This Review is part of a thematic series on Stem Cells, which includes the following articles:


Biomaterials to Enhance Stem Cell Function in the Heart [Circ Res. 2011;109:910–922]

Mesenchymal Stem Cells: Biology, Pathophysiology, Translational Findings, and Therapeutic Implications for Cardiac Disease

Role of Cardiac Stem Cells in Cardiac Pathophysiology: A Paradigm Shift in Human Myocardial Biology

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Cell Therapy of Acute Myocardial Infarction and Chronic Myocardial Ischemia: From Experimental Findings to Clinical Trials

Cell Therapy of Heart Failure: From Experimental Findings to Clinical Trials

Cell Therapy of Peripheral Vascular Disease: From Experimental Findings to Clinical Trials

Methodological Issues in Cell Therapy Studies: What Have We Learned From Clinical Studies

Stefanie Dimmeler, Douglas Losordo, Editors

Mesenchymal Stem Cells

Biology, Pathophysiology, Translational Findings, and Therapeutic Implications for Cardiac Disease

Adam R. Williams, Joshua M. Hare

Abstract: Mesenchymal stem cells (MSCs) are a prototypical adult stem cell with capacity for self-renewal and differentiation with a broad tissue distribution. Initially described in bone marrow, MSCs have the capacity to differentiate into mesoderm- and nonmesoderm-derived tissues. The endogenous role for MSCs is maintenance of stem cell niches (classically the hematopoietic), and as such, MSCs participate in organ homeostasis, wound healing, and successful aging. From a therapeutic perspective, and facilitated by the ease of preparation and immunologic privilege, MSCs are emerging as an extremely promising therapeutic agent for tissue regeneration. Studies in animal models of myocardial infarction have demonstrated the ability of transplanted MSCs to engraft and differentiate into cardiomyocytes and vasculature cells, recruit endogenous cardiac stem cells, and secrete a wide array of paracrine factors. Together, these properties can be harnessed to both prevent and reverse remodeling in the ischemically injured ventricle. In proof-of-concept and phase I clinical trials, MSC therapy improved left ventricular function, induced reverse remodeling, and decreased scar size. This article reviews the current understanding of MSC biology, mechanism of action in cardiac repair, translational findings, and early clinical trial data of MSC therapy for cardiac disease. (Circ Res. 2011;109:923-940.)

Key Words: stem cells ■ regeneration ■ cell differentiation ■ hematopoietic stem cells
Ischemic heart disease is the leading cause of death in developed countries and carries significant morbidity. After an acute myocardial infarction (MI), the heart has limited capacity for self-renewal and undergoes remodeling with resulting depressed left ventricular (LV) function. Over the past decade, there has been tremendous enthusiasm in the quest to find a stem cell capable of regenerating lost myocardium and restoring cardiac function.

Mesenchymal stem cells (MSCs) were first identified and isolated from bone marrow (BM) more than 40 years ago and have emerged as one of the leading candidates in cellular cardiomyoplasty (Figure 1). The unique properties of MSCs (easily isolated and amplified from the BM, immunologically tolerated as an allogeneic transplant, and their multilineage potential) have led to their intense investigation as a cell-based therapeutic strategy for cardiac repair. In this review, we describe the biology of MSCs and discuss the data supporting the translation of MSC therapy to clinical trials for cardiac disease.

**Non-standard Abbreviations and Acronyms**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
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<tbody>
<tr>
<td>BM</td>
<td>bone marrow</td>
</tr>
<tr>
<td>CSC</td>
<td>cardiac stem cell</td>
</tr>
<tr>
<td>DE-MRI</td>
<td>delayed-enhancement cardiac magnetic resonance imaging</td>
</tr>
<tr>
<td>HGF</td>
<td>hepatocyte growth factor</td>
</tr>
<tr>
<td>HSC</td>
<td>hematopoietic stem cells</td>
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<tr>
<td>MSC</td>
<td>mesenchymal stem cell</td>
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**Historical Overview**

In 1970, Friedenstein and colleagues demonstrated that BM contains a population of hematopoietic stem cells (HSCs) and a rare population of plastic-adherent stromal cells (1 in 10,000 nucleated cells in BM). These plastic adherent cells, initially referred to as stromal cells and now commonly called MSCs, were capable of forming single-cell colonies. As the plastic-adherent BM cells were expanded in culture, round colonies resembling fibroblastoid cells formed and were given the name colony forming unit–fibroblasts. Friedenstein was the first investigator to demonstrate the ability of MSCs to differentiate into mesoderm-derived tissue and to identify their importance in controlling the hematopoietic niche. Control of stem cell niches (functional and structural units that spatiotemporally regulate stem cell division and differentiation) is emerging as a key role played by MSCs in a broad array of tissues, including hair follicles and the gut, and recently, MSC ablation was shown to disrupt hematopoesis.

During the 1980s, MSCs were shown to differentiate into osteoblasts, chondrocytes, and adipocytes. Caplan demonstrated that bone and cartilage turnover was mediated by MSCs, and the surrounding conditions were critical to inducing MSC differentiation. In the 1990s, MSCs were shown to differentiate into a myogenic phenotype, and Pittenger and colleagues demonstrated that individual adult human MSCs were capable of being expanded to colonies while retaining their multilineage potential. Also during the late 1990s, Kopen et al described the capacity of MSCs to transdifferentiate into ectoderm-derived tissue.

During the early 21st century, in vivo studies demonstrated that human MSCs transdifferentiate into endoderm-derived...
cells and cardiomyocytes, and in vitro coculturing of ventricular myocytes with MSCs induced transdifferentiation into a cardiomyocyte phenotype. It was also during this time that MSCs were demonstrated to suppress T-lymphocyte proliferation, which paved the way for the application of MSC therapy for allogeneic transplantation and as a potential immunomodulatory therapy. Large-animal preclinical studies of MSC administration in post-MI hearts demonstrated the ability of MSCs to engraft, differentiate, and produce substantial functional recovery. Recently, MSC therapy has been translated to clinical trials for ischemic heart disease.

**Definition of an MSC**

No unique cell surface marker unequivocally distinguishes MSCs from other HSCs, which makes a uniform definition difficult. The International Society for Cell Therapy proposed criteria that comprise (1) adherence to plastic in standard culture conditions; (2) expression of the surface molecules CD73, CD90, and CD105 in the absence of CD34, CD45, HLA-DR, CD14 or CD11b, CD79a, or CD19 surface molecules as assessed by fluorescence-activated cell sorter analysis; and (3) a capacity for differentiation to osteoblasts, adipocytes, and chondroblasts in vitro. These criteria were established to standardize human MSC isolation but may not apply uniformly to other species. For example, murine MSCs have been shown to differ in marker expression and behavior compared with human MSCs. As MSCs are isolated and expanded in culture, it has also been proposed that certain in vivo surface markers may no longer be expressed after explantation, although new markers are acquired during expansion. For example, an MSC line was isolated that uniformly expressed HLA-DR (a marker that should not be expressed on MSCs by the above definition) while also expressing CD90 and CD105, adhering to plastic in culture, and being capable of differentiating into osteoblasts, adipocytes, and chondroblasts. Indeed, MSCs possess species-specific characteristics that make an unequivocal definition difficult to apply to both human and animal models.

Stem cells have classically been defined by their self-renewal capability and multipotency. Some have questioned whether MSCs are true stem cells or rather multipotent precursor cells. MSCs isolated from various adult tissue sources express significantly different morphology, differentiation capabilities, and gene expression. These observations suggest that MSCs from different tissues may not be biologically equivalent and exhibit variable self-renewal and multipotential capabilities. As described above, MSCs express diverse surface markers and are likely a heterogeneous population of cells, which has also raised concern regarding their “stemness.” Further work is required to fully understand the biological differences in MSCs derived from various tissue sources and to characterize tissue-specific MSC self-renewal and multipotency. In view of these considerations, we and others refer to BM-derived cultured MSCs from humans as stem cells, because they have proven capacity for self-renewal and multilineage differentiation.

**Sources, Isolation, and Types of MSCs**

MSCs isolated from BM, adipose tissue, synovial tissue, lung tissue, umbilical cord blood, and peripheral blood are heterogeneous, with variable growth potential, but all have similar surface markers and mesodermal differentiation potential. Furthermore, MSCs have been isolated from nearly every tissue type (brain, spleen, liver, kidney, lung, BM, muscle, thymus, aorta, vena cava, pancreas) of adult mice, which suggests that MSCs may reside in all postnatal organs. MSCs can be derived from many tissue sources, consistent with their broad, possibly ubiquitous distribution. From a translational perspective, because most studies of MSC therapy for cardiac repair have used BM-derived MSCs, we will focus the present review on BM-MSCs.

The BM is the major source of HSCs that continually renew red blood cells, platelets, monocytes, and granulocytes. The HSCs are housed within the BM by non-HSCs that support the microenvironment necessary for development and differentiation of HSCs, and the MSC is one of the most important cells that supports the BM microenvironment, termed the “hematopoietic niche.” Physiologically, MSCs do not migrate easily in the peripheral blood, and available protocols are not very successful in inducing the translocation of this cell pool from the BM to the periphery. Therefore, isolation and culture expansion of MSCs is usually necessary for therapeutic purposes.

MSCs have been isolated from numerous species, and the 3 critical steps that allow MSCs to be isolated from other BM cells are as follows: (1) the use of density gradient centrifugation (Ficoll or Percoll) to separate nonnucleated red blood cells from nucleated cells; (2) the ability of MSCs to adhere to plastic; and (3) the ability of monocytes to be separated from MSCs by trypsinization. Protocols for isolation and expansion are similar among various species, as depicted in Figure 2.

Expansion of plastic adherence MSCs is the most widely used method of obtaining MSCs and represents the most commonly used source of MSCs for cardiac repair. As knowledge evolves regarding MSC biology, attempts have been made to identify the endogenous MSC precursor based on cell surface antigen expression. A subset of MSCs expressing the marker neurotrophic growth factor (CD271) have been shown to have similar differentiation capacity as traditional MSCs, but they secrete higher levels of cytokines and have greater immunosuppressive properties. Another MSC subpopulation is the Stro-3–positive mesenchymal precursor cell, which has extensive capacity for proliferation and differentiation.

**Immunology of MSCs**

Human MSCs express moderate levels of human leukocyte antigen (HLA) major histocompatibility complex class I, lack major histocompatibility complex class II expression, and do not express costimulatory molecules B7 and CD40 ligand. Tolerance of MSCs as an allogeneic transplant is due to this unique immunophenotype coupled with powerful immunosuppressive activity via cell-cell contact with target immune cells and secretion of soluble factors, such as nitric oxide, indoleamine 2,3-dioxygenase, and heme oxygenase-1. MSCs produce an immunomodulatory effect by interacting with both innate and adaptive immune cells (Figure 3).
The innate immune cells (neutrophils, dendritic cells, natural killer cells, eosinophils, mast cells, and macrophages) are responsible for a nonspecific defense to infection, and MSCs have been shown to suppress most of these inflammatory cells. Neutrophils are one of the first cells to respond to an infection, and an important process in their response to inflammatory mediators is the respiratory burst, characterized by large oxygen consumption and production of reactive oxygen species. MSCs have shown to dampen the respiratory burst process by releasing interleukin (IL)-6. Dendritic cells play an important role in antigen presentation to naïve T cells, and MSCs have been shown to inhibit the differentiation of immature monocytes into dendritic cells. Additionally, cocultures of MSCs and dendritic cells inhibit the production of tumor necrosis factor-α, a potent inflammatory molecule. Natural killer cells are important innate cells in the defense against viral organisms and in tumor defense by their secretion of cytokines and cytolysis. MSCs cultured with freshly isolated resting natural killer cells have been shown to inhibit IL-2–induced proliferation and decrease secretion of interferon (IFN-γ) by 80%; however, IL-2–activated natural killer cells can lyse autologous and allogeneic MSCs. The adaptive immune system, composed of T and B lymphocytes, is capable of generating specific immune responses to pathogens with the production of memory cells. Once a T cell is activated by a foreign antigen binding to a specific T-cell receptor, the T cell proliferates and releases cytokines. T cells exist as CD8+ cytotoxic T cells that induce death of target cells or CD4+ helper T cells that modulate the other immune cells, and MSCs have been shown to suppress T-cell proliferation in a mixed lymphocyte culture. Proliferation of cytotoxic and helper T cells are suppressed via soluble factors released by MSCs, such as hepatocyte growth factor (HGF)
and transforming growth factor (TGF)-β1.19 When MSCs are present during naïve T-cell differentiation to CD4+ T-helper cells, there is a marked decrease in the production of interferon-γ and an increase in the production of IL-4,52 which suggests that MSCs alter naïve T cells from a proinflammatory state (heavy production of interferon-γ) to an anti-inflammatory state (greater production of IL-4). The B cell, which produces antibodies, is highly dependent on T cells, and MSC inhibition of T cells likely contributes to the interactions of MSCs with B cells.5 Conflicting data have shown that MSCs can inhibit and promote proliferation of B cells.54,55

In vivo studies have demonstrated that MSCs may play an important role in immune-mediated diseases and rejection of transplanted tissue. Skin allograft transplants survive longer with concomitant intravenous administration of MSCs in a baboon model,56 and systemic infusion of MSCs results in preferential homing to areas of injury.57 Infusion of MSCs in graft-versus-host disease, a life-threatening complication of allogeneic HSC transplantation, has demonstrated encouraging results in steroid-resistant graft-versus-host disease.58

Although MSCs have been shown to suppress innate and adaptive immune cells, in vitro experiments with MSCs induced to acquire a cardiac or vascular phenotype have increased myosin heavy chain Ia and II (immunogenic) expression and decreased myosin heavy chain Ib (immuno-tolerant) expression.59 Furthermore, when allogeneic MSCs were transplanted into post-MI rats, the cells were eliminated from the heart and did not improve cardiac function.59 These data from rats suggest that MSCs may lose their immunoprivileged properties as they differentiate; however, allogeneic MSCs transplanted into pigs were shown to engraft to a large extent as undifferentiated MSCs (~75% of cells identified), with few MSCs differentiated (~25%), and they did not induce an immune response.59 In addition, engraftment of these undifferentiated MSCs in post-MI hearts can stimulate endogenous repair mechanisms.40

Multilineage Potential of MSCs

Stem cells are characterized by their ability to self-renew, clone, and differentiate into multiple tissues.60 The first MSCs isolated more than 40 years ago were observed in culture to form bone and cartilage deposits,61 and this multilineage capability has become a defining feature of MSCs.62 Subsequent experiments in the 1970s using autologous transplantation of pellets of BM-MSCs to the subcapsular region of a rabbit kidney demonstrated the in vivo differentiation capabilities of MSCs to osteocytes.7 Caplan13 expanded on this pioneering work by demonstrating that MSC differentiation into a distinct phenotype (eg, chondrocytes or osteoblasts) is dependent on surrounding conditions. For example, MSC differentiation into osteoblasts is dependent on close proximity to vasculature; however, differentiation to the chondrocyte requires no vasculature.63 Ultimately, these early observations paved the way for experiments that have defined specific conditions to promote MSC differentiation.6

In Vitro Studies

MSCs in the presence of dexamethasone, β-glycerol phosphate, and ascorbic acid express alkaline phosphatase and calcium accumulation, a morphology consistent with osteogenic differentiation.6–66 These osteogenic cells will react with antosteogenic antibodies and form a mineralized extracellular matrix. The osteogenic potential of MSCs is conserved through numerous passages by their increased alkaline phosphatase activity.65 To induce chondrocyte differentiation, MSCs are cultured in a medium that contains dexamethasone and TGF-β3.67 Subsequently, the cells begin secreting an extracellular matrix, which includes type II collagen, aggrecan, and anionic proteoglycans, consistent with articular cartilage formation.6 Glucocorticoids play an important role in the differentiation of MSCs to the chondrocyte lineage by promoting TGF-β–mediated upregulation of collagen type II and inducing the matrix components aggrecan, dermatopontin, and collagen type XL.68 MSC differentiation into an adipogenic lineage can be induced by culturing cells in the presence of dexamethasone, insulin, indomethacin, and 1-methyl-3-isobutylxanthine.6 Cells express markers consistent with adipocytes, such as peroxisome proliferation-activated receptor-γ2, lipoprotein lipase, and fatty acid binding protein p2.6These adipocytes accumulate lipid-rich vacuoles and eventually coalesce.

BM-MSCs also have a capacity for differentiation into other mesoderm-derived tissue, notably myocytes, as shown in some but not all studies.14,69 Rat and human BM-MSCs cultured with 5-azacytidine differentiate into multinucleated myotubes, consistent with a myocyte lineage.14,18 These induced myocytes express β-myosin heavy chain, desmin, and α-cardiac actin and display spontaneous rhythmic calcium fluxes and potassium-induced calcium fluxes.18 Further experiments have also shown that both human and porcine MSCs can be induced to skeletal myogenic differentiation.15,70 Murine BM-MSCs exposed to 5-azacytidine have been shown to spontaneously beat alone, connect to adjoining cells, and after 2 to 3 weeks beat in synchrony.71 Interestingly, electrophysiological evaluation of these cells showed 2 types of morphological action potentials, sinus node–like potentials and ventricular myocyte–like potentials. Reverse-transcription–polymerase chain reaction demonstrated atrial natriuretic peptide, brain natriuretic peptide, α- and β-myosin heavy chain, Nkx2.5, and GATA4 genes. The authors concluded that MSCs are capable of adopting a cardiomyocyte phenotype in vitro.71

Several coculture experiments with cardiac myocytes have shown the ability of MSCs to transdifferentiate into a cardiac phenotype.60,72 When mouse MSCs and rat ventricular myocytes were cocultured, MSCs became α-actin positive and formed gap junctions with native myocytes, and these differentiating MSCs exhibited synchronous contractions with native myocytes. However, MSCs did not become α-actin positive when separated by a semipermeable membrane from myocytes, which suggests that transdifferentiation requires cell-cell contact.72 Contrary to these findings, rat BM-MSCs cocultured with neonatal rat ventricular myocytes separated by semipermeable membrane were shown to transdifferentiate into cardiomyocytes.69 After 1 week, these MSCs were
observed to be contracting, expressed SERCA2 and ryanodine receptor 2 by reverse transcription–polymerase chain reaction, and were positive for cardiac troponin T, sarcomeric α-actinin, and desmin by immunohistochemistry. These studies suggest that physical cell-cell contact, the local cardiac milieu, and factors secreted by myocytes play important roles in MSC transdifferentiation to cardiomyocytes. Functional cardiac differentiation has been described by some to not occur without coculturing of MSCs.73 These interesting findings require further investigation but speak to the concept that factors traverse from cell to cell to facilitate stem cell differentiation, which clearly has an important role in vivo.40 Another key factor is mechanical loading, as shown elegantly by Pijnappels et al.74 This factor may also help explain the increased propensity for MSC differentiation to occur in vivo or in coculture situations that add mechanical forces. MSCs transdifferentiate into nonmesoderm–derived tissue, such as neurons, when exposed to β-mercaptoethanol.75,76 As early as 30 minutes in culture, MSCs express the neuronal markers NSE (neuron-specific enolase) and neurofilament-M (NF-M).75 Importantly, these MSCs that appeared to adopt a neuronal phenotype were not shown to produce an action potential.

Regulation of Differentiation
The molecular regulation of MSC differentiation has mainly focused on 2 pathways, the Wnt canonical pathway and the TGF-β superfamily pathway.62 Wnt glycoproteins are soluble glycoproteins that engage receptor complexes composed of Lrp5/6 proteins to induce a cascade of intracellular events that regulate cell proliferation and differentiation (Figure 4).77 The Wnt pathway has been shown to be critical in skeletogenesis by promoting osteoblast proliferation77 and by suppression of chondrocyte formation via β-catenin, an essential component in transducing Wnt signaling to the nucleus.78 Numerous Wnt ligands and receptors are expressed on MSCs, and regulation of Wnt proteins controls MSC differentiation.79 For example, exposure to Wnt3a inhibited MSC differentiation into osteoblasts while promoting undifferentiated MSC proliferation.80

The other molecular pathway that regulates MSC differentiation is the TGF-β pathway, a family of proteins involved in skeletal tissue growth and regulation of MSC differentiation into chondrocytes.6,62,67 TGF-β3 upregulates gene expression in MSCs to promote chondrogenic differentiation via several intracellular cascades, including extracellular-signal regulated kinase (ERK1/2), SMAD proteins, mitogen-activated protein (MAP) kinases, p38, and JNK (Figure 4).62,81 Several other molecules have been shown to regulate MSC differentiation, such as epidermal growth factor (EGF), platelet-derived growth factor (PDGF), and fibroblast growth factor (FGF).82,83 Indeed, many growth factors likely interact with the Wnt and TGF-β pathways to control MSC differentiation.62

In Vivo Mechanism of Action in Cardiac Repair
Engraftment and Differentiation: Cardiomyocytes and Vasculature
Numerous in vivo rodent and swine studies have demonstrated the ability of MSCs to engraft and differentiate within the heart.17,20,21,84–87 In 2002, Toma and colleagues17 reported the results of human BM-MSCs transfected with β-galactosidase reporter gene injected into immunodeficient adult mouse hearts. The majority of human MSCs were found in the spleen, lung, and liver at 4 days after injection. Only 0.44% of injected human MSCs were identified in the myocardium, and over time, they adopted a morphology indistinguishable from host cardiomyocytes. Immunofluorescence staining with anti-β-galactosidase identified engrafted MSCs in the myocardium,
which colocalized with markers of cardiomyocyte lineage (desmin, α-actin, and cardiac troponin T) as early as 14 days after injection. Shake and colleagues expanded on these early murine studies by surgically injecting autologous porcine Dil-labeled MSCs directly into post-MI swine myocardium. Successful engraftment was demonstrated by observing the labeled MSC engrafting into scarred myocardium, as well as by expression of the cardiomyocyte markers α-actin, tropomyosin, troponin T, myosin heavy chain, and phosphorylamban within 2 weeks after injection.

In a swine model of chronic ischemic cardiomyopathy, our group reported the capacity of allogeneic MSCs to engraft and differentiate into cardiomyocytes, smooth muscle cells, and endothelium. Male BM-MSCs were injected into female swine and identified by colocalization with Y-chromosome fluorescence in situ hybridization. Cardiomyocyte differentiation was present in approximately 14% of Y-positive MSCs as identified by costaining for the cardiac structural proteins α-sarcromere actin and tropomyosin and the transcriptional factors GATA-4 and Nkx2.5. Additionally, differentiated myocytes exhibited the capacity for coupling with host myocytes via connexin-43. MSCs also were shown to participate in coronary angiogenesis (actinin, calponin, and TnT) by immunolocalization for the Y chromosome, but at 8 weeks after transplantation, staining for the Y chromosome was not detected, which suggests failure of prolonged engraftment.

In a swine model of cardiac ischemia, Dixon and colleagues found that combining a statin with intramyocardial injections of BM-MSCs improved the efficiency of MSC engraftment and differentiation in the heart is low compared with the robust functional recovery observed after cell transplantation, which has raised questions as to whether MSC engraftment and differentiation is the predominant mechanism of action. MSCs are known to secrete soluble paracrine factors that have been postulated to contribute to endogenous cardiomyogenesis and angiogenesis.

Table 1. Paracrine Factors Secreted by MSCs

<table>
<thead>
<tr>
<th>Secreted Factor</th>
<th>Function</th>
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<tr>
<td><strong>Proangiogenesis</strong></td>
<td></td>
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<tr>
<td>Fibroblast growth factor-2 (FGF-2)</td>
<td>Induces endothelial and smooth muscle cell proliferation</td>
</tr>
<tr>
<td>Fibroblast growth factor-7 (FGF-7)</td>
<td>Induces endothelial cell proliferation</td>
</tr>
<tr>
<td>Monocyte chemoattractant protein-1 (MCP-1)</td>
<td>Induces angiogenesis; recruits monocytes</td>
</tr>
<tr>
<td>Platelet-derived growth factor (PDGF)</td>
<td>Induces smooth muscle cell proliferation</td>
</tr>
<tr>
<td>Placental growth factor (PIGF)</td>
<td>Promotes angiogenesis</td>
</tr>
<tr>
<td>Transforming growth factor-β (TGF-β)</td>
<td>Promotes vessel maturation</td>
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<tr>
<td><strong>Vascular endothelial growth factor (VEGF)</strong></td>
<td></td>
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<tr>
<td><strong>Remodeling of extracellular matrix</strong></td>
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<tr>
<td>Metalloproteinase-1 (MMP1)</td>
<td>Loosens matrix; tube formation</td>
</tr>
<tr>
<td>Metalloproteinase-2 (MMP2)</td>
<td>Loosens matrix; tube formation</td>
</tr>
<tr>
<td>Metalloproteinase-9 (MMP9)</td>
<td>Loosens matrix</td>
</tr>
<tr>
<td>Plasminogen activator (PA)</td>
<td>Degrades matrix molecules</td>
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<tr>
<td>Tumor necrosis factor-α (TNF-α)</td>
<td>Degrades matrix molecules; cell proliferation</td>
</tr>
<tr>
<td><strong>Stem cell proliferation, recruitment, and survival</strong></td>
<td></td>
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<tr>
<td>Basic fibroblast growth factor (bFGF)</td>
<td>Enhances proliferation of endothelial and smooth muscle cells</td>
</tr>
<tr>
<td>Granulocyte colony stimulating factor (G-CSF)</td>
<td>Increases proliferation and differentiation of neutrophils</td>
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<tr>
<td>Insulin-like growth factor-1 (IGF-1)</td>
<td>Regulates cell growth and proliferation; inhibits apoptosis</td>
</tr>
<tr>
<td>Macrophage colony stimulating factor (M-CSF)</td>
<td>Increases proliferation and differentiation of monocytes</td>
</tr>
<tr>
<td>Thymosin-β4 (Tβ4)</td>
<td>Promotes cell migration</td>
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<tr>
<td>Stem cell-derived factor (SDF)</td>
<td>Progenitor cell homing</td>
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<tr>
<td>Secreted frizzled-related protein-1 (SFRP1)</td>
<td>Enhances cell development</td>
</tr>
<tr>
<td>Secreted frizzled-related protein-2 (SFRP2)</td>
<td>Inhibits apoptosis; enhances cell development</td>
</tr>
<tr>
<td><strong>Immunomodulatory</strong></td>
<td></td>
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<tr>
<td>Heme oxygenase-1 (HO1)</td>
<td>Inhibits T-cell proliferation</td>
</tr>
<tr>
<td>Hepatocyte growth factor (HGF)</td>
<td>Inhibits CD4+ T-cell proliferation</td>
</tr>
<tr>
<td>Indoleamine 2,3-dioxygenase (IDO)</td>
<td>Inhibits innate and adaptive immune cell proliferation</td>
</tr>
<tr>
<td>Inducible nitric oxide synthase (iNOS)</td>
<td>Inhibits inflammation</td>
</tr>
<tr>
<td>Interleukin-6 (IL-6)</td>
<td>Regulates inflammation; VEGF induction</td>
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<tr>
<td>Prostaglandin E2 (PGE2)</td>
<td>Inhibits inflammation</td>
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</table>

Paracrine Signaling

The frequency of MSC engraftment and differentiation in the heart is low compared with the robust functional recovery observed after cell transplantation, which has raised questions as to whether MSC engraftment and differentiation is the predominant mechanism of action. MSCs are known to secrete soluble paracrine factors that have been postulated to contribute to endogenous cardiomyogenesis and angiogenesis. MSCs secrete a wide array of cytokines and growth factors that can suppress the immune system, inhibit fibrosis and apoptosis, enhance angiogenesis, and stimulate differentiation of tissue-specific stem cells (Table 1).

In vitro studies have shown that conditioned medium from hypoxic genetically modified MSCs overexpressing Akt
(Akt-MSCs) inhibits apoptosis and can trigger spontaneous contractions of rat cardiomyocytes.90 When this conditioned medium from Akt-MSCs was injected into post-MI rat hearts, infarct size was reduced and LV function improved.90 Several genes coding for vascular endothelial growth factor, fibroblast growth factor-2, HGF, and insulin-like growth factor-1 are upregulated in hypoxic Akt-MSCs, and these factors could mediate the functional improvements observed.90 Furthermore, these investigators suggest that these improvements are seen within 72 hours, and meaningful engraftment is unlikely to provide such rapid improvements, but secreted paracrine mediators are able to immediately affect the milieu.91 Additionally, Akt-MSCs secrete frizzled-related protein (Sfrp2), a paracrine factor that exerts a prosurvival effect on ischemic myocardium through modulation of Wnt signaling.93

Murine BM-MSC concentrated conditioned media has been shown to consist of vascular endothelial growth factor, basic fibroblast growth factor, placental growth factor, and monocyte chemoattractant protein-1, which enhance proliferation of endothelial and smooth muscle cells.92 Compared with controls, increased local levels of vascular endothelial growth factor and fibroblast growth factor proteins were detected when β-galactosidase–positive MSCs were injected into ischemic murine peripheral muscles. These MSCs were detected in decreasing quantities from days 7 to 28 after transplantation and failed to incorporate into vessels. Administration of the factors secreted by MSCs alone may not have the same impact as administration of the cells. In a swine model of MI, intramyocardial injection of MSCs reduced infarct size by recruiting endogenous c-kit+ cardiac stem cells (CSCs), whereas a single application of concentrated conditioned media did not.40

Cell-Cell Interactions and Niche Reconstitution

Our group has shown the ability of MSCs to stimulate proliferation of endogenous c-kit+ CSCs and enhance cardiomyocyte cell cycling.40 Post-MI female swine were injected with green fluorescent protein (GFP)–labeled allogeneic MSCs to the infarct border zone and on histological examination were shown to exhibit chimeric clusters that contained immature MSCs and c-kit+ CSCs. These niches contained adult cardiomyocytes, GFP+ MSCs, and c-kit+ CSCs that expressed connexin-43–mediated gap junctions and N-cadherin mechanical connections between cells. A 20-fold increase in the endogenous c-kit+ CSCs was observed in MSC-treated animals compared with those given placebo. Additional coculture experiments with c-kit+ CSCs and MSCs demonstrated a 10-fold increase in c-kit+ CSC proliferation compared with c-kit+ CSC cultures without MSCs. These data suggest that MSCs induce endogenous c-kit+ CSC proliferation, and cell-cell interactions play an important role in MSC-based cardiac repair mechanisms.

Using genetically engineered mice that permanently express GFP in all cardiomyocytes after tamoxifen therapy, Loffredo and colleagues99 compared the ability of BM-derived c-kit+ stem cells and BM-MSCs to induce proliferation of endogenous CSCs. Mice were treated with tamoxifen to induce GFP expression in cardiomyocytes, underwent MI, and were given intramyocardial injections of stem cells in the infarct border zone. At 8 weeks, BM c-kit+ stem cells led to a significant reduction in the GFP+ cardiomyocyte pool and parallel increases in β-galactosidase–positive cardiomyocytes compared with control, a finding consistent with increased progenitor activity induced by BM c-kit+ stem cells. However, BM-MSCs did not lead to a reduction in the GFP+ cardiomyocyte pool or increase β-galactosidase–positive cardiomyocytes, which suggests that MSCs may not stimulate endogenous progenitors. These findings in a murine model are clearly different from the body of work conducted with porcine models and additionally reflect species-specific functions of MSCs.

MSC and Cardiomyocyte Fusion

Fusion of BM stem cells with adult cells, including cardiomyocytes, has been proposed as a potential mechanism of action.100,101 BM-MSCs expressing Cre recombinase were injected into Cre reporter gene mice hearts, which can detect fusion of MSCs with mouse cells by LacZ gene expression.102 As early as 3 days after transplantation, rare cellular fusion was detected at injection sites by hematoxylin-and-eosin staining with LacZ gene expressing distinct blue cells, which persisted at 28 days after transplantation. These findings lend support to the potential for MSC fusion with cardiomyocytes, but the paucity of fused cells detected makes it unlikely to be a predominant mechanism of action considering the degree of functional recovery.

Localization of MSCs to In Vivo Niches

One of the key roles of endogenous MSCs is to participate in the regulation of niches, which are clusters of cells that regulate stem cell proliferation and differentiation.103 Niches are found prototypically in the BM,8 hair follicles bulge,104 and the intestinal epithelium.105 In addition, MSCs are located endogenously in mesoderm-derived bone, adipose tissue, and vascular epithelium, which are tissues also affected in Progeria syndrome.106 The presence of MSCs in these tissues and the association with Progeria suggest a role for MSCs in regulating successful aging. Thus, an emerging concept is that MSCs both serve as precursors for certain lineages and regulate the function of other lineages through participation in niches. The presence of MSCs or stromal cells in the heart supports the concept that this principle may be operative there as well.106

Preclinical Trials of MSC Therapy: Effects on Cardiac Structure and Function

Several large-animal species, including swine, sheep, and dogs, have been used to investigate the effects of MSC therapy in models of chronic and acute post-MI left ventricular dysfunction (summarized in Table 2). Stem cells can be delivered to the heart via peripheral intravenous infusion, via direct surgical injection during open heart surgery, or via catheter-based intracoronary infusion, retrograde coronary venous infusion, or transendocardial injection.114,115 Using radiolabeled BM stem cells, γ-emission counting of harvested organs 1 hour after stem cell delivery demonstrated that intramyocardial injection had the highest retention rate of
Table 2. Results of MSC Therapy in Large-Animal Models

<table>
<thead>
<tr>
<th>Source</th>
<th>Species</th>
<th>Model</th>
<th>Type of BM-MSC</th>
<th>Delivery Method</th>
<th>Follow-Up Imaging</th>
<th>Effects of MSC Therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Shake et al</td>
<td>Swine</td>
<td>Subacute MI</td>
<td>Autologous</td>
<td>Surgical</td>
<td>Sonomicrometry crystals</td>
<td>↑ Systolic wall thickening</td>
</tr>
<tr>
<td>Quevedo et al</td>
<td>Swine</td>
<td>Chronic MI</td>
<td>Allogeneic</td>
<td>TESI</td>
<td>CMR</td>
<td>↓ Scar size; ↑ EF; ↑ regional contractility; ↑ myocardial perfusion</td>
</tr>
<tr>
<td>Schuleri et al</td>
<td>Swine</td>
<td>Chronic MI</td>
<td>Autologous</td>
<td>Surgical</td>
<td>CMR</td>
<td>↓ Scar size; ↑ EF; ↑ regional contractility; ↑ myocardial perfusion</td>
</tr>
<tr>
<td>Hatzistergos et al</td>
<td>Swine</td>
<td>Acute MI</td>
<td>Allogeneic</td>
<td>TESI</td>
<td>CMR</td>
<td>↑ EF; ↓ scar size</td>
</tr>
<tr>
<td>Hattamoto et al</td>
<td>Sheep</td>
<td>Acute MI</td>
<td>Allogeneic</td>
<td>Surgical</td>
<td>Echocardiography</td>
<td>↑ EF; ↓ EDV</td>
</tr>
<tr>
<td>Amado et al</td>
<td>Swine</td>
<td>Acute MI</td>
<td>Allogeneic</td>
<td>TESI</td>
<td>CMR</td>
<td>↑ Diastolic function; ↑ mechanoenergetics; ↑ EF; ↓ scar size</td>
</tr>
<tr>
<td>Makkar et al</td>
<td>Swine</td>
<td>Chronic MI</td>
<td>Allogeneic</td>
<td>Surgical</td>
<td>Echocardiography; LV angiography</td>
<td>Preserved EF; no change in LV chamber dimensions</td>
</tr>
<tr>
<td>Price et al</td>
<td>Swine</td>
<td>Subacute MI</td>
<td>Allogeneic</td>
<td>IC/TESI</td>
<td>Echocardiography</td>
<td>↑ EF; ↓ EDV; ↓ ESV</td>
</tr>
<tr>
<td>Halos et al</td>
<td>Swine</td>
<td>Acute MI</td>
<td>Allogeneic</td>
<td>IV</td>
<td>LV angiography</td>
<td>No change in EF</td>
</tr>
<tr>
<td>Hashemi et al</td>
<td>Swine</td>
<td>Chronic MI</td>
<td>Allogeneic</td>
<td>TESI</td>
<td>CMR</td>
<td>↓ Scar size</td>
</tr>
<tr>
<td>Schuleri et al</td>
<td>Swine</td>
<td>Acute MI</td>
<td>Allogeneic</td>
<td>TESI</td>
<td>CMR</td>
<td>↑ Myocardial perfusion</td>
</tr>
<tr>
<td>Gyongyosi et al</td>
<td>Swine</td>
<td>Subacute MI</td>
<td>Allogeneic</td>
<td>TESI</td>
<td>CMR</td>
<td>↓ Scar size</td>
</tr>
<tr>
<td>Qi et al</td>
<td>Swine</td>
<td>Acute MI</td>
<td>Allogeneic</td>
<td>IC</td>
<td>CMR</td>
<td>↑ EF; ↓ scar size</td>
</tr>
</tbody>
</table>

TESI indicates transendocardial stem cell injection; CMR, cardiac magnetic resonance imaging; IC, intracoronary infusion; ESV, end-systolic volume; IV, intravenous infusion; ↑, increase; and ↓, decrease.

cells. Interestingly, most cells delivered to the heart by any method are not retained in the myocardium and commonly are found in the lungs and spleen. Positron emission tomographic tracking of MSCs delivered by catheter-based transendocardial injection showed retention of approximately 6% of injected cells in the myocardium at 10 days after injection, with increased uptake in the pericardium and pleura. Although stem cell retention in the myocardium appears to be low by any delivery route, preclinical data of MSC therapy for cardiac disease have shown highly promising results. Here, we will review the preclinical trials using allogeneic and autologous MSCs delivered to the heart via the 4 most common techniques: peripheral intravenous infusion, intracoronary infusion, catheter-based transendocardial injection, and direct surgical injection.

**Intravenous MSC Therapy**

Intravenous infusion of MSCs is the easiest and most practical method for delivery because it only requires peripheral venous access; however, for the cells to reach the myocardium, they must travel through the pulmonary circulation, where entrapment of cells is a concern. Intravenous infusion of MSCs in a swine model of acute MI was conducted at 15 minutes after left anterior descending (LAD) coronary artery occlusion with swine randomized to vehicle or various doses (1, 3, or 10 million cells/kg) of allogeneic BM-MSCs. At 12 weeks after MI, LV ventriculography showed no difference in ejection fraction (EF) between MSC-treated animals versus placebo. Pressure-volume loop analysis demonstrated a significant improvement in the end-systolic pressure-volume relationship and preload recruitable stroke work in MSC-treated animals compared with placebo. Histological analysis of postmortem hearts showed significantly greater density of von Willebrand factor–positive blood vessels and vascular endothelial growth factor expression in MSC-treated animals. Steady state coronary blood flow reserve was similar among groups, but adenosine-recruited coronary blood flow reserve was improved in MSC-treated animals.

In a swine model of acute MI, hemodynamic and electrophysiological effects of intravenous infusion of allogeneic MSCs were studied. Using transthoracic echocardiography, EF improved, and less eccentric hypertrophy in MSC-treated animals was detected at 3-month follow-up than with placebo. Confocal spectral imaging of explanted organs confirmed DiI fluorescence in the lungs and rarely in the hearts of MSC-treated animals. Electrophysiological studies at 3 months after MI showed shortened epicardial effective refractory periods in MSC-treated animals compared with placebo. Shortened effective refractory periods may induce ventricular tachycardia, which raises the possibility that MSCs may induce proarrhythmic remodeling.

**Intracoronary Infusion of MSCs**

Intracoronary infusion of stem cells is delivered with a standard over-the-wire balloon angioplasty catheter placed into the target coronary artery. After the angioplasty catheter is positioned, the balloon is inflated at a low pressure to block blood flow (which allows adhesion and potential transmigration) while the cells are infused through the distal lumen. Concern about inducing ischemia during coronary artery occlusion and the lack of vessels in chronically occluded areas of scar tissue may not allow effective cell
delivery. However, intracoronary infusion may be able to effectively deliver cells to ischemic tissue after full reperfusion therapy after an acute MI. Advantages of this technique are the familiarity of angioplasty techniques to interventional cardiologists and the ability to deliver cells during percutaneous intervention for acute MI.118

Most preclinical studies of intracoronary infusion of MSCs have focused on models of acute MI.107,113,119,120 Cardiac magnetic resonance imaging (MRI) was used to investigate the effects of intracoronary infusion of iron oxide–labeled MSCs or placebo in swine at approximately 5 days after LAD MI.113 Delayed-enhancement (DE) cardiac MRI showed an 8% reduction in scar size in MSC-treated swine compared with increased scar size in placebo-infused animals. EF was unchanged in placebo-infused animals, whereas a 15-point improvement in EF was observed in MSC-treated swine. At 8 weeks after infusion, Prussian blue staining identified engraftment of iron oxide–labeled MSCs in the peri-infarct zone.

In an ischemia-reperfusion canine model of acute MI, intracoronary infusion was compared with electroanatomic guided transendocardial injection of allogeneic MSCs (100 million cells) at 7 days after MI with a no-infusion placebo control arm.107 Two animals died in the intracoronary infusion arm, 1 because of microvascular plugging associated with MSC infusion and another of intestinal ischemia, with no deaths in the other groups. Echocardiography at 21 days after transplantation showed no difference in EF or LV chamber sizes in the MSC intracoronary infusion group compared with placebo, whereas EF, end-diastolic volume (EDV), and end-systolic volume improved in the transendocardial MSC injection group. Histological analysis showed that intracoronary infusion produces a more uniformly distributed pattern of MSCs, concentrated in the border zone and normal myocardium. Transendocardial injection was also used to deliver MSCs to the border zone and normal myocardium and achieved higher MSC concentration per square micron than intracoronary delivery.

Catheter-Based Intramyocardial Injection of MSCs

Transendocardial stem cell injection delivers MSCs directly into the myocardium with a catheter navigated in the LV by fluoroscopic guidance or electroanatomic mapping.118 Transendocardial stem cell injection uses a needle deployed from the tip of a catheter that engages and infuses cells into the myocardium. Perforation of myocardium, with the potential for cardiac tamponade, and induction of arrhythmias must be monitored with transendocardial stem cell injection, but the minimally invasive ability to directly inject cells into scar and border zones makes it an attractive delivery technique.112,115

In a series of acute MI studies in swine, our group demonstrated the ability of transendocardial MSC injection to improve cardiac function and reduce scar size (Figure 5).40,86,121 Allogeneic MSCs were administered to the border and infarct zones at 3 days after MI, and DE cardiac MRI showed approximately 50% reduction in scar size at 8 weeks after transplantation, whereas control animals had no change in scar size.86 On gross pathological examination, MSC-treated animals had infarct confined to the midmyocardium, with visible myocardium present at both the subendocardial and subepicardial zones. The subendocardial tissue, also present on DE-MRI, was consistent with new tissue growth and was not evident on control animals. Cardiac MRI demonstrated an increase in EF from 25% to 42% at 8 weeks after injection, whereas the nontreated animals had minimal change in EF. Pressure-volume loops showed improved LV relaxation (LV end-diastolic pressure and relaxation time) and systolic compliance (end-systolic pressure-volume relationship) in MSC-treated animals compared with placebo. Additional work by our group has also shown that transendocardial MSC injection improves resting myocardial blood flow by first-pass gadolinium perfusion MRI at 8 weeks after injection compared with placebo.121 Vessel density assessed by von Willebrand factor expression was similar in MSC- and placebo-treated swine, but MSC-injected animals had significantly larger vessels that likely contributed to improved tissue perfusion.

Figure 5. Impact of allogeneic MSC therapy on infarct size. DE-MRI images of (A) swine with chronic ischemic cardiomyopathy injected with 200 million allogeneic MSCs before and 3 months after therapy, showing a 17% reduction in scar size, and (B) a control animal showing no change in scar size (red arrows indicate gadolinium-enhanced scar).
In another study, 3 allogeneic MSC doses (2.4×10^7, 2.4×10^8, or 4.4×10^8) versus placebo delivered by electroanatomic mapping–guided transendocardial stem cell injection were compared at 3 days after MI. DE-MRI at 12 weeks after injection showed reduced infarct size in all MSC dosage groups, whereas placebo-injected animals had increased infarct size. Interestingly, there was no dose-dependent effect on scar size, and EF at 12 weeks was similar in all groups. In another study, transendocardial injection of autologous MSCs also produced a significant reduction in DE-MRI–derived scar size at 10 days after injection compared with placebo injection.

To investigate the impact of MSCs on healed scars, our group studied transendocardial allogeneic MSC injection in swine with 3-month-old anterior wall infarcts. At 3 months after MI, remodeling in swine is nearly complete and results in depressed LV function. DE-MRI at 12 weeks after injection showed that MSC therapy resulted in a 30% reduction in scar size, whereas the placebo-injected animals had unchanged scars. Furthermore, the circumferential extent of scar decreased approximately 14% in MSC-treated swine and increased slightly in placebo-injected animals. When tagged MRI was used to assess regional LV function, peak Eulerian circumferential shortening demonstrated improved contractility of the infarct and border zones in MSC-treated animals, whereas further declines in function were evident in the placebo group. Scar size reduction and increased regional function led to improved global LV function in MSC-treated animals, whereas EF remained depressed in placebo-injected swine. These findings are consistent with the ability of intramyocardial injection of MSCs to lead to reverse remodeling in the chronically injured heart.

**Direct Surgical Intramyocardial Injection of MSCs**

The largest preclinical experience with allogeneic and autologous MSC therapy for cardiac disease used direct surgical intramyocardial injection. Surgical injection is the most invasive delivery technique because it requires either a thoracotomy or sternotomy for cell delivery, but it allows direct visualization of scarred myocardium and needle engagement. If perforation occurs, it can be controlled at the time of injection with sutures. Direct surgical injection of MSCs has been studied in large animals with acute MI, subacute MI, and chronic ischemic cardiomyopathy.

In a sheep model of acute LAD coronary artery occlusion without reperfusion, BM-derived STRO-3–positive mesenchymal precursor cells were injected directly into the infarct border zone 1 hour after MI. As discussed above, STRO-3–positive mesenchymal precursor cells are a subset of BM-MSCs with extensive capacity for proliferation and differentiation. Animals were allocated to 1 of 4 different cell dosages (25, 75, 225, or 450 million cells) or placebo. Echocardiography at 8 weeks after injection demonstrated improved EF in all mesenchymal precursor cell–treated animals compared with placebo. Compared with placebo-treated animals, EDV was significantly smaller in the 2 low-dose groups (25 and 75 million cells), but it was unchanged in the 2 high-dose groups (225 and 450 million cells). EF improved significantly in all cell-treated animals compared with placebo. Echocardiography-derived MI length was significantly smaller in the 3 low-dose groups (25, 75, and 225 million) compared with placebo but was not significantly different in the high-dose (450 million)–treated group compared with placebo. These data suggest that there may be a threshold effect with this particular subpopulation of MSCs.

In an ischemia-reperfusion swine model of subacute MI, autologous MSCs (60 million cells) were injected directly into 2-week-old infarcts via a midline sternotomy. Sonomicroscopy crystals were placed in the infarct region during exposure of the heart to assess wall thickness and regional contractility. At 4 weeks after injection, a trend toward improved end-diastolic wall thickness and improved systolic wall thickening was seen in MSC-treated animals compared with placebo.

Our group reported the results of direct surgical injections of autologous MSCs in a swine model of ischemic cardiomyopathy. Animals underwent LAD coronary artery occlusion followed by reperfusion, and at 12 weeks after MI, they received either 20 million or 200 million autologous MSCs or placebo injections to the infarct and border zone. Serial cardiac enzymes (troponin I, creatine kinase, and creatine kinase-MB) and white blood cells obtained after injections showed no difference between groups. Cardiac MRI was used to assess LV function, scar size, and myocardial blood flow. DE-MRI conducted at 12 weeks after injection demonstrated a trend toward decreased infarct size in low-dose MSC–treated animals and a significant reduction in infarct size in high-dose MSC-treated animals. Furthermore, decreased circumferential extent of scar was evident in MSC-treated animals, whereas there was a trend in scar expansion in placebo-treated animals. Regional contractility as assessed by tagged MRI–derived peak Eulerian circumferential shortening showed improved contractility of the infarct border zones of low- and high-dose MSC–treated animals. The high-dose MSC–treated animals also had significant improvements in regional contractility of the infarct zone. First-pass myocardial perfusion MRI showed improved myocardial blood flow in both high- and low-dose MSC–treated animals compared with no change in placebo. The reduction in scar size, improved regional contractility, and increased myocardial blood flow resulted in increased EF of MSC-treated animals compared with unchanged EF in placebo-treated animals.

In a swine model of LAD coronary artery occlusion without reperfusion, allogeneic BM MSCs (200 million cells) or placebo was surgically injected during thoracotomy at 30 days after MI. Sixty days after injection, global cardiac function assessed by contrast LV angiography showed deterioration in EF of placebo-treated animals, whereas MSC-treated animals had preserved EF. Echocardiography-derived LV chamber dimensions were not different between MSC or placebo-treated animals. Infarct size determined by planimetry imaging showed no difference between treated and placebo groups. This is a particularly important study because it stands in contrast to the totality of large-animal experience with reperfusion models, in which MI size is reduced by allogeneic and autologous MSC injection.

MSC therapy has also been studied in dogs with chronic ischemic hibernating myocardium. Dogs underwent amer-
Heme oxygenase-1 (HO-1) is an inducible enzyme that rapidly degrades heme, which results in antiapoptotic and anti-inflammatory activity, and in vitro studies have shown HO-1 to enhance the tolerance of MSCs to hypoxia-reoxygenation injury.\textsuperscript{126} BM-MSCs transfected to overexpress HO-1 were administered to cells or placebo via intracoronary infusion after acute MI in a swine model of ischemia-reperfusion.\textsuperscript{125} Cardiac MRI at 3 months after injection demonstrated improved EF in HO-1–transfected MSCs compared with placebo- and plasmid-transfected MSCs. EDV was similar among groups, whereas end-systolic volume was significantly lower in the HO-1–transfected MSC group. Western blot demonstrated significantly enhanced myocardial expression of vascular endothelial growth factor and reduced expression of tumor necrosis factor-\textalpha{} and IL-6 in HO-1–transfected MSC-treated hearts compared with control. Histological analysis demonstrated increased capillary and arteriolar density in HO-1 MSC-treated hearts. Taken together, HO-1 overexpression in MSCs appears to be associated with decreased inflammatory cytokine levels and improved angiogenesis when transplanted to post-MI swine hearts.

In another genetically modified MSC study, BM-MSCs were transfected with Akt, an enzyme that has been shown to protect cardiomyocytes against apoptosis after ischemia-reperfusion injury.\textsuperscript{127,128} Transfected Akt-MSCs, MSCs alone, or placebo was administered via intracoronary infusion 3 days after MI. Coronary angiograms after intracoronary MSC infusion showed several cases of slow coronary flow that required administration of intracoronary adenosine, nitroglycerin, or nicorandil. At 4 weeks after transplantation, SPECT showed significantly improved EF in Akt-MSC–treated animals compared with MSC alone, whereas both showed improved LV function compared with placebo. Infarct size by SPECT was significantly less in both MSC-only and Akt-MSC–treated animals compared with placebo. Furthermore, the infarct size was significantly smaller in Akt-MSC–treated animals than with MSC alone, which suggests that Akt-MSCs may provide additional scar size reduction.

**Safety of MSC Therapy**

Although evidence continues to accrue that MSCs have great potential as a new therapy to treat damaged myocardium, it is crucial to keep safety concerns in mind. Two key concerns that warrant mention include proarrhythmia and tumor formation.\textsuperscript{129}
Intravenous infusion of MSCs in swine was shown to alter the electrophysiological properties of the myocardium contrast to these findings in swine, intravenous infusion of allogeneic MSCs in humans with acute MI demonstrated fewer ventricular arrhythmias than with placebo infusion. Furthermore, other clinical trials have not shown increased arrhythmias after MSC therapy. In the setting of ischemic cardiomyopathy, programmed electric stimulation did not detect a higher level of inducibility in MSC-treated pigs.

Several reports have raised concerns about tumor formation by use of BM-cultured MSCs. Mouse-derived BM-MSCs were used in these studies and were shown to undergo chromosomal abnormalities that resulted in tumor formation in numerous organs. As discussed previously, in a series of large-animal preclinical studies by our group and others, MSCs were shown to be safe in large animals, with no evidence of tumor formation or ectopic tissue growth. Furthermore, there have been no reports of ectopic tissue growth in the early-phase human studies using MSCs. Nonetheless, these reports of tumorigenesis in murine models highlight the importance of continued long-term surveillance of patients treated with MSCs.

Clinical Trials of MSC Therapy for Cardiac Repair

Acute MI
On the basis of rigorous preclinical testing highlighted above that demonstrated the safety of MSC delivery to patients with cardiac disease, clinical trials have been initiated for both acute MI and ischemic cardiomyopathy. Phase II clinical data have been reported with intravenous therapy, intracoronary infusion, and intramyocardial injection. Chen and colleagues investigated the effects of intracoronary infusion of autologous BM-MSCs (8–10^6) or saline (n=34) in patients with subacute MI. Positron emission tomographic imaging showed improvement in perfusion defects at 3 months after BM-MSC therapy, and left ventriculography demonstrated improved EF and LV chamber sizes in MSC-treated patients compared with placebo. Importantly, this study showed that MSC infusion was safe, with no deaths reported during follow-up, and electrocardiographic monitoring showed no arrhythmias.

Our group was part of a randomized study investigating allogeneic MSCs (n=39) versus placebo (n=21) administered intravenously after acute MI. This dose-ranging study showed that intravenous allogeneic MSCs are safe in post-MI patients, with no difference in adverse events between groups. Interestingly, follow-up electrocardiograms demonstrated a reduction in ventricular arrhythmias and improved pulmonary function in MSC-treated patients. Additionally, echocardiography showed MSC-treated patients experienced a 6% increase in EF at 3 months.

Ischemic Cardiomyopathy
On the basis of the large amount of mechanistic data available and the extensive translational findings that MSCs can stimulate reverse remodeling in porcine models of ischemic cardiomyopathy, clinical trial programs are under way to test the safety and efficacy of MSCs and MSC precursor cells for patients with cardiac injury due to previous MI. Our group directly tested the hypothesis that transendocardial, intramyocardial injection of MSCs (and/or whole BM) produced a cardiac phenotype of reverse remodeling by use of cardiac MRI imaging. In this study, 8 patients with chronic ischemic cardiomyopathy were administered BM-MSCs (n=4) or mononuclear cells (n=4) to the scar and border zone. We demonstrated reverse remodeling and improved regional contractility of the treated scar, which was evident as early as 3 months after injection and persisted at 12 months (Figure 6). The improved regional contractility strongly correlated with the reduction in both EDV (r^2=0.69, P=0.04) and end-systolic volume (r^2=0.83, P=0.01). Importantly, we used serial whole-body computed tomography scans that showed no evidence of ectopic tissue growth at 1 year after transplantation, and serial Holter electrocardiographic recordings showed no sustained arrhythmias. Taken together, the early-phase clinical trial data demonstrate that MSC therapy for post-MI is safe and has favorable effects on cardiac structure and function.

To follow up on this finding, 2 larger studies are under way: the Transendocardial Autologous Cells in Ischemic Heart Failure Trial (TAC-HFT) and Percutaneous Stem Cell Injection Delivery Effects on Neomyogenesis (POSEIDON). TAC-HFT is a randomized, double-blinded, placebo-controlled trial comparing BM-MSCs versus mononuclear cells, and POSEIDON is comparing the effects of allogeneic versus autologous MSC therapy in patients with ischemic cardiomyopathy. Additionally, we have completed enrollment in the Prospective Randomized Study of Mesenchymal Stem Cell Therapy in Patients Undergoing Cardiac Surgery (PROMETHEUS), a trial investigating MSC therapy delivered during coronary artery bypass surgery; and recently we initiated POSEIDON-DCM to study MSC therapy for the treatment of idiopathic dilated cardiomyopathy. The next few years will witness the results of these early clinical trial efforts.

Several other clinical trial efforts are also under way, including the use of mesenchymal precursor cells and MSCs treated ex vivo with cytokines to enhance cardiopoiesis. The results of the C-CURE clinical trial (Safety, Feasibility and Efficacy of Guided Bone Marrow–Derived Mesenchymal Cardiopoietic Cells for the Treatment of Heart Failure Secondary to Ischemic Cardiomyopathy) were recently provisionally reported, which included 45 patients with ischemic heart failure randomized to standard medical care (n=24) or guided cardiopoietic-MSC therapy (n=21) delivered to viable but dysfunctional myocardium by electromechanical guidance. At 6-month follow-up, there was no evidence of cardiopoietic-MSC–induced arrhythmias or toxicity. In the cardiopoietic-MSC arm, there was a significant improvement in clinical performance as assessed by the 6-minute walk test. In addition, EDV and end-systolic volume were decreased by cardiopoietic-MSC therapy compared with controls, and a significant improvement in EF of approximately 5% was detected in the cardiopoietic-MSC group.

Cardiac Repair
Strategies and Future Directions

Retention of stem cells in the heart is low by any method of delivery, and strategies to enhance engraftment and differentiation are needed. Guided cardiopoiesis has been proposed as a technique to enhance the cardiac phenotype of MSCs and has been shown to increase engraftment in murine hearts. The exact mechanism of action of MSCs (whether via paracrine signaling, cell fusion, cell-cell interaction, or differentiation to cardiomyocytes and vascular cells) is still highly debated and unresolved. The mechanisms of action of MSCs in cardiac repair are likely multifaceted, and the data accumulated to date in large-animal models and humans have shown that MSC therapy for cardiac disease is safe and provides substantial improvements in cardiac structure and function.

In summary, understanding of the endogenous roles of MSCs and their therapeutic potential has evolved substantially over the past few decades. Initially considered bystanders of uncertain significance, MSCs are now appreciated as essential cells that govern tissue homeostasis by regulating niches. This property, along with a capacity for multipotential differentiation, has greatly facilitated a role for these cells as a cell-based therapeutic agent. Enhanced by ease of preparation and immunoprivilege, MSCs have been tested in preclinical models and proof-of-concept clinical trials for their ability to both prevent and reverse ventricular remodeling. The early demonstrations of these effects, coupled with a remarkable safety profile, have set the stage for pivotal testing of these cells as a therapeutic agent. Should these trials be positive and start to reveal clinical benefits for MSC-based therapy, a truly transformative approach to cardiac therapeutics will have been achieved.

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References

6. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, Moorman MA, Simonetti DW, Craig S, Marshak DR. Multilineage...


23. Hare JM, Mckenzie J, Larson BL, Hall BM, Gibson LF. Prockop DJ. Adult stem cells from bone marrow (MSCs) isolated from different strains of inbred mice vary in surface epitopes, rates of proliferation, and differentiation potential. Blood. 2004;103:1662–1668.


34. Nardi NB, Cannarsa M. Isolation and culture of rodent bone marrow-derived multipotent mesenchymal stem cells. Methods Mol Biol. 2011;698:151–160.


60. Day TF, Guo X. Guo-Gonzalez L, Yang Y. Wnt/beta-catenin signaling in mesenchymal progenitors controls osteoblast and chondrocyte differentiation during vertebrate skeletogenesis. Dev Cell. 2005;8:739–750.


63. Arita NA, Pelaez D, Cheung HS. Activation of the extracellular signal-regulated kinases 1 and 2 (ERK1/2) is needed for the TGFbeta-induced chondrogenic and osteogenic differentiation of mesenchymal stem cells. Biochem Biophys Res Commun. 2011;405:564–569.


68. Amado LC, Salilatt AR, Schulter KH, St John M, Xie JS, Cattaneo S, Durand DJ, Fitton T, Kuang JJ, Stewart G, Lehrke S, Baumgartner


Gnecci M, He H, Noisneux N, Liang OD, Morello F, Mu H, Melo LG, Pratt RE, Ingwall JS, Dzav J. Evidence supporting paracrine hypothesis for Akt-modified mesenchymal stem cell-mediated cardiac protection and functional improvement. FASEB J. 2006;20:661–669.

Gnecci M, He H, Liang OD, Melo LG, Morello F, Mu H, Noisneux N, Zhang L, Pratt RE, Ingwall JS, Dzav J. Paracrine action accounts for marked protection of ischemic heart by Akt-modified mesenchymal stem cells. Nat Med. 2005;11:367–368.


Mirotou M, Zhang Z, Deb A, Zhang L, Gnecci M, Noisneux N, Mu H, Pachori A, Dzav. Secreted frizzled related protein 2 (Sfrp2) is the key Akt-mesenchymal stem cell–released paracrine factor mediating myocardial survival and repair. Proc Natl Acad Sci U S A. 2007;104:1643–1648.


Kinnaird T, Stabile E, Burnett MS, Lee CW, Barr S, Fuchs S, Epstein SE. Marrow–derived stromal cells express genes encoding a broad spectrum of arteriogenic cytokines and promote in vitro and in vivo arteriogenesis through paracrine mechanisms. Circ Res. 2004;94:678–685.


Dufourcq P, Descamps B, Tojais NF, Leroux L, Oses P, Duret D, Mereau C, Lamaziere JM, Couffinhal T, Duplaa C. Secreted frizzled-related protein-1 enhances mesenchymal stem cell function in angiogenesis and contributes to neovessel maturation. Stem Cells. 2008;26:2991–3001.
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