novo and/or replacement cells for many tissues. Embryonic stem cells can be obtained from the inner cell mass of the embryonal blastocyst. Although it was recently shown that human embryonic stem cells can differentiate into cardiomyocytes, because of the immunogenicity and rejection, as well as ethical considerations, these cells may be restricted to experimental in vitro studies and their therapeutic potential remains to be determined. Also, these cells may act as an unanticipated arrhythmogenic source after intramyocardial transplantation. Clinical application of these cells is most likely years ahead (Table).

In contrast, adult human stem cells (hematopoietic, mesenchymal) are found in mature tissues, eg, the bone marrow. Plasticity of adult stem cells can probably generate lineages of cells different from their original organ of origin. Thus, these cells can be used for organ regeneration and for cellular repair in various species, as well as in humans.

Ethical problems for adult autologous stem cells do not exist, and although much experimental work remains to be done, their clinical relevance and therapeutic benefit in heart disease have recently been shown for the first time.

Except for hematopoietic and mesenchymal stem cells, many other bone marrow-related cell types may participate in organ repair of infarction models; bone marrow hemangio blasts take part in neovascularization, mesodermal progenitor cells are contained within the mononuclear bone marrow cell fraction that differentiates to endothelial cells, and endothelial progenitor cells can transdifferentiate into cardiomyocytes. Primitive bone marrow cells mobilized by stem cell factor and granulocyte-colony stimulating factor are capable of homing to infarct regions, replicating, differentiating, and promoting myocardial repair. Ultimately, a variety of different cell types from the mononuclear bone marrow cell fraction contribute to the regeneration of necrotic myocardium and damaged vessels. In this regard, therapeutic use of mononuclear cell populations of bone marrow may be more useful and promising than single isolated cell fractions alone. The effect manifested by more heterogenous bone marrow cell populations that contain very small numbers of stem cells may also suggest the importance of an entire array of bone marrow-derived growth factors and cytokines that may also regulate cellular growth and regeneration via cellular secretion mechanisms.

Stem Cells and Angiogenesis
The complex cellular and molecular mechanisms by which endothelial and smooth-muscle cells interact with each other to form blood vessels are now better understood. Endothelial cells alone can initiate the formation and sprouting of
endothelium-lined channels, namely angiogenesis, in response to a physiological or pathological stimulus. Peri-endothelial cells are required for vascular maturation. Recruitment of smooth muscle cells provides these vessels with essential viscoelastic and vasomotor properties and enables accommodating the changing needs in tissue perfusion. This later stage is called arteriogenesis and has a major role in collateral growth. Endothelial progenitor cells could be isolated from peripheral blood and/or bone marrow and showed incorporation into sites of physiological and pathological neovascularization in vivo after either systemic injection or using direct intramyocardial transplantation. In contrast to differentiated endothelial cells, transplantation of progenitor cells successfully enhanced vascular development by in situ differentiation and proliferation within ischemic organs. Recent results in mouse experiments suggest the potency of extracardiac progenitor cells for transdifferentiation into new cardiomyocytes after acute experimental myocardial infarction. Bone marrow cells cultured with 5-azacytidine differentiated into cardioid-like muscle cells in culture and in vivo in ventricular scar tissue in pigs and improved myocardial function. In clinical myocardial infarction, evidence has been provided that autologous bone marrow stem cells may regenerate in infarcted myocardium and improve myocardial perfusion of the infarct zone. Studies with transplanted human hearts have shown that adult humans have extracardiac progenitor cells capable of migrating to and repopulating damaged myocardium, a process occurring at very low levels. Recently, cases have been described in which a male patient receives a heart from a female donor, which provided an opportunity to test whether progenitor cells translocate from the recipient to the graft on the basis of Y chromosome labeling. Results showed that myocytes, coronary arterioles, and capillaries that had a Y chromosome made up 7% to 10% of those in the donor hearts and were proliferative. This indicates a regenerative capacity of the transplanted myocardium. Thus, there is growing evidence for a repair function of extracardiac cells, eg, from bone marrow in the case of cardiac lesion and the necessity of myocardial healing, although these results are not unanimously approved.

### Stem Cell Differentiation to Muscle Cells

The principal aim is to transplant cells of primarily noncardiac origin, such as human bone marrow-derived mononuclear cells containing human stem cells. These cells may operate as a precursor of heart muscle tissue and of coronary blood vessel cells. Human bone marrow contains hematopoietic (1% to 2%) and mesenchymal stem cells (<0.05%). Both types of stem cells may contribute to heart muscle repair. Hematopoietic stem cells are progenitor cells for many types of cells, eg, endothelial cells, which may also differentiate to heart muscle cells. Mesenchymal stem cells are progenitor cells for types of cells such as heart muscle cells, as well as for a variety of cells of noncardiac concern. Recent results in

### Milieu-Dependent Differentiation and Enhanced Environment

Studies from several species demonstrate that bone marrow-derived stem cells are stem cells for various mesenchymal tissues. The cells are therefore not simply stromal precursors, but precursors of peripheral tissues, such as heart muscle. Normal growth and ultimate stem cell fate depend on engraft-
ment in an appropriate “niche.” Nonetheless, the mechanisms by which the local milieu influences stem cell differentiation are as yet undetermined. Thus, it seems that the fate of bone marrow stem cells is determined by the environment in which they engraft rather than by an intrinsically programmed fate. Therefore, enhancement of functional activity of the specific organ’s niche for heart muscle, eg, by positive inotropic (pharmacologic augmentation of contractility) or by positive chronotropic stimuli (heart rate increase by exercise), may promote and intensify the transdifferentiation of bone marrow-derived stem cells to the cardiomyocyte phenotype. After an injury, eg, myocardial infarction, or a cellular damage, eg, in severe pressure or volume overload of the heart, specific factors, including cytokines, stem cell factor, and various growth factors, that stimulate cell replication and substitution in the injured tissue are released by the surrounding cells. In addition, transplanted stem cells, differentiating to cardiomyocytes, become indistinguishable over time from the surrounding cardiomyocytes, and they begin to express the contractile proteins specific for striated heart muscle, including desmin, α-myosin, heavy chain, α-actinin, and phospholamban at levels that are the same as in the host cardiomyocytes. This transdifferentiation process is more pronounced in injured tissue than in healthy organs and may be intensified when the heart as the recipient organ contributes to its enhanced environment by high chronotropic and inotropic activity. Thus, regionally large concentrations of stem cells and increased mechanical activity of the recipient heart muscle may provide a favorable environment for successful engraftment of stem cells after cardiac injury.

Route of Cell Administration

The appropriate route of cell administration to the damaged organ is an essential prerequisite for the success of organ repair (Figure). High cell concentrations within the area of interest and prevention of homing of transplanted cells into other organs are desirable. Therefore, targeted and regional administration and transplantation of cells should be preferred. Below, several special routes of administration are described.

- In regional heart muscle disease, as in myocardial infarction, selective cell delivery by intracoronary catheterization techniques leads to an effective accumulation and concentration of cells within the infarcted zone. This can be realized in humans with bone marrow-derived cells. With intracoronary administration, all cells must pass the infarct and peri-infarct tissue during the immediate first passage. Accordingly, with the intracoronary procedure, the infarct tissue can be enriched with the maximum available number of cells at all times. Further developments of catheterization systems for various clinical studies are needed.

- The transendocardial and transpericardial route of application has been used in large animal experiments and was also recently tested in patients. The main potential advantage of the surgical procedure is injection under visualization, which allows anatomic identification of the target area and even distribution of the injections. The safety and feasibility of catheter-based transendocardial injection was demonstrated in large animal studies and initial clinical experience in 19 patients using intramyocardial gene trans-
fer showed similar safety profiles. Current clinical experience is limited to one injection system, using electromechanical mapping to generate 3-dimensional left ventricular reconstruction before the injection. Intraventricular catheter manipulation, however, can injure the myocardium, so intraventricular premature beats and short runs of ventricular tachycardia. In certain cases, this precludes injection to the more arrhythmogenic zones, and it may extend the duration of the procedure and should always be carefully monitored. Each injection catheter is tested for cell biocompatibility to assure no mechanical or functional damage to cells being propelled under pressure through the narrow injection needle. Future developments with steerable transcendocardial injection and delivery systems with mapping of the injured zone are needed. Transendocardial injection of autologous bone marrow cells has also been performed as part of several pilot and phase I studies. Safety and feasibility data are still pending and efficacy parameters need large randomized clinical trials.

- The intravenous route of administration is easiest. The main disadvantage, however, is that approximately only 3% of normal cardiac output will flow per minute through the left ventricle, and it is also limited because of transpulmonary first-pass attenuation effect on the cells. Therefore, this administration technique will require many circulation passages to enable infused cells to come into contact with the infarct-related artery. During that time, homing of infused cells to other organs will considerably reduce the number of cells that will populate the infarcted area.

- Some major cell types, such as skeletal myoblasts, have the disadvantage of an embolicogenic potency when delivered systemically. Therefore, intramyocardial injection during open-heart surgery has been tested. This procedure has also been used in humans. However, the therapeutic effect is limited because of severe arrhythmogenic complications. Another approach implanted autologous bone marrow cells during open-heart surgery and could show improvement in myocardial perfusion in 3 of 5 treated patients.

### Detection of Transplanted Stem Cells

An important clinical problem will be the identification and localization of transplanted autologous stem cells within the injured area of the heart. The transplanted cell or cell population is a single unit in a complex biological network of other cells. Therefore, for both localization and fate mapping of stem cells within the target organ, specific cell markers are desirable. Thus, analysis of stem cell behavior will presume (1) in situ labeling of a single cell or a transplanted cell population or (2) transplantation of already in vitro labeled cells or cell populations. For labeling in animal experiments, retroviral transduction with a marker gene or labeling with thymidine or bromodeoxyuridine (BrdU) have been used. For clinical detection of stem cells, magnetic labeling and in vivo tracking of bone marrow cells by the use of magnetodendrimers or radioactive detection methods may be useful. Myocardial biopsies in humans hardly will be justifiable under these circumstances. Thus, localization and fate mapping of stem cells in the region of myocardial injury will represent an important task for experimental and clinical stem cell research in the future, as well as for the assessment of time course of proliferation in the recipient new cell homes and for the evaluation of proper cell function after full transdifferentiation. First results through the detection of the reporter gene LacZ, by identification of β-galactosidase–positive cells in tissue section and chromosome analysis by Florescence in situ hybridization (FISH) techniques are encouraging.

### Stem Cells for Cardiac Wound Repair: A Joint Clinical and Experimental Approach

In the regenerating tissues, stem cells and progenitor cells in the microenvironment both take part in the renewal process. Bone marrow cells injected or mobilized to the damaged myocardium were shown to behave as cardiac stem cells with remarkable plasticity, giving rise to myocytes, endothelial cells, and smooth muscle cells. In the case of human infarcted tissue, autologous bone marrow cells have shown to be highly effective in wound repair in terms of regenerating heart muscle and improving perfusion in the infarcted and border zone area. Clinical studies therefore are necessary — in parallel to basic and experimental investigations — analyzing the promising prerequisites for clinical wound repair, preferably the optimum cell administration to the region of interest of the heart, eg, the infarcted tissue, and their optimum concentration and accumulation by different catheter-based techniques.

Moreover, catheter-guided cell transfer to the human heart has the unique advantages of being safe under local anesthesia and during routine cardiac catheterization, being fast, taking between 20 to 40 minutes for the whole procedure, and allowing the administration of bone marrow cells in abundance, selected or non-selected, from bone marrow puncture to the region of interest, which permits a much greater availability of stem cells for the heart than the normal wound healing in various heart diseases or in cardiac transplantation models per se would bring about.

Experimental studies will be needed simultaneously to differentiate between the therapeutically most successful kinds of bone marrow cells:

- Global bone marrow containing all mononuclear bone marrow cells or specifically selected subfractions, as isolated cell fractions containing preferably CD34+ or CD34-, CD45-, or AC133+ cells.

- Analysis of the transdifferentiation of bone marrow cells to muscle cells and their contribution to the remodeling process in various heart diseases, including cardiac transplantation models.

- Cardiac lesions may be multifactorial and include myocardial infarction, myocarditis, cardiomyopathy or cardiac remodeling due to severe pressure, and volume overload. It is
uncertain whether the same therapeutic approach and the same type of cells will be suitable for all of these different diseases. However, organ repair by stem cells represents a general biological mechanism. Thus, it will be one of the future tasks to find the most practical and specific way of evolving and targeting the healing potency of stem cells for selected cardiovascular diseases.

Therapeutic Alternatives in Advanced Heart Failure
Except for pharmacotherapeutics and other measures, the therapy of severe global heart failure and of advanced regional contraction insufficiency is based on nonpharmacological interventions. These are aimed at unloading the heart (cardiac assist device), harmonizing the electrical and mechanical course of contraction and relaxation (ventricular synchronization), restoring ventricular geometry by ventricular size diminution (myocardial left ventricular resection), or abolishing detrimental volume overload in mitral incompetence (repair of the mitral valve).26,27 The clinical limitations of all of these approaches, which are aimed at reducing systolic wall stress and myocardial oxygen consumption,28 justify the search for alternative therapeutic options that may beneficially modify the natural course of the disease. By stem cell-derived de novo restoration of damaged cells, replacement of destroyed and scarred tissue with the consecutive improvement of ventricular performance may be possible. It may be speculated that future therapeutic options of combined therapeutical strategies, eg, ventricular resynchronization together with myocardial stem cell repair, may result in additive therapeutical benefit.

Conclusions and Open Questions
Stem cell therapy represents a fascinating new approach for the management of heart diseases. Recent clinical results have shown the feasibility of adult autologous cell therapy in acute myocardial infarction in humans. However, many unresolved questions about experimental and clinical cardiology are still open for future research, especially many basic problems concerning, among others, the following issues:

- The long-term fate of transplanted stem cells in the recipient tissue.
- The ability of transplanted stem cells to find their optimum myocardial “niche.”
- The potency of stem cells to transdifferentiate into heart muscle cells.
- The optimal angiogenic milieu needed for transplanted cells in hypoperfused tissue.
- The capability of the recipient tissue to enable an enhanced environment to offer optimum, milieu-dependent differentiation of engrafted cells.
- Specific detection of engrafted cells or cell populations by labeling techniques.
- The optimal time course of availability and application for stem cell replacement therapy in cardiovascular disease.
- The arrhythmogenic potential of implanted cells.
- The specific characterization of the progenitor cells that should be measured to predict therapeutic effect of transplanted cells.
- Development of safe and reproducible catheter-based delivery systems for depositing stem cells to recipient heart muscle.

Additional research is needed to explore the therapeutic merits of cell transplantation techniques while accepting the likelihood that possible adverse side effects may occur. With regard to the clinical practicability, ethical problems, and hazards of immunogenity, actual and future research will focus preferably on adult stem cells, whereas research on embryonic stem cells may emerge presumably into comparable clinical relevance in several years to come.

References


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