

Adult Stem Cells for Myocardial Tissue Repair

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The prospect of using adult stem cells for myocardial tissue repair has caused understandably great excitement among cardiovascular physicians and scientists, because it may all but revolutionize treatment of the sequels of ischemic heart disease. The traditional definition of a stem cell requires the capacity for “asymmetric” cell division (i.e., the stem cell divides into one stem cell and one differentiated cell), whereas a classic progenitor cell divides in two differentiated daughter cells. The cells that are used in myocardial regeneration attempts do not always fulfill these criteria; it may therefore be more appropriate to talk about cardiac cell therapy in a more general way. In this context, some biologic principles of stem cells may be worth reiterating: Whereas embryonic stem cells are uncommitted and pluripotent in their differentiation capability, adult stem cells are believed to be committed to differentiate only into specialized cells of the organ or tissue they are derived from. The function of adult stem cells seems to be maintenance and repair of their tissue of origin; they are therefore also termed somatic stem cells. Understanding of adult/somatic stem cells has been upset by recent experimental data indicating that adult stem cells derived from hematopoietic tissue can give rise to non-hematopoietic cells such as cardiomyocytes, hepatocytes, endothelial, and epithelial cells. Initially, this was interpreted to represent trans-differentiation of hematopoietic stem cells (HSCs) by crossing lineage boundaries, the so-called “stem cell plasticity.”¹ Alternatively, the

existence of non-HSCs or even more immature multipotent types of stem cells in the various transplanted cell sources as well as the phenomenon of fusion of transplanted cells with resident cells in the damaged organ have been taken into consideration.^{2,3} To date, the mechanisms underlying adult stem cell-mediated organ regeneration are not clear (Figure 2.1). Various types of progenitors and stem cells with myocardial regenerative potential have been derived from skeletal muscle and myocardium as well as different hematopoietic cell sources including bone marrow (BM), peripheral blood (PB), and umbilical cord blood.

In the following text, we will discuss the pathophysiologic background of ischemic heart failure and the rationale for the use of adult stem cells to regenerate ischemic myocardium. We will further highlight information on contractile muscle-derived regenerative cells as well as adult stem cells from hematopoietic tissue to build a basis for a critical discussion of the ongoing clinical trials.

Ischemic Heart Disease

Despite a better understanding of its etiology, the prevalence of ischemic heart disease remains exceedingly high in industrialized countries, and is on the rise in developing countries. Risk factors for coronary atherosclerosis have long been established, but it remains unclear whether

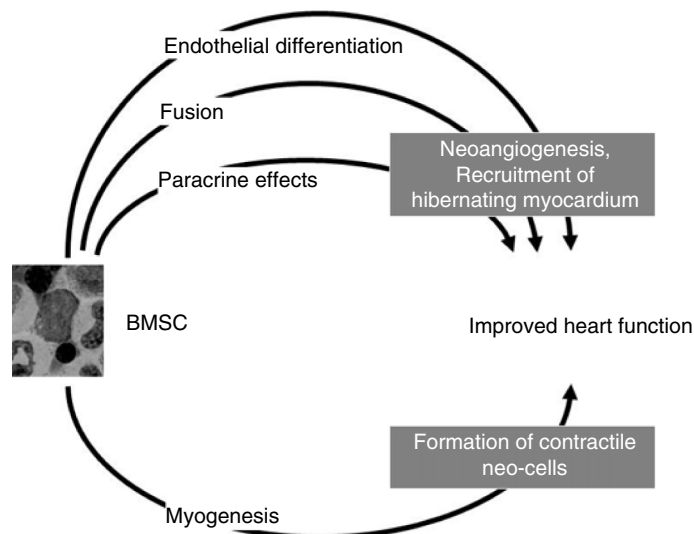


Figure 2.1. Several mechanisms for cell therapy-induced improvement of left ventricular contractility are possible. Neoangiogenesis does not necessarily require true differentiation of adult stem cells in cells of endothelial phenotype. Paracrine effects of transplanted stem cells have been shown to be at least partly responsible for vasculogenesis, and fusion with host tissue cells might also have beneficial effects. In turn, improved perfusion of ischemic myocardium may translate into better contractility via recruitment of hibernating myocardium. However, formation of new contractile tissue would likely require myogenic differentiation of stem or progenitor cells. BMSC, bone marrow stem cell.

there is one unifying mechanism by which atherosclerotic narrowing of the coronary arteries develops. In many patients, sudden rupture of the endothelial surface of a localized atherosclerotic plaque leads to thrombus formation with acute occlusion of the coronary vessel. The resulting myocardial ischemia induces immediate biochemical changes and loss of myocardial contractility. Irreversible necrosis of cardiomyocytes takes approximately 30 minutes to develop, and further extends with time for several hours. Thanks to improvements in primary and secondary prevention as well as therapeutic interventions, both incidence and mortality of acute myocardial infarction (AMI) recently decreased, but the number of individuals suffering AMI is still estimated to exceed 1.5 million per year in the United States and 2 million per year in Western and Central Europe. Many patients develop diffuse atherosclerotic disease of the entire coronary artery tree, and repeated episodes of AMI may result in severely impaired myocardial contractility and heart failure, often described as “ischemic cardiomyopathy.” The most problematic consequence – besides the clinical symptoms of angina pectoris – is a net loss of contractile tissue. The myocardium consists of terminally differentiated cells without a clinically relevant potential for regeneration,

although the existence of cardiac stem cells has recently been indicated. Hence, large numbers of cardiomyocytes that were subject to necrotic or apoptotic cell death cannot sufficiently be replaced by new contractile cells. Instead, remodeling processes ultimately lead to diffuse interstitial myocardial fibrosis or formation of a transmural fibrous scar. Without invasive treatment, survival of patients with myocardial infarction and considerably reduced left ventricular (LV) contractility [e.g., LV ejection fraction (LVEF) <30%] is less than 40% after 5 years, which illustrates the urgent need for novel therapeutic measures. Surgical or interventional restoration of blood supply to ischemic myocardium effectively treats angina, prevents myocardial infarction, improves function of the remaining viable myocardium, but viability and function of necrotic myocardium cannot be restored with current therapeutic means.

Recently, transplantation of cells into infarcted myocardium has evolved as a strategy to restore myocardial viability and contractility. Initial reports on cell therapy for myocardial tissue repair described the implantation of differentiated cells (i.e., cardiomyocytes) or defined progenitor cells [i.e., skeletal myoblasts or endothelial progenitor cells (EPCs)].^{4–6} Contractile cell types such as allogenic cardiomyocytes or skeletal myoblasts

have been shown to survive in areas of myocardial necrosis and to improve local contractile function.⁴ Because of their ease of isolation, cells from hematopoietic tissue are considered the best available source of adult stem cells.⁷ Finally, embryonic stem cells have been used experimentally in the context of myocardial cell therapy, but will not be reviewed in this chapter. The potential to induce both neoangiogenesis and neomyogenesis in infarcted myocardium by transplanting adult stem cells attracted tremendous attention by clinicians and basic researchers. The current understanding of the underlying mechanisms has recently been reviewed in great detail.^{8–10} Herein, we will briefly portray the various cell types and their experimental applications, summarize the current clinical experience with myocardial cell therapy, and discuss some of the problems arising in the process of rapid bench-to-bedside translation.

Contractile (Progenitor) Cells

Among the first cells that were implanted in experimental models of myocardial infarction were allogenic or syngenic *cardiomyocytes*. After direct injection of cardiomyocytes into postinfarction scar tissue, they survived, appeared to be integrated into the myocardial syncytium as evidenced by formation of intercalated disks and expression of specific gap-junction proteins, and led to improvement of myocardial contractility.¹¹ Even though the initial reports were promising, cardiomyocyte transplantation will probably not reach clinical significance for several reasons: First, availability is limited. Neonatal rodent cardiomyocytes can be isolated and cultivated, but adult cardiomyocytes in higher mammals have virtually no capacity for proliferation in cell culture. Theoretically, allogenic or xenogenic cardiomyocytes could be obtained from donors, but would be subject to rejection, unless immunosuppression would be induced as in any other organ transplant patient. Second, transplanted cardiomyocytes are as susceptible to ischemia as the native cardiomyocytes. Implanted in ischemic myocardium, they are therefore prone to succumb to tissue hypoxia just as the native host cells did. Third, ventricular cardiomyocytes spontaneously produce action potentials and contract, albeit slowly.

This quality is potentially lifesaving in a patient with bradycardia, but may result in serious ventricular arrhythmia when such cells form an arrhythmogenic focus that cannot be effectively suppressed by the surrounding host myocardium. In fact, cardiomyocytes have been shown to act as pacemakers after implantation in dog hearts once the native conduction system was destroyed.¹² In contrast, *smooth muscle cells* do not contract spontaneously in vitro or after implantation in the heart. They can be isolated from intestine, blood vessel wall, or genitourinary organs, and may readily be expanded in cell culture. Their potential usefulness for restoration of contractile tissue after AMI has been demonstrated in rodent models,¹³ but they are currently not as intensively studied as other cell types. The largest body of knowledge has most likely been collected regarding *skeletal myoblasts*. These so-called “satellite cells” reside in the periphery of skeletal muscle fibers and serve to regenerate skeletal muscle tissue after injuries. Skeletal myoblasts can be readily isolated from a small muscle biopsy; in humans, the thigh musculature is often used. The isolation process involves enzymatic digestion and mechanic destruction of myofibers, and the myoblasts are collected and enriched by filtration and plating. No specific surface marker-based cell selection is necessary. There is usually some fibroblast contamination, which can be sufficiently controlled by frequent culture replating, and purity of the final product should exceed 80%. Myoblasts have a robust proliferation capacity and multiply in high-serum concentration for numerous passages without changes in phenotype. Once the serum concentration in the medium is lowered, they rapidly differentiate and fuse to form multinucleated myotubes. Besides their intrinsically preprogrammed differentiation in a myocyte phenotype, their most intriguing quality is resistance to hypoxia, and it has been assumed that myoblasts are able to survive in ischemic myocardium better and longer than cardiomyocytes. Doubt remains, however, as to their ability to functionally integrate into the myocardial syncytium, i.e., to express cardiac-specific connexins and form functioning gap junctions with surrounding viable cardiomyocytes. In principle, undifferentiated myoblasts can express connexin 43 (Cx43), the predominant gap-junction protein in ventricular myocardium, and at least some of the myoblasts appear to be able to form functioning

cell–cell communications with cocultured cardiomyocytes *in vitro*.¹⁴ Once they have differentiated and formed myotubes in conventional two-dimensional (2-D) culture, the Cx43 expression is markedly down-regulated but may persist to some degree. However, when myoblasts are grown and differentiate in 3-D culture under mechanical stimulation such as longitudinal strain, Cx43 expression is preserved or even up-regulated. The situation *in vivo* after implantation in infarcted myocardium is naturally more difficult to assess. Many reports indicated that skeletal myotubes are not morphologically integrated in the host myocardium; instead they appear to form distinct islets in postinfarct tissue. Cx43 expression in transplanted myoblasts has been described in several animal models, but has not been detected in patients who underwent myoblast injection and postmortem histologic studies. In a careful study of myoblast transplantation in mice, Rubart et al.¹⁵ found that the majority of the intramyocardial myoblasts/myotubes are functionally isolated from the surrounding myocardium, and suggested that the remaining cells connect with host cardiomyocytes as a result of cell fusion. Their observations might not only explain the conflicting results of other studies, but also provide an explanation of a significant clinical problem: The durations of calcium transients recorded from intramyocardial skeletal myoblasts were heterogeneous compared with those in neighboring host cardiomyocytes, which may interfere with the propagation of excitation across the ventricular myocardium and pose the heart at risk of ventricular arrhythmia. Rhythm disturbances have indeed been observed in several patients who underwent skeletal myoblast implantation. Whereas the very first patient who underwent myoblast implantation together with a coronary artery bypass grafting (CABG) operation in a pioneering undertaking by Menasché and colleagues¹⁶ had an uneventful postoperative course, several other patients developed life-threatening ventricular arrhythmia a few days after the procedure. Surgical patients were still under close observation and could be treated, but elsewhere patients had undergone transcatheter myoblast implantation with early discharge from the hospital and could not be saved. Therefore, at least in well-structured trials, clinical myoblast implantation for heart failure is currently limited to patients who have an automatic defibrillator device implanted. As to the

efficacy of myoblast implantation, numerous small and large animal studies have shown a significant improvement of LV contractility. Results of the early clinical pilot trials were also encouraging, but results of large-scale controlled clinical trials are not yet available.

Marrow, Blood, and Cord Blood Cells

Blood and bone are attractive, readily accessible sources of stem cells (Table 2.1). These cell reservoirs harbor various populations of candidate regenerative cells including hematopoietic progenitor cells (HPCs)⁴ and HSCs, EPCs,⁵ mesenchymal stem cells (MSCs),^{6–8} and multipotent adult progenitor cells (MAPCs).^{9,10} In the context of cardiac cell therapy, the term “adult stem cells” usually refers to the application of mixed cell populations obtained from marrow, PB, or cord blood. Other sources of cells with the capacity for asymmetric division and plasticity such as fatty tissue or pancreas have, so far, only occasionally been used in myocardial regeneration attempts. Contradictory findings regarding the regenerative potency of these cells are, at least in part, attributed to the heterogeneity of stem cells present in different sources and preparations. Within BM, at least two distinct populations of progenitor and stem cells have so far been clearly recognized. These are (i) CD34⁺/45⁺/133⁺ HPCs including a small fraction of HSCs as the traditional source of hematopoietic progeny, and (ii) CD34[−]/45[−]/133[−] MSCs with the capacity to renew marrow stroma, support hematopoiesis, and to differentiate into various mesodermal cell types including connective tissue (chondrocytes, osteocytes), adipocytes, and myocytes.^{17,18} The phenotype of *in vitro* cultured MSCs shows a typical combination of marker molecules CD73 (SH3, SH4), CD90 (Thy1), and CD105 (SH2) with a lack of the HSC markers CD34 and CD133. The homogeneous expression of the otherwise endothelial-specific molecule CD146 (Muc18) may allow for speculations about a certain relation between MSCs and the endothelial lineage (Figure 2.2). In fact, MSCs cultured under standard conditions readily form vascular-like networks after implantation into a 3-D Matrigel matrix [Figure 2.3A (see color section)], although these structures are less complete than that formed by primary vascular endothelial cells

Table 2.1. Adult progenitor and stem cells for myocardial repair*

Cord blood	Bone marrow	Peripheral blood	Mobilized peripheral blood	Tissue
Hematopoietic stem cells ⁵²	Hematopoietic stem cells ⁵²	Hematopoietic stem cells ⁵²	Hematopoietic stem cells ⁵²	Side population ⁵²
Hemangioblast ⁵³	Hemangioblast ⁵³	Blood outgrowth endothelial cell ²³	Hemangioblast ⁵⁴	Endothelial progenitor cells ⁸
Circulating endothelial progenitor ⁵⁵	Marrow endothelial progenitor ²¹	Circulating endothelial progenitor ²⁴	Circulating endothelial progenitor ⁵⁵	Mesenchymal stem cells ⁵⁶
Universal somatic stem cell ⁵⁷	Mesenchymal stem cell ⁵⁸	Endothelial progenitor cell ⁶	Mesenchymal stem cell ⁵⁹	Skeletal myoblast ⁴
	Multipotent adult progenitor cell ²¹	Circulating endothelial cell ²⁵		

*Candidate adult stem cell types and possible sources for cell transfer-induced myocardial regeneration. Numerous subpopulations of cells with regenerative capacity have been described. They are mainly defined by expression of surface markers, and some by functional properties such as adhesion to plastic surfaces. Although the list of cell types is growing steadily, the ideal cell has not yet been found. The references are by no means exhaustive. They have merely been selected to allow for a first orientation in the rapidly growing field of adult stem cell research.

[Figure 2.3B (see color section)]. Another hallmark of EPCs, the formation of cord-like structure in liquid cultures, can also regularly be found in early MSC-containing BM explants [Figure 2.3C (see color section)]. Almost complete acquisition of endothelial phenotype and function under optimized culture conditions has recently been reported.¹⁹ A minute subpopulation of cells that copurify with MSCs has been originally described as mesodermal progenitor cells and, because of the wide spectrum of differentiation, renamed as multipotent adult progenitor cells (MAPCs).²⁰ These CD34⁻/133⁺/Flk1⁺ cells have been postulated to comprise the principle endothelial progenitor in human postnatal BM.²¹ Human MAPCs can differentiate into

adipocytes, osteoblasts, endothelial cells, and hepatocytes in vitro and, after injection into the tibialis anterior muscle of NOD/SCID mice in vivo, into myocytes.²²

Endothelial cells can definitively be derived from BM²³ but a conclusive description of an endothelial stem cell in adult BM is still a matter of debate. CD133⁺/VEGFR2⁺ BM-derived cells are currently the most widely used functional EPC.⁶ Putative BM-derived progenitors of endothelial cells have been originally recovered from normal PB as CD34⁺/45⁺ cells.²⁴ The relation between these cells and circulating endothelial cells could not be clarified so far.²⁵ Various types of cells related to the endothelial lineage have thus far been described in most hematopoietic

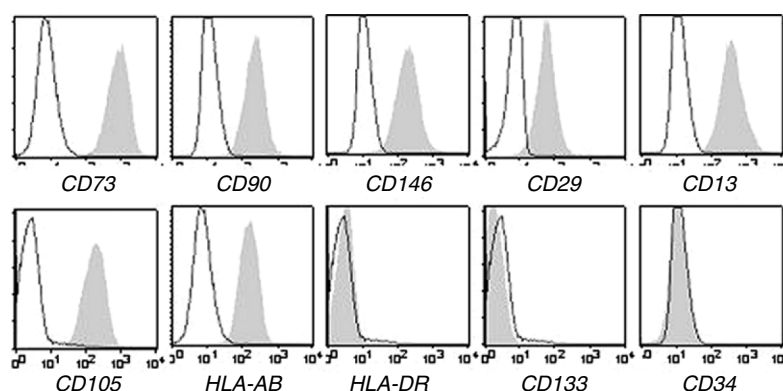


Figure 2.2. Immune phenotype of in vitro cultured adult human bone marrow MSCs. Flow cytometric analysis of typical marker molecules on short-term cultured human adult MSCs. (Courtesy of Christina Malischnik, BSc.)

and nonhematopoietic tissues (Table 2.1). The differences in frequency, phenotype, and function of these endothelial lineage cells clearly need to be analyzed in greater detail. The reactivity of several of these cell types with CD133 and the coexpression of CD133 on the greater part of CD34⁺ HPCs and HSCs have been taken as a strong argument in favor of positive selection to enrich adult stem cells for clinical use.

Adult Stem Cells for Myocardial Regeneration

Ideally, a stem cell that has been implanted into infarcted myocardium would give rise to new blood vessels *and* new contractile cells (i.e., angiogenesis and myogenesis). Proliferation and differentiation would be guided by local humoral and/or cellular control mechanisms, and no neoplastic growth or differentiation in unwanted noncardiac cell types would occur. So far, neither the ideal source and type of stem cell nor the critical cell number and mode of application have been defined. In 2001, two experimental studies of myocardial repair by adult stem cells from hematopoietic sources after experimental myocardial infarction promoted an unparalleled boost of clinical and experimental regenerative stem cell therapy studies. They also mirror the variety of adult stem cells used for myocardial repair. Kocher et al.²⁶ found that systemic intravenous infusion of purified human CD34⁺ cells from granulocyte colony-stimulating factor (G-CSF) mobilized PB can improve rat heart function by generating new blood vessels within the infarct area ("neovasclogenesis"). Our own group has reproduced this phenomenon using human cord blood cells [Figures 2.4 and 2.5 (see color section)]. Orlic et al.^{27,28} reported on both neovasclogenesis and transdifferentiation of transplanted cells into cardiomyocytes by either using intramyocardial application of mouse BM-derived Lin^{NEG}/c-Kit⁺ stem cells or cytokine-induced stem cell mobilization. Many of the initial clinical pilot trials were fueled by the suggestion that early hematopoietic stem cells have a plasticity high enough to enable differentiation into contractile cells of cardiomyocyte phenotype. In fact, such cells were considered to be able to truly regenerate infarcted myocardium by promoting both neoangiogenesis and neomyogenesis. The initial enthusiasm, however, has largely

faded. Whereas *in situ* neoangiogenesis induction by hematopoietic cells, associated with functional improvements, is consistently observed, it proved difficult to find corroborating evidence for true cardiomyocyte differentiation. In fact, two independent groups reported early in 2004 that they did not detect any meaningful evidence of cardiomyocyte differentiation of HSCs in mouse models that were designed to confirm the earlier findings.^{29,30} In contrast, the myogenic potential of stroma cell-derived MSCs is much better documented. Several years ago, Wakitani et al.³¹ reported the *in vitro* development of myogenic cells from rat BM MSCs exposed to the DNA-demethylating agent 5-azacytidine, and Makino et al.³² isolated a cardiomyogenic cell line from murine BM stromal cells that were treated with 5-azacytidine and screened for spontaneous beating. Those cells connected with adjoining cells, formed myotube-like structures, and beat spontaneously and synchronously. They expressed various cardiomyocyte-specific proteins, had a cardiomyocyte-like ultrastructure, and generated several types of sinus node-like and ventricular cell-like action potentials. When isogenic marrow stromal cells are implanted in rat hearts, they appear to become integrated in cardiac myofibers, assume the histologic phenotype of cardiomyocytes, express connexins, and form gap junctions with native cardiomyocytes.^{33,34} Again, pretreatment with 5-azacytidine is believed to facilitate differentiation toward a cardiomyocyte phenotype *in vivo*.³⁵ Human MSCs derived from the marrow of volunteers have also been injected in hearts of immunodeficient mice, and again it was observed that they assume cardiomyocyte morphology and express various cardiomyocyte-specific proteins.³⁶

Taken together, there is quite convincing evidence that MSCs derived from marrow stroma may assume a myocyte-like phenotype under certain conditions, whereas hematopoietic stem and progenitor cell types are primarily involved in angiogenic processes (Figure 2.6). Nevertheless, numerous questions remain to be answered before cell therapy for neovascularization of ischemic myocardium can become clinical routine: First, is the angiogenic potential of human cells sufficient for relevant neovascularization of ischemic myocardial tissue? Second, what are the best surface markers of human adult stem cells with angiogenic potential? Third, how are such cells best delivered to ischemic myocardium? Fourth, what is the molecular mechanism of stem

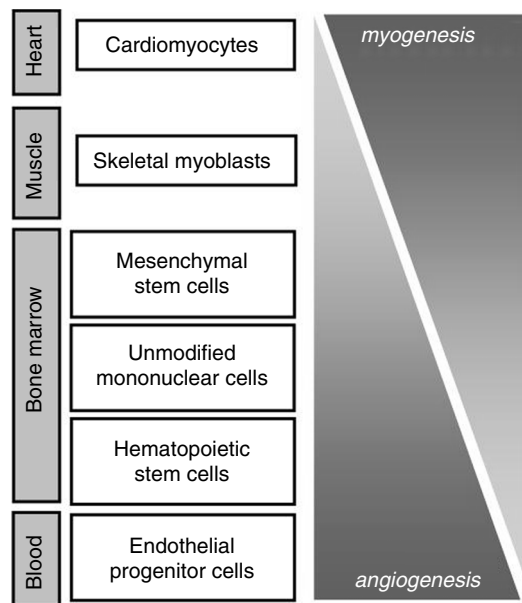


Figure 2.6. Of the clinically relevant cell types, some are primarily myogenic cells, and others have angiogenic characteristics. Some investigators argue that the angiogenic and myogenic properties are mutually exclusive; others claim that several cell types have capacity for both.

cell-endothelial differentiation? Fifth, are there risks of cell-induced neovascularization that outweigh the potential benefit? Finally, can the natural angiogenesis-inducing capacity of adult stem cells, which is obviously not sufficient in many situations, be further augmented?

Cell Survival

When cells are injected into ischemic myocardium, it is likely that most of them do not survive. The cell preparation process is usually well tolerated. Skeletal myoblasts or other ex vivo expanded cells, as well as processed primary cells normally have viability rates higher than 90%. It is often argued that injection of cell suspension through a needle or a catheter significantly compromises cell viability. In our experience, this is not the case. Even injection through a long cardiac catheterization device has very little effect on cell survival. Once the cells have entered the myocardial interstitium, however, many of them appear to succumb to necrotic or apoptotic death. Its magnitude is difficult to determine, but

the suggested survival rate over days or weeks ranges between 0.1% and 10%. The causality is probably multifactorial. First of all, the ischemic myocardium is obviously a hostile environment, because of local hypoxia, acidosis, lack of substrates, and accumulation of metabolites. Skeletal myoblasts are known to be quite resistant to ischemia, but little is known about the energy requirements of hematopoietic or mesenchymal stem cells. Second, necrotic myocardium is subject to infiltration with phagocytic cells that remove cell debris and initiate the scarring process. Even though the stem/progenitor cells used clinically are autologous, many are probably lost in this “clean-up” process. Third, the mechanic forces that are present in the myocardium may have a role. Transmural pressure is high during systole, and there are shear forces between contracting myofibers and layers. Again, a skeletal myoblast might be able to tolerate this, but a marrow cell is certainly not well equipped to withstand such stress. Fortunately, the rate of cell death can be slowed by targeted manipulation of the cells to be injected. Transfection of marrow stromal cells with a gene encoding for the anti-apoptotic protein AKT has been shown to greatly improve cell survival and regenerative capacity upon injection in infarcted myocardium. Pretreatment of EPCs with endothelial nitric oxide synthase-enhancing substances also seems to have a beneficial conditioning effect. Simple hypoxic preconditioning before cell injection might also help, because it has been shown to activate both antiapoptotic and nitric oxide-related signaling pathways.

Functional Effects

To the clinician, the functional effects of myocardial cell therapy matter so much more than histologic evidence of neoangiogenesis or neomyogenesis. The reliability of LV function measurements in experimental models depends both on the animal model and on the method used. Much of the basic experimental work on cell therapy for myocardial regeneration has been done in mouse models. The main reasons for that are the availability of immunodeficient mice (SCID mice) that allow for the use of human cell transplants with immunologic response, the use of GFP, lac-Z, or otherwise labeled cells from genetically modified animals,

and the ready availability of antibodies and nucleic acid probes for detailed expression analysis *in situ*. The possibilities of histologic analysis of mouse heart tissue after cell transfer are virtually unlimited. The problem, however, is the functional analysis. Echocardiographic as well as magnetic resonance imaging of mouse hearts are indeed possible, but given the tiny dimensions and tremendously high heart rate, reliable determination requires highly experienced investigators. The most robust parameter of global LV contractility in mice may be shortening fraction (the extent by which the diameter of the LV cavity decreases during systole), but reproducible data on regional contractility are almost impossible to obtain. The next larger model, the rat, has been used for cardiac cell transplantation with good results. The availability of athymic rats allows for xenogenic transplantation of human cell populations without T cell-mediated acute rejection, and functional studies are much easier. Serial echocardiographic analysis of global LV function and dimensions can be performed with good reproducibility. Moreover, cardiac catheterization is feasible, and by using a multielectrode electromagnetic conductance catheter supplied with a pressure transducer, LV pressure-volume curves can be recorded. Those allow for detailed functional analysis of myocardial contractility and relaxation properties. The rabbit as a model for myocardial cell transfer experiments is being used quite rarely. There is no clear advantage with respect to functional analysis as compared with the rat, and the problems with antibodies and other tools for expression profiling and histology are notorious. Thus, truly reliable analysis of myocardial function after cell therapy requires the use of a large animal model, *i.e.*, porcine, canine, or ovine. Here, both echocardiography and magnetic resonance imaging allow for serial studies of global as well as regional myocardial contractility in the specific area of interest. In acute experiments or set-ups with chronic instrumentation, very reliable sonomicrometry data can be obtained after cardiac implantation of ultrasound crystals. Scintigraphic perfusion scans and coronary angiography can be performed. A major disadvantage of many large animal models is the deficiency of antibodies against stem cell surface markers, and the inevitable immune response when xenogenic, *i.e.*, human cells are used.

The baseline result of most of the published work is that there is some improvement of global and – if appropriate – regional contractility after adult stem/progenitor cell implantation in ischemic myocardium. This has been described for skeletal myoblasts, unfractionated mononuclear BM cells, stroma cell-derived cell lines, and cells selected using markers of HSCs or EPCs. Most frequently, an improvement of regional wall movement and/or systolic wall thickening in the cell-treated area has been described. The improvement of global LV contractility is usually in the range of 5% to 20%, and the size of the infarct area is somewhat smaller on histology than in untreated animals. In several small animal studies, a reduction of postinfarct mortality has also been observed. One particularly important lesson learned from large animal experiments is the notion that one should aim at injecting cells that work primarily via angiogenesis induction into the infarct border zone, rather than in the center of the infarct area. Only by doing so, a relevant improvement of myocardial contractility and perfusion is likely to be induced.³⁷ When primarily contractile cells, *i.e.*, myoblasts, are used in sufficiently vascularized tissue, this may be less important. To date, no clear advantage of one cell type over another can be seen. Several studies attempted a direct comparison between two or more different cell populations, *i.e.*, skeletal myoblasts versus BM cells, or marrow cells versus EPCs, but the data are not conclusive, yet. The same is to be said regarding the dose-response relationship of a given cell population, as well as the host-related issues such as the infarct size or the interval between myocardial infarction and cell treatment.

Clinical Application

Translation of experimental cell-therapy approaches for myocardial regeneration into the clinical setting has just begun (Table 2.2). Whether the first clinical pilot trials were initiated too early remains subject to very controversial debate. The earliest clinical application was reported by Menasché and colleagues.¹⁶ A patient with significantly reduced LV contractility after myocardial infarction had to undergo CABG to optimize myocardial blood supply. Two weeks before, a muscle sample was

Table 2.2. Clinical cell therapy for myocardial tissue repair*

Reference	Disease	Cell type/Source	Application mode	Patients (n)	Effects	Readout	Additional readout
BOOST Wollert et al. ⁴⁴	AMI	BM-MNC (80–120 mL) vs. CTRL; prospective, randomized (30 + 30)	PCI Intracoronary	60	EF↑ Coronary flow reserve↑	Cardiac MRI i.c. Doppler	LVA FCM
TOPCARE-AMI Assmus et al. ⁶⁰	AMI	BM-MNC (50 mL) vs. adher. cult. PB-MNC (250 mL) vs. CTRL	PCI Intracoronary	59 (200)	Global and regional EF↑ Viability↑ LVESV↓	LVA, TTE, MRI PET, MRI LVA	FCM
Univ. Düsseldorf Strauer et al. ⁶¹	AMI	BM-MNC (40 mL) vs. CTRL	PCI Intracoronary	60	EF unchanged Hypokinetic area↓ Contract. infarct region↑	MUGA – RNV LVA LVA	DSE RVA
MAGIC Kang et al. ⁴⁹	AMI	G-CSF PB-MNC (10 ⁹ ~ Aph.) vs. G-CSF (s.c. 10 µg/mL) vs. CTRL – stopped	PCI Intracoronary vs. s.c. G-CSF mobilization	27	Aggravated restenosis† EF↑ Perfusion defect↓ LVESV↓ (P = 0.05) Exercise capacity↑	LVA D-SPECT Treadmill	i.c. Doppler DSE FCM
NIH Hill et al. ⁵¹	CAD (iAP)	G-CSF (10 µg/kg/d) Pilot study – closed	s.c.	12	2 AMI 1 Cardiac death		
Univ. Rostock Stamm et al. ⁴⁰	CHF post-AMI	CD133+ BM-MNC (85–195 mL)	i.my./CABG	33	EF↑ Perfusion defect↓ LVEDV↓	SPECT	24 h ECG FCM
Texas Heart Perin et al. ⁴¹	CHF	BM-MNC (50 mL) vs. CTRL; prospective, nonrandomized (14 + 7)	i.my./NOGA (15 × 0.2 cc)	30	EF↑ LVESV↓ Perfusion defect↓ NYHA/CCSAS↓	LVA SPECT	Clinical/lab. eval.; treadmill; 2-D Doppler; 24 h ECG
Washington, DC; New York; and Israel Fuchs et al. ^{42,43}	CHF	BMC (20 mL) Pilot study	i.my./NOGA (12 × 0.2 cc)	27	Stress perfusion defect↓ CCSAS↓ (Exercise capacity non sign↑) No arrhythmia	D-SPECT Treadmill	ECG Clinical/lab. eval. ELISA (VEGF; MCP1)
Univ. Hong Kong Tse et al. ³⁹	CHF	BM-MNC (40 mL)	i.my./NOGA	8	Wall motion and thickening↑ Perfusion defect↓; no arrhythmia EF unchanged NYHA↓	MRI (7/8) SPECT; 24 h ECG	ECG; medication use Clinical/lab. eval. CFU-GM; FCM
Paris Menasché et al. ^{16,62}	CHF post-AMI EF <35%	Cultured skeletal muscle cells (myoblasts ~8.7 × 10 ⁸)	i.my./CABG	10	EF↑; wall thick. ↑; NYHA↓ 4 Delay tachycardia → ICD 1 Unrelated death	TTE 24 h ECG	Clinical/lab. eval.

(Continued)

Table 2.2. Clinical cell therapy for myocardial tissue repair*—Cont'd

Reference	Disease	Cell type/Source	Application mode	Patients (n)	Effects	Readout	Additional readout
Univ. Pamplona Herreros et al. ⁶³	dHF post-AMI EF <35%	Cultured skeletal muscle cells (myoblasts ~1.8 × 10 ⁸)	i.my./CABG	12	Perfusion defect↓; no arrhythmia EF↑; wall thick. ↑	¹⁸ F FDG PET TTE	ECG; Clinical/lab. eval. ¹³ N PET; FCM

*Published studies more than patients all showing feasibility; update AHA 2004 if available.
†Coronary restenosis observed.
dHF, chronic ischemic heart failure; LVA, RVA, left/right ventricular angiography; LVESV, left ventricular end systolic volume; BMC, filtered heparinized whole bone marrow; DSE, dobutamine stress echo; Aph., apheresis; D-SPECT, dipyridamole/Persantine-SPECT; CAD, severe coronary artery disease; iAP, intractable angina pectoris.

obtained from the thigh, and skeletal myoblasts were cultivated and grown for several passages. During the bypass operation, these cells were directly injected into the infarcted myocardium. The patient tolerated the procedure well, and a distinct improvement of wall thickening and motion in the area of cell injection was noted postoperatively. This procedure has been repeated worldwide ever since, and the reports quite uniformly describe a mild improvement in contractility. Analogous to the experimental experience, however, the myofibers that originated from the transferred myoblasts appear to survive in the infarct tissue but do not fully integrate into the myocardial syncytium (see above). The CABG operation represents a unique opportunity to directly access the infarcted myocardium, but, at the same time, conceals the effects of the cell therapy. Analysis of regional contractility may give some hints regarding the cell-induced functional improvement, but, inevitably, there is always some overlap of the effects of myocardial revascularization and those of the cell injection. Shortly after the advent of combined CABG and myoblast injection, a procedure was developed that enables the cardiologist to deliver myoblasts directly to the myocardium as a stand-alone treatment. By using a novel cardiac catheterization device, the infarcted myocardium can be quite precisely located based on its electrophysiologic properties [Figure 2.7 (see color section)]. A map of the endocardial surface of the LV myocardium is then constructed that clearly depicts the localization of the infarct tissue. A needle is advanced through the endocardial layer into the myocar-

dium at a preset depth, and the cell suspension is injected. This procedure has been performed in patients with severely impaired LV function, and, again, a mild improvement of LV contractility was noted, together with some relief of the symptoms of ischemic heart failure. The myoblast injection, however, seemed to result in a transient period of electrical instability a few days after the injection, which repeatedly led to sustained ventricular arrhythmia. Therefore, myoblast injection as a stand-alone treatment is currently limited to patients who have an automatic defibrillation device implanted. It should also be noted that it is still controversial to what extent the preexisting ischemic heart disease, rather than the cell transfer, is responsible for the observed rhythm disturbances.

Although clinical skeletal myoblast transfer is mainly limited to patients with chronic heart failure, cell therapy with BM or blood-derived cells may evolve in a novel treatment option for both chronic ischemic heart disease and AMI (Table 2.3). In either situation, the angiogenic potential of certain adult stem/progenitor cell types is probably the key to functional improvements, whereas true neomyogenesis is, at present, rather unlikely. Around 2001, a number of clinical trials were initiated, and among the first published was the work by Hamano et al.,³⁸ who injected BM mononuclear cells intramyocardially during a CABG operation, Tse et al.,³⁹ who used a catheter-based system for direct intramyocardial delivery of mononuclear cells, and our own group,⁴⁰ who injected a purified population of AC133+ BM cells, again in conjunction with a CABG operation. Several other groups have

Table 2.3. Principal “Pros” (+) and “Cons” (–) of clinically usable stem cell sources

	+	–
Skeletal myoblasts	Myocyte phenotype	Arrhythmia Preparation Angiogenesis ?
Crude bone marrow	Instant preparation	Stem cell content ? Inflammation ?
Marrow stroma cells/mesenchymal stem cells	Myogenic potential	Preparation Angiogenesis ?
Hematopoietic marrow stem cells	Rapid preparation Approval (GMP/GLP) Angiogenesis	Myogenesis ?
Circulating progenitor cells	Angiogenesis	Preparation Myogenesis ?
Mobilization (G-CSF)	“Noninvasive”	Systemic inflammation
This simplified summary is based on data and anecdotal experience that are constantly being revised and updated. To date, there is no “ideal” cell type for myocardial regeneration.		

reported similar studies since then.^{41–43} What these trials have in common is that patients with chronic ischemic heart disease are addressed. The most recent myocardial infarction in those patients usually dates back several weeks, months, or even years. In the infarcted or chronically ischemic myocardium, a substantial net loss of contractile tissue mass has occurred, increased collagen deposition has led to a more or less pronounced, diffuse, or localized scar formation, and blood supply to the myocardium remains impaired although there may have been some collateral vessel growth. Usually, the ischemic myocardium in those patients is not a complete transmural fibrous scar, which would eventually progress into an LV aneurysm, but still vital cardiomyocytes are dispersed within the fibrous network. Theoretically, such “hibernating” cardiomyocytes can be re-recruited for contractile work once sufficient supply of oxygen and nutrients has been reestablished, and stem/progenitor cell-induced growth of microvessels in the infarct borderzone may thus translate into improved myocardial contractility. Nevertheless, it should be kept in mind that the chances to resuscitate hibernating cells in a functionally relevant manner are likely to decrease with time. The longer the interval between myocardial infarction and cell treatment is, the smaller the chance to achieve a beneficial effect becomes. This notion, however, is largely intuitive, and presently not supported by clear-cut data.

The situation in AMI studies is fundamentally different. Here, the onset of myocardial ischemia was usually between several hours and

a few days previous. Typically, a patient is admitted with acute chest pain and electrocardiogram shows signs of myocardial ischemia. Laboratory tests indicate the onset of myocardial necrosis and liberation of cardiomyocyte-specific intracellular proteins (i.e., CK-MB, troponin) after loss of cell membrane integrity. If possible, the blocked coronary artery is immediately reopened by emergency catheterization, balloon dilation, and stent placement. The extent of myocardial necrosis and thus the impairment in contractility largely depend on the time that has passed until the infarct vessel is reopened. There is no way to predict the ultimate infarct size in a given patient. Ideally, cardiomyocyte necrosis is completely prevented because the coronary artery has been quickly reopened. In many patients, the necrotic myocardium does not extend across the entire wall thickness (nontransmural infarction), and in some the entire myocardium downstream to the arterial obstruction is subject to complete necrosis (transmural infarction). Analogous to the chronically hibernating myocardium, acutely ischemic cardiomyocytes can still be vital but have temporarily lost much of their capacity for contractile work (myocardial stunning). Tissue infiltration with inflammatory cells is beginning, but fibrous scarring has not yet occurred. In this situation, direct injection of cell suspension into the weakened myocardium is prohibitive, but infusion of stem/progenitor cells into the reopened coronary artery is currently being evaluated.

So far, mainly BM mononuclear cell preparations have been used in such trials. A few days

after the onset of myocardial infarction, a second cardiac catheterization is performed and the cell suspension is injected into the infarct vessel while blood is temporarily interrupted by balloon inflation. Pilot studies have demonstrated feasibility and safety of this approach, and controlled efficacy trials are currently on the way. In one of the first of those trials, there was a difference in LV ejection fraction of 6% at 6 months follow-up between 30 patients who received intracoronary cell injection and 30 patients who only had standard infarct treatment. On average, LVEF improved by 6.7% in cell-treated patients as compared with immediately after myocardial infarction, whereas LVEF in control patients remained largely unchanged.⁴⁴ Other clinical trials based on the same principle are currently underway, but it is too early to make a definitive judgment about long-term functional efficacy and possible side effects. One of the most interesting questions is how, and to what extent, the intracoronary cell injection leads to stem/progenitor cell extravasation and migration into the myocardial interstitium. Our group has recently established a mouse model that allows for direct visualization of stem cell–endothelial cell interaction and interstitial migration using intravital fluorescence microscopy. This technique will hopefully help to better understand and to optimize the stem cell trafficking to ischemic myocardium.

Stem Cell Mobilization

Another, fundamentally different approach aims at circumventing any invasive procedure for cell delivery while minimizing the interval between the onset of myocardial infarction and cell therapy by mobilizing marrow cells using G-CSF. The idea is that stem/progenitor cells mobilized from marrow will be attracted to the ischemic heart and initiate regeneration events or at least modulate remodeling processes. That the number of circulating progenitor cells can be greatly enhanced by G-CSF stimulation has been well established. However, the number of mature leukocytes also increases markedly, and this has already raised principle concerns regarding the safety of G-CSF treatment. The pathophysiologic equivalent of such reasoning may be the mobilization of EPCs in patients with AMI or various degrees of congestive heart fail-

ure.^{45–47} Because stem cell mobilization did not result in a favorable outcome in a nonhuman primate model of myocardial infarction, this practicable pharmacologic approach has been viewed with some restraint.⁴⁸ Despite safety of G-CSF mobilization with or without consecutive apheresis and favorable short-term results of intracoronary infusion of G-CSF mobilized PB in patients with myocardial infarction after coronary stenting, an unexpectedly high rate of in-stent restenosis has been observed in association with G-CSF.⁴⁹ Moreover, restenosis has at least been mentioned in another (TACT) trial for adult stem cell therapy for peripheral occlusive artery disease with unfractionated BM.⁵⁰ In a third study in 12 patients with intractable angina, the administration of G-CSF was associated with two AMIs and one cardiac death.⁵¹ Nevertheless, several other clinical pilot studies are currently on the way.

Summary

The fundamentals of myocardial tissue repair based on adult progenitor or stem cells are far from being “textbook knowledge.” Knowledge is progressing rapidly, but it is not uncommon that the assumed progression is quickly followed by regression. The unresolved issues are too manifold to list. Despite the surge in cell therapy-related publications, the “facts” that are largely undisputed are few: 1. Contractile cells or their immediate progenitors, obtained from whatever source, can be transferred into ischemic myocardium, and some of them survive and prosper. In many experimental models, this cell transfer indeed leads to an improvement of LV function. 2. Cell types belonging to the hematopoietic–angiogenetic complex, obtained from whatever source, can enhance the vascularization of ischemic tissue, including infarcted myocardium. Again, in many experimental models, this is associated with some improvement of heart function. Having accepted the limitations of our current knowledge, it does not seem inappropriate to state that adult stem/progenitor cell therapy for myocardial tissue repair holds exceedingly great promise. If the translation of experimental approaches in clinical medicine succeeds (Figure 2.8), we will, for the first time, be able to offer patients with heart failure a true cure.

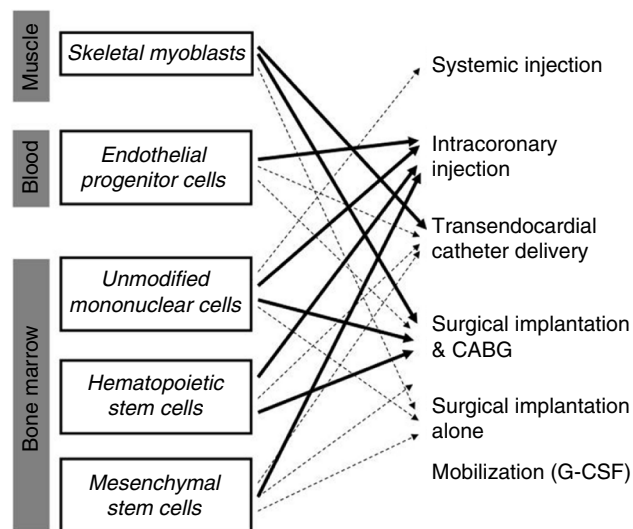


Figure 2.8. Several adult stem cell types can at least theoretically be used in clinical trials of cell therapy for myocardial regeneration. There are also numerous clinically applicable ways to deliver cells to the infarcted heart. The result is a confusing variety of possible trial designs. The solid arrows indicate trials that are currently underway; the results of clinical trials indicated by interrupted arrows have not been reported to date.

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