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Mesenchymal stem cells: Biology, patho-physiology, translational findings, and therapeutic implications for cardiac disease

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Abstract

Mesenchymal stem cells (MSCs) are a prototypic 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 mesodermal and non-mesodermal 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 (MI) demonstrate 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 improve LV function, induces reverse remodeling, and decreases 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.

Keywords

Stem Cells; Regeneration; Differentiation; Niches

Introduction

Ischemic heart disease is the leading cause of death in developed countries and carries significant morbidity.[1] After an acute myocardial infarction (MI), the heart has limited capacity for self-renewal and undergoes remodeling with resulting depressed left ventricular (LV) function.[2] 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 the bone marrow (BM) more than 40 years ago[3], and have emerged as one of the leading candidates in

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cellular cardiomyoplasty (Figure 1). The unique properties of MSCs—easily isolated and amplified from the BM,[4] immunologically tolerated as an allogeneic transplant[5], and multilineage potential[6]—have lead to intense investigation as a cell-based therapeutic 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.

Historical Overview

In 1970, Friedenstein and colleagues[3] demonstrated that bone marrow (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-shaped colonies resembling fibroblastoid cells formed and were given the name Colony Forming Unit – fibroblasts (CFU-f). Friedenstein was the first investigator to demonstrate the ability of MSCs to differentiate into mesodermal derived tissue as well as identify their importance in controlling the hematopoietic niche[7]. Control of stem cell niches – functional and structural units that spatiotemporally regulate stem cell division and differentiation[8, 9] – 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 hematopoiesis.[10]

During the 1980s, MSCs were shown to differentiate into osteoblasts, chondrocytes, and adipocytes.[11, 12] Caplan demonstrated that bone and cartilage turnover was mediated by MSCs, and the surrounding conditions were critical to inducing MSC differentiation.[13] In the 1990s, MSCs were shown to differentiate into a myogenic phenotype,[14] and Pittenger and colleagues demonstrated that individual adult human MSCs were capable of being expanded to colonies while still retaining their multilineage potential.[6] Also during the late 1990s, Kopen et al. described the capacity of MSCs to transdifferentiate into ectodermal derived tissue.[15]

During the early 21st century, in-vivo studies demonstrated that human MSCs transdifferentiate into endodermal derived cells and cardiomyocytes;[16, 17] and in-vitro co-culturing of ventricular myocytes with MSCs induced transdifferentiation into a cardiomyocyte phenotype.[18] It was also during this time that MSCs were demonstrated to suppress T-lymphocyte proliferation, paving the way for the application of MSC therapy for allogeneic transplantation and as a potential immunomodulatory therapy.[19] Large animal preclinical studies of MSC administration in post-MI hearts demonstrated the ability of MSCs to engraft, differentiate, and produce substantial functional recovery.[20–22] Recently, MSC therapy has been translated to clinical trials for ischemic heart disease.[23–25]

Definition of an MSC

No unique cell surface marker unequivocally distinguishes MSCs from other HSCs, making a uniform definition difficult. The International Society for Cell Therapy (ISCT) proposed a criteria[26] that comprises: (1) adherence to plastic in standard culture conditions; (2) expression of the surface molecules CD73, CD90, CD105 in the absence of CD34, CD45, HLA-DR, CD14 or CD11b, CD79a or CD19 surface molecules as assessed by FACS analysis; (3) capacity for differentiation to osteoblasts, adipocytes, and chondroblasts in vitro. These criteria were established to standardize human (h)MSC isolation but may not uniformly apply to other species. For example, murine MSCs have been shown to differ in marker expression and behavior compared to hMSCs.[27] 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, while 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 were capable of differentiating into osteoblasts, adipocytes, and chondroblasts.[28] Indeed, MSCs possess species specific characteristics, which make an unequivocal definition difficult to apply to both human and animal models.

Sources, Isolation, and Types of MSCs

MSCs isolated from bone marrow,[6] adipose tissue,[29] synovial tissue,[30] lung tissue,[31] umbilical cord blood,[32] and peripheral blood[33] are heterogeneous with variable growth potential, but all have similar surface markers and mesodermal differentiation potential.[34] Furthermore, MSCs have been isolated from nearly every tissue type (brain, spleen, liver, kidney, lung, bone marrow, muscle, thymus, aorta, vena cava, pancreas) of adult mice, suggesting MSCs may reside in all post-natal organs.[35] MSCs can be derived from many tissue sources, consistent with their broad, possibly ubiquitous distribution. From a translational perspective, as most studies of MSC therapy for cardiac repair have utilized BM derived MSCs, we will focus this review on BM-MSCs.

The BM is the major source of HSCs that continually renew red blood cells (RBCs), 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,[36] and the MSC is one of the most important cells that supports the BM microenvironment, termed the hematopoietic niche.[37] MSCs have been isolated from numerous species,[6, 38, 39] and the three critical steps that allow MSCs to be isolated from other BM cells are: (1) the use of density gradient centrifugation (Ficoll or Percoll) to separate non-nucleated RBCs 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.[4] Protocols for isolation and expansion are similar among various species, as depicted in Figure 2.

Expansion of plastic adherence MSCs is the most widely employed 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 upon cell surface antigen expression. A subset of MSCs expressing the marker neurotrophic growth factor (CD271) has been shown to have similar differentiation capacity as traditional MSCs, but secrete higher levels of cytokines and have greater immunosuppressive properties.[40] Another MSC subpopulation is the Stro-3-positive mesenchymal precursor cell (MPC), which has extensive capacity for proliferation and differentiation.[41]

Immunology of MSCs

Human MSCs express moderate levels of human leukocyte antigen (HLA) major histocompatibility complex (MHC) class I, lack MHC class II expression, and do not express costimulatory molecules B7 and CD40 ligand.[42–44] 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-dioxygnease, and heme oxygenase-1.[45–48] MSCs produce an immunomodulatory effect by interacting with both innate and adaptive immune cells (Figure 3).

The innate immune cells—neutrophils, dendritic cells (DCs), natural killer (NK) cells, eosinophils, mast cells, and macrophages—are responsible for non-specific 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.[5] MSCs have shown to dampen the respiratory burst process by releasing interleukin (IL)-6.[49] DCs play an important role in antigen presentation to naïve T cells, and MSCs have been shown to inhibit the differentiation of immature monocytes into DCs.[50] Additionally, co-cultures of MSCs and DCs inhibit the production of tumor necrosis factor (TNF-α), a potent inflammatory molecule.[51] NK cells are important innate cells in defending against viral organisms and in tumor defense by secreting cytokines and cytolysis.[5] MSCs cultured with freshly isolated resting NK cells have been shown to inhibit IL-2 induced proliferation and decrease secretion of interferon (IFN-γ) by 80%; however, IL-2 activated NK cells can lyse autologous and allogeneic MSCs.[51, 52]

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.[5] T-cells exist as CD8+ cytotoxic T-cells that induce death of target cells, or CD4+ helper T-cells which modulate the other immune cells, and MSCs have shown to suppress T-cell proliferation in a mixed lymphocyte culture.[19, 51] Suppression of lymphocyte proliferation is mediated through cytokines released by MSCs, such as rhHGF and rhTGF-β1, that equally suppress the proliferation of cytotoxic and helper T cells.[19] When MSCs are present during naïve T cell differentiation to CD4+ T helper cells, there is marked decrease in the production of IFN-γ and increase in the production of IL-4,[51] suggesting that MSCs alter naïve T cells from a pro-inflammatory state (heavy production of IFN- γ) to an anti-inflammatory state (more production of IL-4). The B cell, which produce antibodies, are highly dependent on T-cells and MSC inhibition of T cells likely contributes to the interactions of MSCs with B cells.[5] Conflicting data has shown that MSCs can inhibit and promote proliferation of B cells.[53, 54]

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,[55] and systemic infusion of MSCs preferentially home to areas of injury.[56] Infusion of MSCs in graft-versus-host disease (GVHD), a life threatening complication from allogeneic HSC transplant, has demonstrated encouraging results in steroid resistant GVHD.[57]

While 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 MHC Ia and II (immunogenic) expression and decreased MHC Ib (immunotolerant) expression.[58] Furthermore, when allogeneic MSCs were transplanted into post-MI rats, the cells were eliminated from the heart and did not improve cardiac function.[58] This data from rats suggests 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 cell identified) with few MSCs differentiated (~25%), and did not induce an immune response.[21] In addition, engraftment of these undifferentiated MSCs in post-MI hearts can stimulate endogenous repair mechanisms.[39]

Multilineage Potential of MSCs

Stem cells are characterized by their ability to self renew, clone, and differentiate into multiple tissues.[59] The first MSCs isolated more than 40 years ago were observed in culture to form bone and cartilage deposits;[3, 60] and this multilineage capability has become a defining feature of MSCs.[61] Subsequent experiments in the 1970s using autologous transplantation of pellets of BM-MSCs to the sub-capsular region of a rabbit kidney

demonstrated the in-vivo differentiation capabilities of MSCs to osteocytes[7]. Caplan expanded on this pioneering work by demonstrating that MSC differentiation into a distinct phenotype (e.g. chondrocytes or osteoblasts) is dependent on surrounding conditions.[13] For example, MSC differentiation into osteoblasts is dependent on close proximity to vasculature; however, differentiation to the chondrocyte requires no vasculature.[62] 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, 63–65] These osteogenic cells will react with antiosteogenic antibodies and form a mineralized extracellular matrix. The osteogenic potential of MSCs is conserved through numerous passages by their increased alkaline phosphatase activity.[64] To induce chondrocyte differentiation, MSCs are cultured in a medium containing dexamethasone and transforming growth factor-beta3 (TGF-β3).[66] Subsequently, the cells begin secreting an extracellular matrix, including type II collagen, aggrecan, 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 matrix components aggrecan, dermatopontin, and collagen type XI.[67] 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 (PPAR γ2), lipoprotein lipase (LPL), and fatty acid binding protein aP2.[6] These adipocytes accumulate lipid rich vacuoles and eventually coalesce.

BM-MSCs also have capacity for differentiation into other mesodermal derived tissue, notably myocytes, as shown in some but not all studies.[14, 68] 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 (β-MHC), 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.[17, 69] Murine BM-MSCs exposed to 5-azacytidine have been shown to spontaneously beat alone, connect to adjoining cells, and at after 2–3 weeks beat in synchrony.[70] Interestingly, electrophysiological evaluation of these cells showed two types of morphological action potentials, sinus node like potentials and ventricular myocyte like potentials. Reverse- transcription-polymerase chain reaction (RT-PCR) demonstrated atrial natriuretic, 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.[70]

Several co-culture experiments with cardiac myocytes have shown MSCs' ability to transdifferentiate into a cardiac phenotype.[68, 71] When mouse MSCs and rat ventricular myocytes were co-cultured, MSCs became α-actin positive, 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 semi-permeable membrane from myocytes, suggesting that transdifferentiation requires cell-cell contact.[71] Contrary to these findings, rat BM-MSCs co-cultured with neonatal rat ventricular myocytes separated by semi-permeable membrane were shown to transdifferentiate into cardiomyocytes.[68] After one week, these MSCs were observed to be contracting; expressed SERCA2 and RyR2 by RT-PCR; and were positive for cardiac troponin T, sarcomeric α-actinin, and desmin by immunohistochemistry. These studies

suggest that physical cell-cell contact as well as the local cardiac milieu and the factors secreted by myocytes play important roles in MSC transdifferentiation to cardiomyocytes. Future studies will be required to establish the relative roles of these two factors in MSC differentiation.

MSCs transdifferentiate into non-mesodermal derived tissue, such as neurons, when exposed to β-mercaptoethanol (BME).[72, 73] As early as thirty-minutes in culture MSCs express the neuronal markers NSE and neurofilament–M (NF-M).[72] Importantly, these MSCs which 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 two pathways, the Wnt canonical pathway and the TGF-β superfamily pathway.[61] Wnt are soluble glycoproteins which engage receptor complexes composed of Lrp5/6 proteins to induce a cascade of intracellular events that regulate cell proliferation and differentiation (Figure 4).[74] The Wnt pathway has been shown to be critical in skeletogenesis by promoting osteoblast proliferation[74] as well as suppression of chondrocyte formation via beta-catenin, an essential component in transducing Wnt signaling to the nucleus.[75] Numerous Wnt ligands and receptors are expressed on MSCs, and regulation of Wnt proteins controls MSC differentiation.[76] For example, exposure to Wnt3a inhibited MSC differentiation into osteoblasts while promoting undifferentiated MSC proliferation.[77]

The other molecular pathway regulating MSC differentiation is the TGF-β pathway, a family of proteins involved skeletal tissue growth and regulation of MSC differentiation into chondrocytes.[6, 61, 66] 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).[61, 78] Several other molecules are have been shown to regulate MSC differentiation, such as epidermal growth factor (EGF), platelet derived growth factor (PDGF), and fibroblast growth factor (FGF).[79, 80] Indeed, many growth factors likely interact with the Wnt and TGF-β pathways to control MSC differentiation.[61]

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, 81–84] In 2001, Toma and colleagues reported the results of human BM-MSCs transfected with β-galactosidase reporter gene injected into immunodeficient adult mouse hearts.[17] The majority hMSCs were found in the spleen, lung, and liver at 4 days post-injection. Only 0.44% of injected hMSCs were identified in the myocardium, and over time adopted a morphology indistinguishable from host cardiomyocytes. Immunofluorescence staining with anti-β-galactosidase identified engrafted MSCs in the myocardium, which co-localized with markers of cardiomyocyte lineage (desmin, α-actin, and cardiac troponin T) as early as 14 days post-injection. Shake and colleagues expanded on these early murine studies by surgically injecting autologous porcine Di-I-labeled MSCs directly into post-MI swine myocardium.[20] Successful engraftment was demonstrated by observing the labeled MSC engrafting into scarred myocardium, and also expressing the cardiomyocyte markers α-actin, tropomyosin, troponin-T, myosin heavy chain, and phospholamban within 2-weeks post-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.[21] Male BM-MSCs were injected into female swine, and identified by co-

localization using Y-chromosome FISH. Cardiomyocyte differentiation was present in approximately 14% of Y-positive MSCs as identified by co-staining for the cardiac structural proteins α-sarcomeric actinin and tropomyosin, and 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, smooth muscle protein 22-α expression) and accounted for approximately 10% of Y-positive cells identified, with the remaining 76% of engrafted cells identified as clusters of immature cells in the interstitial compartment. In another swine study, Makkar and colleagues also demonstrated the ability of allogeneic MSCs to engraft and differentiate into vascular cells and cardiomyocytes that formed connexin-43 gap junctions with adjacent host cardiomyocytes.[84] Yang and colleagues found that combining a statin with intramyocardial injections of BM-MSCs improves the efficiency of cardiomyocyte differentiation by approximately 4-fold in a swine model of acute myocardial infarction.[81] In a dog model of chronic ischemic cardiomyopathy, Silva and colleagues showed that canine MSCs engraft and differentiate into cells with a vascular phenotype, but were unable to show MSC differentiation into cardiomyocytes using troponin I co-localization techniques, suggesting important species differences.[82]

In contrast to numerous reports of engraftment, Dixon and colleagues transplanted male mesenchymal precursor cells (a subpopulation of MSCs expressing STRO-3) into post-MI female sheep and were unable to demonstrate engraftment.[85] At one-hour post injection successful implantation was confirmed by immunolocalization for the Y-chromosome, but at 8 weeks post-transplantation staining for the Y-chromosome was not detected, suggesting failure of prolonged engraftment. Furthermore, PCR confirmed the lack of engraftment by the inability to detect sex determining region genes in any female swine hearts at 8 weeks post-injection.

Paracrine Signaling

The frequency of MSC engraftment and differentiation in the heart is low compared to 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.[86–91] MSCs secrete a wide array of cytokines and growth factors, which can suppress the immune system, inhibit fibrosis and apoptosis, enhance angiogenesis, and stimulate differentiation of tissue specific stem cells (Table 1).[89, 92–94]

In-vitro studies have shown that conditioned medium from hypoxic akt-MSCs inhibits apoptosis and can trigger spontaneous contractions of rat cardiomyocytes.[87] When this conditioned medium from akt-MSCs was injected into post-MI rat hearts, infarct size reduced and LV function improved.[87] Several genes coding for VEGF, FGF-2, HGF, IGF-I are upregulated in hypoxic akt-MSCs, and these factors could mediate the functional improvements observed.[87] 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.[88] Additionally, Akt-MSCs secrete frizzled related protein (Sfrp2), a paracrine factor that exerts a pro-survival effect on ischemic myocardium through modulating Wnt signaling.[90]

Murine BM-MSC concentrated conditioned media (CCM) has been shown to consist of vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF), placental growth factor (PlGF), and monocyte chemoattractat protein-1 (MCP-1), which enhance proliferation of endothelial and smooth muscle cells.[89] Increased local levels of VEGF and FGF proteins were detected when β-galactosidase labeled MSCs were injected into ischemic

murine peripheral muscles compared to controls. These MSCs were detected in decreasing quantities from days 7 to 28 post-transplantation and failed to incorporate into vessels. Administering the factors secreted by MSCs alone may not have the same impact as administering 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 CCM did not.[39]

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.[39] Post-MI female swine were injected with GFPlabeled allogeneic MSCs to the infarct borderzone, and on histological examination were shown to exhibit chimeric clusters containing immature MSCs and c-kit+ CSCs. These niches contained adult cardiomyocytes, GFP+ MSCs, and c-kit+ CSCs expressing 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 to placebo. Additional co-culture experiments with c-kit+ CSCs and MSCs demonstrated a 10-fold increase in c-kit+ CSC proliferation compared to 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 colleagues compared the ability of BM derived c-kit+ stem cells and BM-MSCs to induce proliferation of endogenous CSCs.[95] Mice were treated with tomoxifen to induce GFP expression in cardiomyocytes, underwent MI, and administered intramyocardial injections of stem cells to the infarct borderzone. At 8 weeks, BM c-kit+ stem cells led to a significant reduction in the GFP+ cardiomyocyte pool and parallel increases in β-galactosidase+ cardiomyocytes compared to control, a finding consistent with increased progenitor activity induced by BM c-kit+ stem cells. However, BM-MSCs did not lead to reduction in the GFP+ cardiomyocyte pool or increase β-galactosidase+ cardiomyocytes, suggesting that MSCs may not stimulate endogenous progenitors. These findings in a murine model are clearly different from the body of work conducted using 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.[96, 97] 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.[98] As early as three days after transplant, rare cellular fusion was detected at injection sites by H&E staining with LacZ gene expressing distinct blue cells, which persisted at 28 days post-transplant. 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.

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.[20, 21, 41, 82–85, 99–108] Stem cells can be delivered to the heart via peripheral intravenous infusion; direct surgical injection during open heart surgery; or via catheter-based intracoronary infusion, retrograde coronary venous infusion, or

transendocardial injection.[109, 110] Using radiolabeled BM stem cells, γ-emission counting of harvested organs 1-hour after stem cell delivery demonstrated that intramyocardial injection has the highest retention rate of cells.[110] Interestingly, most cells delivered to the heart by any method are not retained in the myocardium and found commonly in the lungs and spleen.[110] Positron emission tomographic (PET) 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 pluera.[111] While stem cell retention in the myocardium appears to be low by any delivery route, preclinical data of MSC therapy for cardiac disease has shown highly promising results. Here, we will review the preclinical trials using allogeneic and autologous MSCs delivered to the heart via the four 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 as it only requires peripheral venous access; however, for the cells to reach the myocardium they have to transit through the pulmonary circulation where entrapment of cells is a concern.[112] Intravenous infusion of MSCs in a swine model of acute MI was conducted at fifteen minutes following 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.[101] At 12 weeks post-MI, LV ventriculography showed no difference in EF between MSC treated animals versus placebo. Pressure-volume (PV) loop analysis demonstrated a significant improvement in the end systolic pressure volume relationship (ESPVR) and preload recruitable stroke work (PRSW) in MSC treated animals compared to placebo. Histological analysis of post-mortem hearts showed significantly greater density of von Willebrand Factor (vWF) positive blood vessels and vascular endothelial growth factor (VEGF) expression in MSC treated animals. Steady state coronary blood flow reserve was similar among groups, but adenosine recruited coronary blood reserve was improved in MSC treated animals.

In another acute MI swine model, hemodynamic and electrophysiological effects of intravenous infusion of allogeneic MSCs were studied.[100] Using transthoracic echocardiography, EF improved and less eccentric hypertrophy in MSC treated animals was detected at 3 months follow-up compared to 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 post-MI showed shortened epicardial effective refractory periods (ERP) in MSC treated animals compared to placebo. Shortened ERP may induce ventricular tachycardia,[113] raising the possibility that MSCs may induce pro-arrhythmic remodeling.

Intracoronary infusion of MSCs

Intracoronary infusion of stem cells is delivered using a standard over-the-wire balloon angioplasty catheter placed into the target coronary artery.[114] After positioning the angioplasty catheter, the balloon is inflated at a low pressure to block blood flow (allowing adhesion and potential transmigration), while the cells are infused through the distal lumen.[114, 115] Concern for inducing ischemia during coronary artery occlusion and the lack of vessels in scar tissue may not allow effective cell delivery. 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.[114]

Most preclinical studies of intracoronary infusion of MSCs have focused on models of acute MI.[99, 102, 103, 115] Cardiac MRI was used to investigate the effects of intracoronary infusion of iron oxide labeled MSCs or placebo in swine at approximately 5 days post LAD MI.[115] Delayed enhancement (DE) cardiac MRI showed an 8% reduction in scar size in MSC treated swine compared to increased scar size in placebo infused animals. EF was unchanged in placebo infused animals, while a 15-point improvement in EF was observed in MSC treated swine. At 8 weeks post-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 to electroanatomical guided transendocardial injection of allogeneic MSCs (100 million cells) at 7 days post-MI with a no-infusion placebo control arm.[99] Two animals died in the intracoronary infusion arm, one due to microvascular plugging associated with MSC infusion and another due to intestinal ischemia, with no deaths in the other groups. Echocardiography at 21-days post transplant showed no difference in EF or LV chamber sizes in the MSC intracoronary infusion group compared to placebo, while EF, EDV, and ESV improved in the transendocardial MSC injection group. Histological analysis showed that intracoronary infusion produces a more uniformly distributed pattern of MSCs, concentrated in the borderzone and normal myocardium. Transendocardial injection was also used to deliver MSCs to the borderzone and normal myocardium, and achieved higher MSC concentration per square micron compared to intracoronary delivery.

Catheter-based intramyocardial injection of MSCs

Transendocardial stem cell injection (TESI) delivers MSCs directly into the myocardium using a catheter navigated in the LV by fluoroscopic guidance or electroanatomical mapping.[114] TESI 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 inducing arrhythmias need to be monitored with TESI, but the minimally invasive ability to directly inject cells into scar and borderzones make it an attractive delivery technique.[110, 111]

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).[39, 83, 107] Allogeneic MSCs were administered to the border and infarct zones at 3 days post-MI, and DE cardiac MRI showed approximately 50% reduction in scar size at 8 weeks post-transplant while control animals had no change in scar size.[83] On gross pathological examination, MSC treated animals had infarct confined to the mid-myocardium with visible myocardium present at both the subendocardial and subepicardial zones. The subendocardial tissue, also present on DEMRI 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 post injection, while the non-treated 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 to placebo. Additional work by our group has also showed that transendocardial MSC injection improves resting myocardial blood flow by first-pass gadolinium perfusion MRI at 8 weeks post-injection compared to placebo.[107] Vessel density assessed by vWF expression was similar in MSC and placebo treated swine, but MSC injected animals had significantly larger vessels that likely contributed to improved tissue perfusion.

In another study, three allogeneic MSC doses (2.4×10^7, 2.4×10^8, or 4.4×10^8) versus placebo delivered by electroanatomical mapping guided TESI were compared at three days post-MI.[104] DE-MRI at 12-weeks post-injection showed reduced infarct size in all MSC

dosage groups, while 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 post-injection compared to placebo injection.[111]

To investigate the impact of MSCs on healed scars, our group studied transendocardial allogeneic MSC injection in swine with three-month old anterior wall infarcts.[21] At three months post MI, remodeling in swine is nearly complete and results in depressed LV function.[116] DE-MRI at 12-weeks post-injection showed that MSC therapy resulted in a 30% reduction in scar size, while the placebo injected animals had unchanged scars. Furthermore, the circumferential extent of scar decreased approximately 14% in MSC treated swine and slightly increased in placebo injected animals. Using tagged MRI to assess regional LV function, peak Eulerian circumferential shortening (Ecc) demonstrated improved contractility of the infarct and border zones in MSC treated animals while further declines in function were evident in the placebo group. Scar size reduction and increased regional function resulted 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 has utilized direct surgical intramyocardial injection.[20, 22, 41, 82, 84, 85, 117] Surgical injection is the most invasive delivery technique as it requires either a thoracotomy or sternotomy for cell delivery, but 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, sub-acute 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 (MPC) were injected directly into the infarct borderzone one-hour following MI.[41, 85] As discussed above, STRO-3 positive MPCs are a sub-set of BM-MSCs with extensive capacity for proliferation and differentiation.[118] Animals were allocated to one of four different cell dosages (25 million; 75 million; 225 million; or 450 million cells) or placebo. Echocardiography at 8-weeks post-injection demonstrated improved EF in all MPC treated animals compared to placebo. Compared to placebo treated animals, EDV was significantly smaller in the two low dose groups (25 million and 75 million cells), but was unchanged in the two high dose groups (225 million and 450 million cells). EF significantly improved in all cell treated animals compared to placebo. Echocardiography derived MI length was significantly smaller in the three low dose groups (25 million, 75 million, and 225 million) compared to placebo, but was not significantly different in the high dose (450 million) treated group compared to placebo. This data suggests that there may be threshold effect with this particular subpopulation of MSCs.

In an ischemia-reperfusion swine model of sub-acute MI, autologous MSCs (60 million cells) were directly injected into two-week old infarcts using a midline sternotomy.[20] Sonomicrometry crystals were placed in the infarct region during exposure of the heart to assess wall thickness and regional contractility. At 4-weeks post injection a trend towards improved end diastolic wall thickness and improved systolic wall thickening was seen in MSC treated animals compared to placebo.

Our group reported the results of direct surgical injections of autologous MSCs in a swine model of ischemic cardiomyopathy.[22] Animals underwent LAD coronary artery occlusion followed by reperfusion, and at 12-weeks post-MI received either 20 million or 200 million autologous MSCs or placebo injections to the infarct and borderzone. Serial cardiac enzymes (troponin-I, creatine kinase, and creatine kinase MB) and white blood cell (WBC) 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 post-injection demonstrated a trend towards 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 while there was a trend in scar expansion in placebo treated animals. Regional contractility as assessed by tagged MRI derived peak Ecc showed improved contractility of the infarct borderzones 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 to 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 to unchanged EF in placebo treated animals.

In a swine model of LAD coronary artery occlusion without reperfusion, allogeneic bone marrow MSCs (200 million cells) or placebo were surgically injected during thoracotomy at 30 days post-MI.[84] Sixty days after injection, global cardiac function assessed by contrast LV angiography showed deterioration in EF of placebo treated animals, while 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 as it stands in contrast to the totality of large animal experience with reperfusion models, in which MI size is reduced by MSC injection.

MSC therapy has also been studied in dogs with chronic ischemic hibernating myocardium.[82] Dogs underwent ameroid constrictor placement around the LAD coronary artery to induce chronic ischemia, and thirty days later direct surgical injections of allogeneic BMMSCs (100 million cells) or placebo were administered to the ischemic territory during thoracotomy. Serial cardiac enzymes (creatine kinase – MB and troponin I), c-reactive protein (CRP), and WBC obtained after injections showed no comparable levels between groups. Cardiac function assessed by echocardiography showed significant attenuation in EF in MSC treated animals compared to placebo at 30 days post-injection. Quantitative morphometry assessment of scar size showed a trend towards reduced fibrosis in the anterolateral wall of MSC treated animals compared to controls.

In summary, MSC therapy improves LV function, reduces scar size and increases myocardial tissue perfusion in post-MI large animal models regardless of delivery method or species. Intramyocardial injection appears to have the highest retention rate, and when compared to intracoronary infusion accordingly has a greater impact on LV function.[99, 110] The optimal time to deliver MSCs in the post-MI setting, whether acutely during the remodeling phase or in the chronic setting after a healed scar is developed, needs better and more rigorous definition in future studies. Other important lessons from cell injection trials include the importance of reperfusion.

Genetically and Pharmacologically Modified MSCs

Several preclinical studies have investigated the effects of genetically modified MSCs and concomitant pharmacological therapy on cardiac repair.[81, 119, 120] In an acute MI swine

model of ischemia-reperfusion, direct surgical injections of autologous MSCs (30 million cells) alone or in combination with oral simvastatin were compared to placebo injected swine with and without oral simvastatin therapy. At 6 weeks post-injection, myocardial single photon emission tomography (SPECT) assessment of myocardial perfusion showed a reduction in the perfusion defect in both the simvastatin alone and combination (simvastatin + MSC) groups compared to control; however, there was no added benefit to improving myocardial perfusion by adding MSC therapy to simvastatin alone. Cardiac MRI showed improvement in EF in the combination (simvastatin + MSC) group compared to control. There was no difference in EDV between groups, and ESV was significantly less in the combination (simvastatin + MSC) group compared to control. DE-MRI showed reduced scar size in both simvastatin alone and combination (simvastatin and MSC) groups compared to placebo, but not between groups. Systolic wall thickening improved only in the combination (simvastatin + MSC) group compared to control. Histology showed increased engraftment of MSCs with concomitant treatment with simvastatin compared to MSC therapy alone, suggesting that a statin may improve retention and survival of MSCs in post-MI hearts.

In a swine model of chronic ischemic cardiomyopathy, the effect of adding hepatocyte growth factor (HGF) to BM-MSC therapy was investigated.[119] Four weeks after permanent LAD occlusion, swine were administered autologous MSCs (5 million cells) alone, combination HGF/MSCs (5 million cells), or placebo via intracoronary infusion to the non-infarct related artery. Gated myocardial SPECT showed no change in myocardial perfusion of placebo treated swine; however, both the MSC alone and MSC/HGF groups had significant improvements at 4 weeks with no difference in myocardial perfusion between MSC alone and MSC/HGF groups. SPECT derived EF should significant improvement in MSC alone and MSC/HGF treated animals compared to no change in controls. Histological analysis showed increased vessel density in MSC alone and MSC/HGF treated animals compared to placebo, but no difference between treated groups. Taken together this study suggests that adding HGF to MSC therapy does not provide additional benefit over MSC therapy alone.

Heme oxygenase-1 (HO-1) is an inducible enzyme that rapidly degrades heme resulting in anti-apoptotic and anti-inflammatory activity, and in-vitro studies have been shown HO-1 to enhance the tolerance of MSCs to hypoxia-reoxygenation injury.[121] BM-MSCs transfected to overexpress heme oxygenase-1 (HO-1) were administered to cells or placebo via intracoronary infusion following acute MI in a swine model of ischemia-reperfusion.[120] Cardiac MRI at 3 months post-injection demonstrated improved EF in HO-1 transfected MSCs compared to placebo and plasmid transfected MSCs. EDV was similar among groups, while ESV was significantly lower in the HO-1 transfected MSC group. Western blot demonstrated significantly enhanced myocardial expression of VEGF and reduced expression of TNF-α and IL-6 in Ho-1 transfected MSC treated hearts compared to 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.[122, 123] Transfected Akt-MSCs, MSCs alone, or placebo were administered via intracoronary infusion three days post-MI. Coronary angiograms following intracoronary MSC infusion showed several cases of slow coronary flow requiring administration of intracoronary adenosine, nitroglycerin, or nicorandil. At 4 weeks post-transplant, SPECT showed significantly improved EF in akt-MSC treated animals compared to MSC alone, while both showed LV function compared to placebo. Infarct size by SPECT

was significantly less in both MSC alone and akt-MSC treated animals compared to placebo. Furthermore, the infarct size was significantly smaller in akt-MSC treated animals compared to MSC alone, suggesting that akt-MSCs may provide additional scar size reduction.

Safety of MSC Therapy

Whereas, 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 pro-arrhythmia and tumor formation.[124] Intravenous infusion of MSCs in swine was shown to alter the electrophysiological properties of the myocardium;[100] but contrary to these findings in swine, intravenous infusion of allogeneic MSCs in humans with acute MI demonstrated less ventricular arrhythmias compared to placebo infusion.[125] Furthermore, other clinical trials have not shown increased arrhythmias after MSC therapy.[126, 127] In the setting of ischemic cardiomyopathy, programmed electrical stimulation did not detect a higher level of inducibility in MSC treated pigs.

Several reports have raised concern for tumor formation using BM cultured MSCs.[124, 128] Murine derived BM-MSCs were used in these studies and shown to undergo chromosomal abnormalities that result in tumor formation in numerous organs. As previously discussed, in a series of large animal preclinical studies by our group and others, MSCs were safe in large animals with no evidence of tumor formation or ectopic tissue growth.[22, 39, 41, 82–85, 99–104, 106, 107] Furthermore, there have been no reports of ectopic tissue growth in the early phase human studies using MSCs.[23, 126, 127] Nonetheless, these reports of tumorgenesis in murine models highlights the importance of continued long-term surveillance of patients treated with MSCs.

Clinical Trials of MSC Therapy for Cardiac Repair

Acute MI

Based upon 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 as well as ischemic cardiomyopathy. Phase I/II clinical data has been reported using intravenous therapy, intracoronary infusion, and intramyocardial injection.[23, 126, 127] Chen and colleagues investigated the effects of intracoronary infusion of autologous BM-MSCs (8 to 10 × 10^9, n=34) or saline (n=35) in patients with sub-acute MI.[126] PET 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 to 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 following acute MI.[23] This dose ranging study showed that IV 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

Based upon 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 underway testing 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 bone marrow) produced a cardiac phenotype of reverse remodeling by employing cardiac MRI imaging. In this study, 8 patients with chronic ischemic cardiomyopathy were administered bone marrow MSCs (n=4) or mononuclear cells (MNCs, n=4) to the scar and border zone.[127] We demonstrated reverse remodeling and improved regional contractility of the treated scar, which was evident as early as 3 months post-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 ESV (r^2=0.83, p=0.01). Importantly, we used serial whole body computed tomography (CT) scans that showed no evidence of ectopic tissue growth at one year after transplantation, and serial Holter ECG recordings showed no sustained arrhythmias. Taken together, the early phase clinical trial data demonstrates that MSC therapy for post-MI is safe and has favorable effects on cardiac structure and function.

To follow-up on this finding, two larger studies are underway: 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 bone marrow MSCs versus MNCs, and POSEIDON is comparing the effects of allogeneic versus autologous MSC therapy in patients with ischemic cardiomyopathy.[24] 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 studying MSC therapy for the treatment of idiopathic dilated cardiomyopathy.

Several other clinical trial efforts are also underway including the use of MPCs and MSCs treated ex-vivo with cytokines to enhance cardiopoiesis. The next few years will witness the results of these early clinical trial efforts.

Strategies and Future Directions

Retention of stem cells in the heart is low by any method of delivery,[110] and strategies to enhance engraftment and differentiation are needed. Guided cardiopoiesis has been a proposed technique to enhance the cardiac phenotype of MSCs and was shown to increase engraftment in murine hearts.[129] 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.[21, 39, 86] The mechanism of action of MSCs in cardiac repair are likely multifaceted and the data accumulated to date in large animal models and humans has shown that MSC therapy for cardiac disease is safe and provides substantial improvements in cardiac structure and function.

In summary, understanding of endogenous roles of MSCs and their therapeutic potential has substantially evolved over the past few decades. Initially considered bystanders of uncertain significance, MSCs are now appreciated as essential cells governing tissue homeostasis by regulating niches. This property, along with a capacity for multipotential differentiation, has greatly facilitated a therapeutic role for these cells as a cell-based therapeutic. Enhanced by ease of preparation and immunoprivilege, MSCs have been tested in preclinical models and proof-ofconcept clinical trials for 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. 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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Non-standard abbreviations and acronyms

MSC	mesenchymal stem cell
HSCs	hematopoietic stem cells
BM	bone marrow
MPC	mesenchymal precursor cell
CFU-f	Colony Forming Unit – fibroblasts
SPECT	single photon emission tomography
MI	myocardial infarction
LV	left ventricle
EDV	end-diastolic volume
ESV	end-systolic volume

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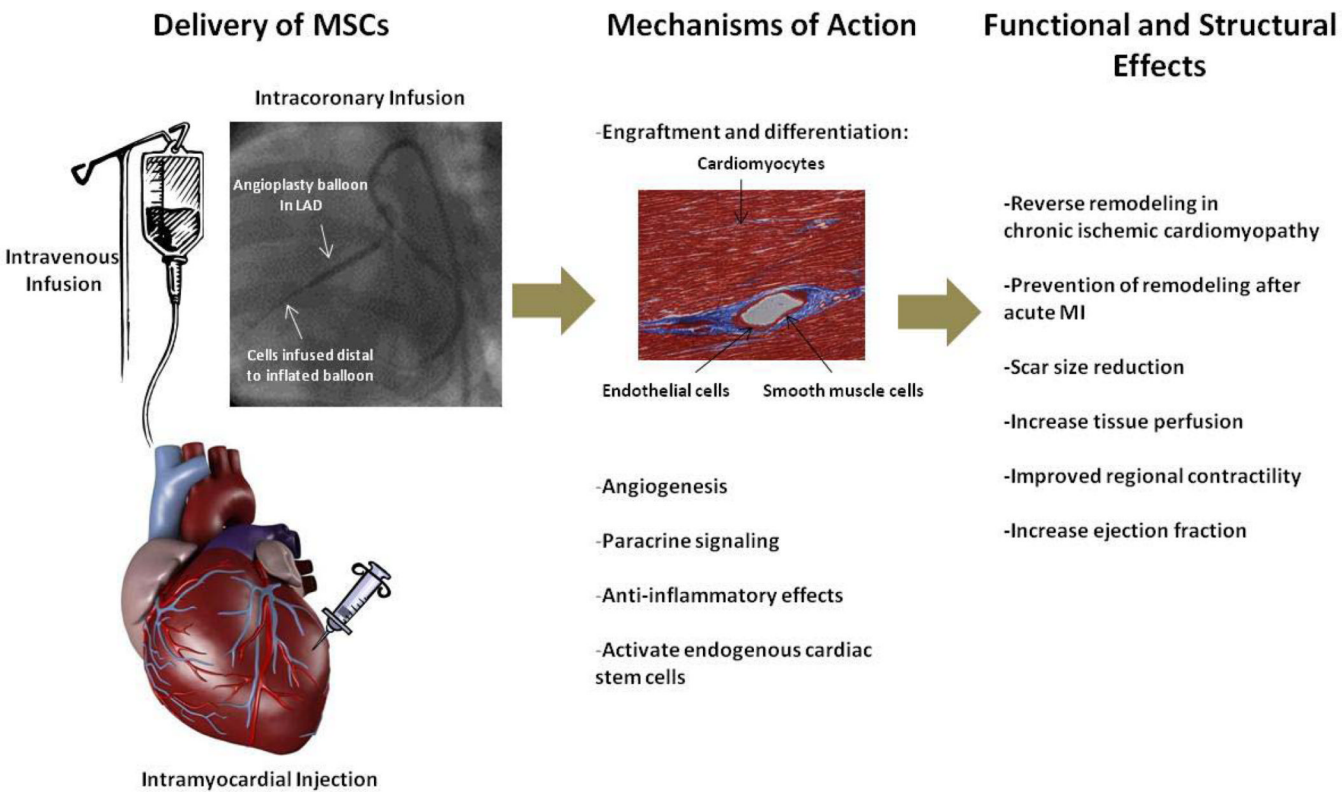

Figure 1. Delivery and potential effects of MSC therapy in cardiac disease

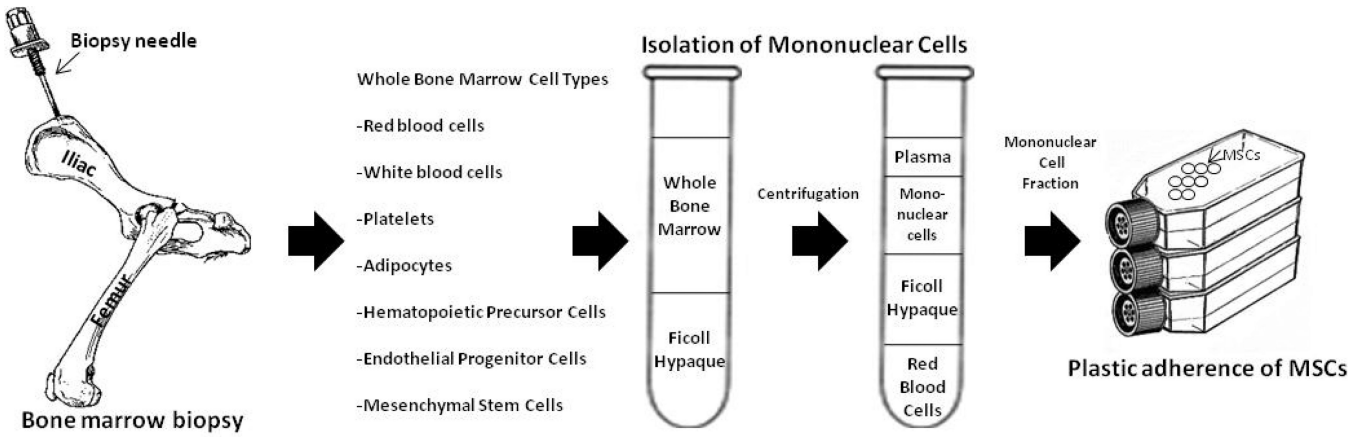

Figure 2. Isolation of mesenchymal stem cells from a bone marrow biopsy

Many stem cells and maturing cells exist in the bone marrow cavity. The mononuclear cells are isolated from red blood cells using Ficoll density centrifugation and the MSCs are separated from the MNCs by plastic adherence in culture.

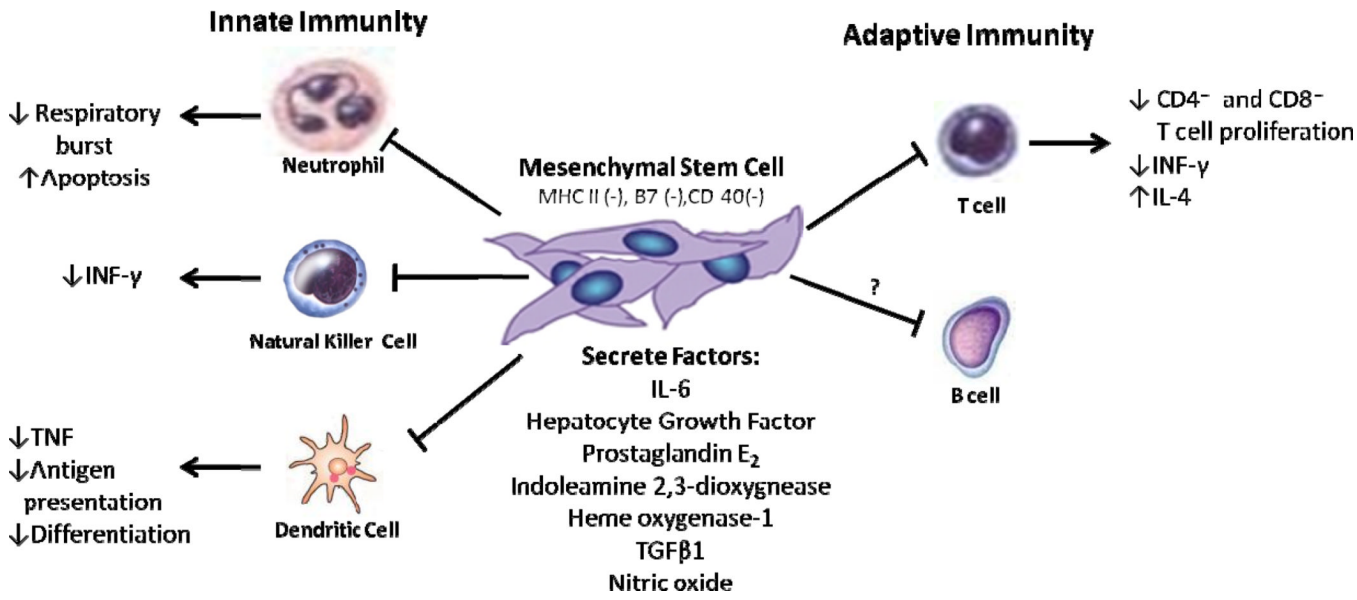

Figure 3. MSC interactions with immune cells

MSCs are immunoprivileged cells inhibit both innate (neutrophils, dendritic cells, natural killer cells) and adaptive (T cells and B cells) immune cells.

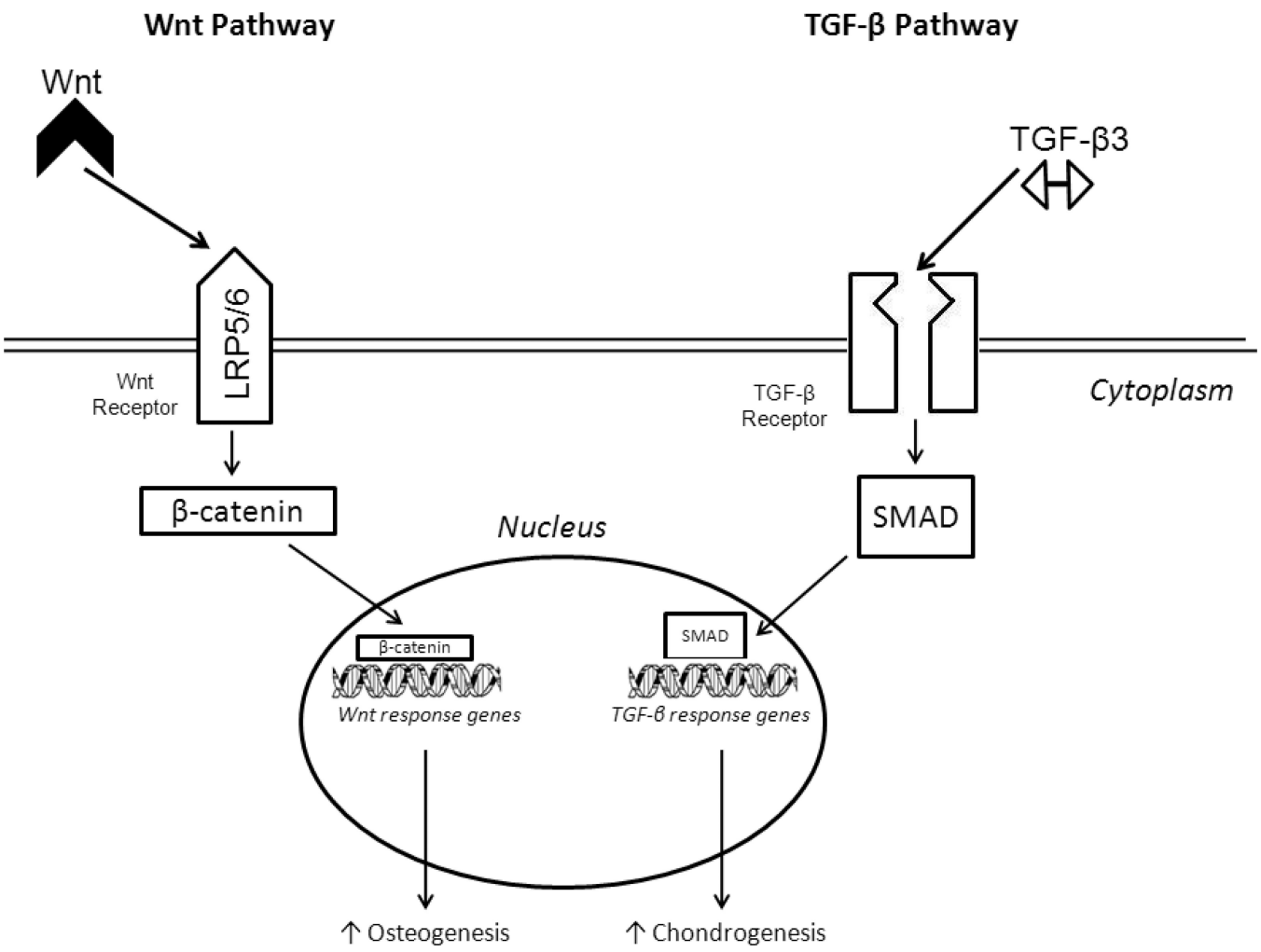

Figure 4. Molecular regulation of MSC differentiation

Wnt signaling and TGF-β induce intracellular signaling regulate differentiation of MSCs.

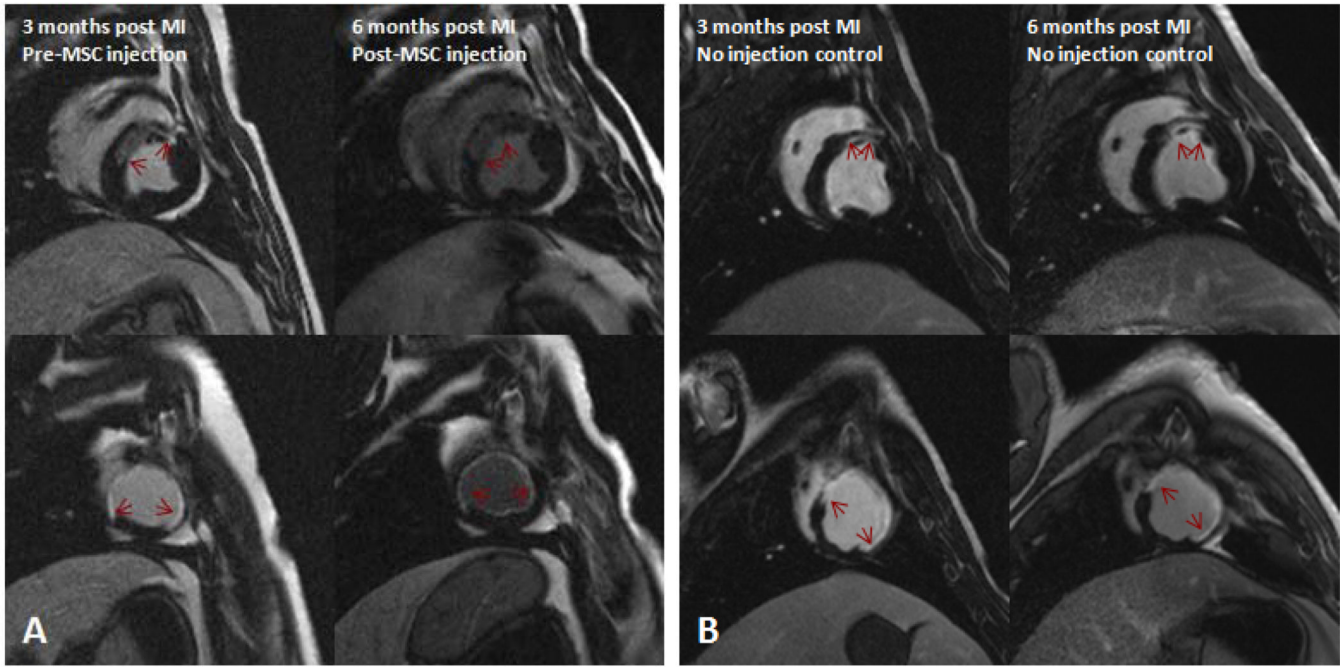

Figure 5. Impact of allogeneic MSC therapy on infarct size

DE-MRI images of swine with chronic ischemic cardiomyopathy (A) injected with 200M 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).

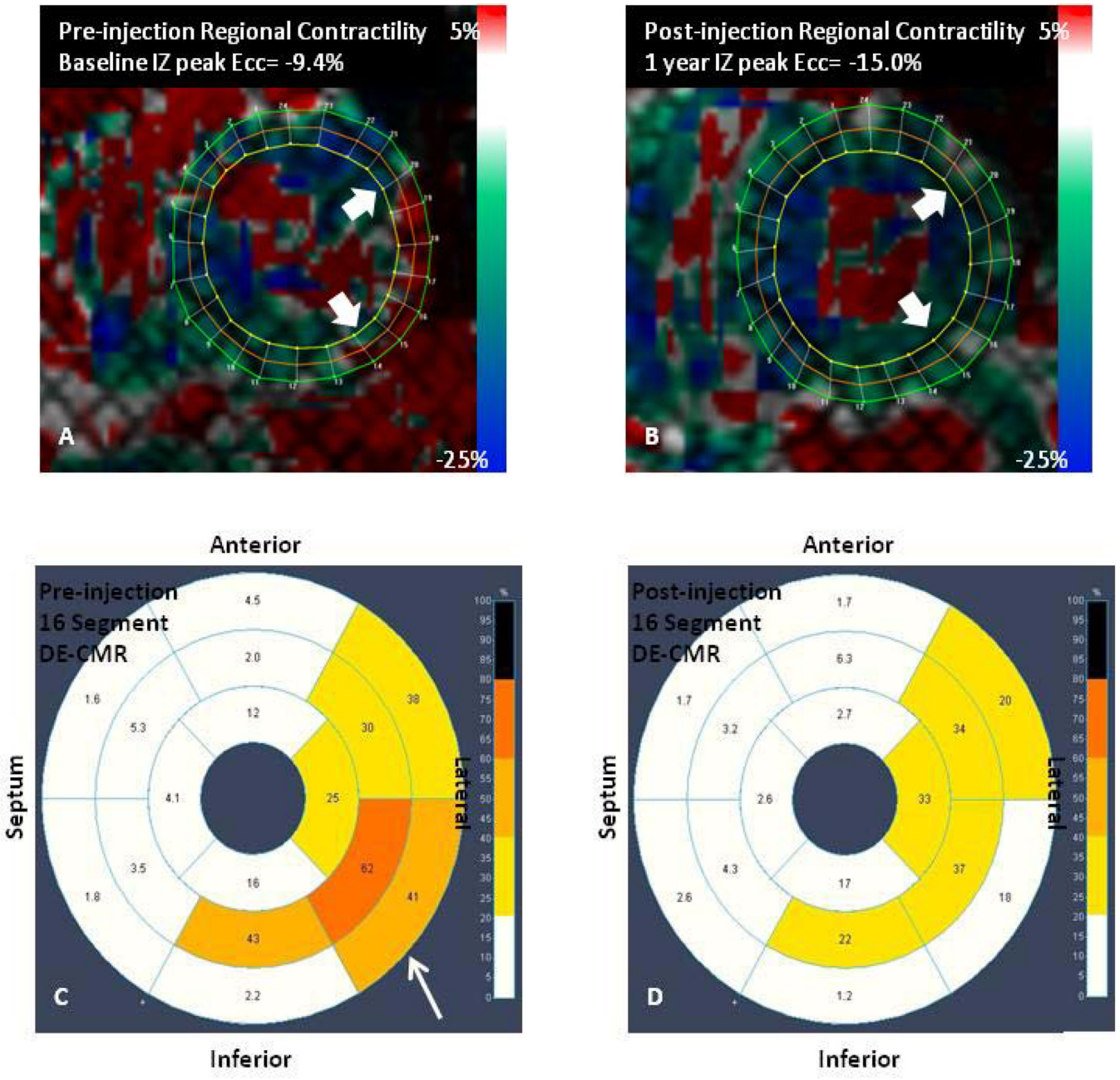

Figure 6. Intramyocardial injection of autologous MSCs improves regional contractility

Tagged CMR strain maps (A) before MSC injection showing a lateral infarct (white arrows) with decreased contractility (red) and (B) post-injection showing improved contractility (green/blue). Corresponding DE-CMR 16 segment model of infarct size (A) before MSC injection and (D) 1 year after injection (less transmularity depicted by decreased orange/yellow). (Panels A and B are reproduced from Williams et al. Circ Res. 2011;108:792–796, courtesy of the American Heart Association)

Table 1

Paracrine factors secreted by MSCs

Secreted Factor	Function
Pro-angiogenesis:	
Fibroblast growth factor-2 (FGF-2)[87, 93]	Induces endothelial and smooth muscle cell proliferation
Fibroblast growth factor-7 (FGF-7)[93]	Induces endothelial cell proliferation
Monocyte chemoattractanat protein-1 (MCP-1)[89]	Induce angiogenesis; recruit monocytes
Platelet derived growth factor (PDGF)[93]	Smooth muscle cell proliferation
Placental growth factor (PlGF)[89, 93]	Promotes angiogenesis
Transforming growth factor-β (TGF-β)[93]	Vessel maturation
Vascular endothelial growth factor (VEGF)[87, 89]	Endothelial cell proliferation, migration, tube formation
Remodeling of extracellular matrix::	
Metalloproteinase-1 (MMP1)[93]	Loosens matrix; tubule formation
Metalloproteinase-2 (MMP2)[93]	Loosens matrix; tubule formation
Metalloproteinase-9 (MMP9)[93]	Loosens matrix
Plasminogen activator (PA)[93]	Degrades matrix molecules
Tumor necrosis factor -α (TNF-α)[93]	Degrades matrix molecules; cell proliferation
Stem cell proliferation, recruitment, & survival:	
Basic fibroblast growth factor (bFGF)[89]	Enhance proliferation of endothelial and smooth muscle cells
Granulocyte colony stimulating factor (G-CSF)[94]	Increases proliferation and differentiation of neutrophils
Insulin –like growth factor-1 (IGF-1)[87]	Regulates cell growth and proliferation; inhibits apoptosis
Macrophage colony stimulating factor (M-CSF)[94]	Increases proliferation and differentiation of monocytes
Thymosin-β4 (Tβ4)[87]	Promotes cell migration
Stem cell-derived factor (SDF)[93]	Progenitor cell homing
Secreted frizzled-related protein-1 (SFRP1)[130]	Enhance cell development
Secreted frizzled-related protein-2 (SFRP2)[90]	Inhibit apoptosis; enhance cell development
Immunomodulatory:	
Heme oxygenase-1(HO1)[47]	Inhibits T cell proliferation
Hepatocyte Growth Factor (HGF)[87]	Inhibit CD4+ T cell proliferation
Indoleamine 2,3-dioxygenase (IDO)[45]	Inhibits innate and adaptive immune cell proliferation
Inducible nitric-oxide synthase (iNOS)[48]	Inhibits inflammation
Interleukin-6 (IL-6)[94]	Regulates inflammation; VEGF induction
Prostaglandin E_2 (PGE_2)[52]	Inhibits inflammation