Stem cell-based therapies to promote angiogenesis in ischemic cardiovascular disease

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Hou L, Kim JJ, Woo YJ, Huang NF. Stem cell-based therapies to promote angiogenesis in ischemic cardiovascular disease. Am J Physiol Heart Circ Physiol 310: H455–H465, 2016. First published December 18, 2015; doi:10.1152/ajpheart.00726.2015.—Stem cell therapy is a promising approach for the treatment of tissue ischemia associated with myocardial infarction and peripheral arterial disease. Stem and progenitor cells derived from bone marrow or from pluripotent stem cells have shown therapeutic benefit in boosting angiogenesis as well as restoring tissue function. Notably, adult stem and progenitor cells including mononuclear cells, endothelial progenitor cells, and mesenchymal stem cells have progressed into clinical trials and have shown positive benefits. In this review, we overview the major classes of stem and progenitor cells, including pluripotent stem cells, and summarize the state of the art in applying these cell types for treating myocardial infarction and peripheral arterial disease.

stem cell therapy; pluripotent stem cell; angiogenesis; myocardial infarction; peripheral arterial disease; hindlimb ischemia

CARDIOVASCULAR DISEASE (CVD) is the leading cause of mortality and morbidity among Americans and Veterans, accounting for one in every three deaths and a hospital burden of $196 billion (63). In particular, tissue ischemia associated with coronary heart disease and peripheral arterial disease (PAD) accounts for more than half of all CVDs. Although the quality of life of the patients with CVDs has improved over recent decades, owing to drug treatment and organ transplantation, there is still a tremendous need to develop improved therapies for the treatment of tissue ischemia. Accordingly, stem cell-based approaches to promote angiogenesis for the improvement of tissue function and/or blood perfusion are attractive. A number of stem and progenitor cells have been examined for boosting angiogenesis within ischemic CVDs, including adult bone marrow-derived mononuclear cells (BM-MNCs), endothelial progenitor cells (EPCs), and pluripotent stem cell-derived endothelial cells (PSC-ECs). In this review, we will discuss the state of stem cell therapy for the treatment of peripheral arterial disease (PAD) and myocardial infarction (MI) in the preclinical and clinical settings.

Classes of Stem and Progenitor Cells

To describe the therapeutic efficacy of stem cells in preclinical models, here we first define and characterize these various cell populations. Stem and progenitor cells are generally identified by specific phenotypic markers, although no single marker is sufficient to uniquely distinguish them from other cells. A phenotypic signature can be empirically defined based on the expression of surface receptors, transcription factors, and cytosolic proteins, in addition to morphological or functional characteristics. Below is an overview of the major classes of stem and progenitor cells used in the treatment of CVDs (Table 1).

Mononuclear cells. BM-MNCs and peripheral blood MNCs (PB-MNCs) are the most commonly used therapeutic cells for clinical treatment of CVDs, owing to their ease of isolation by bone marrow aspiration or venipuncture and subsequent purification by density gradient centrifugation (23). Another advantage is that these cells can be harvested with short turn-around time and implanted into the patient without in vitro expansion. BM-MNCs and PB-MNCs represent heterogeneous populations composed of hematopoietic stem cells, mesenchymal stem cells (MSCs), and EPCs. As a result, they are primarily characterized by having a single nucleus. The cellular composition appears to vary depending on the purification procedure. Comparison of MNC isolation approaches demonstrated that Ficoll gradient centrifugation separation resulted in higher cell recovery than using Lymphoprep separation (85). Furthermore, Lymphoprep separation resulted in a reduction in CD34+ and CD133+ hematopoietic stem cells, compared with the use of Ficoll gradient centrifugation. These results underscore the importance of the purification approach on cellular composition and demonstrate the potential advantages of Ficoll gradient centrifugation for the isolation of MNCs.

Endothelial progenitor cells. First described by Asahara and colleagues (2), EPCs derived from the bone marrow or PB are known to home to sites of tissue injury to regenerate new blood vessels. Representing less than 1% of the cell population in PB, EPCs can be purified from blood by density gradient centrifugation.
uigation for MNCs, followed by attachment onto tissue culture plastic dishes (104). Although there is no consensus in the phenotypic marker expression of EPCs, they are generally characterized by the positive expression of FLK1 (VEGF receptor 2), CD133, and CD34 at early stages after attachment onto tissue culture plastic dishes (3, 34, 78). After expansion for several weeks, the late-outgrowth EPCs functionally express endothelial markers such as vascular endothelial (VE) cadherin and von Willebrand factor (vWF). EPCs also express C-X-C chemokine receptor type 4 receptors that bind stromal-derived factor-1, which is released at sites of tissue ischemia (11). EPCs home to these sites of tissue injury and participate by directly forming new vessels and indirectly by secreting proangiogenic cytokines and growth factors (4).

**Mesenchymal stem cells.** MSCs are a class of stromal cells with spindle-shaped morphology (10, 42, 73, 75). They can be derived from numerous organs including bone marrow, liver, adipose tissue, blood, and liver (7, 9). MSCs within bone marrow occupy only 0.01% of the total population but can expand over one million-fold (73). As a multipotent stem cell, MSCs have limited self-renewal capacity and can give rise to specialized cells such as adipocytes, chondrocytes, and osteocytes (73, 74). From bone marrow aspirates, MSCs can be isolated by density gradient centrifugation techniques, followed by plating onto tissue culture plastic for adherent cells. Although MSC phenotype varies within different organs and species, they generally express the surface markers STRO-1 (a stromal cell surface antigen), CD29 (integrin-β1), CD44 (receptor for hyaluronic acid and matrix proteins), CD105 (endoglin), and CD166 (cell adhesion molecule) (18, 89). MSCs predominantly give rise to cells of musculoskeletal and fat lineages but have been shown to differentiate into endothelial lineage by stimulation with growth factors (110), shear stress (106), and extracellular matrix compliance (109, 110). Besides differentiating into cells of the endothelial lineage, MSCs can also participate in angiogenesis by secreting angiogenic paracrine factors including VEGF, basic fibroblast growth factor (bFGF), and PDGF (22, 45).

**Pluripotent stem cells.** Human embryonic stem cells (ESCs) are PSCs derived from the inner cell mass of the blastocyst (97). They are capable of infinite expansion, while being capable of giving rise to cells from all three germ layers. Morphologically, ESCs grow in compact colonies formed on top of murine embryonic fibroblast feeder cells or on Matrigel basement membrane extract (114). Phenotypically, ESCs express pluripotent markers such as transcriptional factors Oct3/4 and Sox2, homeodomain protein Nanog, and surface markers SSEA-1 and TRA-1-81 (28, 79, 111). Limiting the translational relevance of ESCs are the ethical concerns of embryo destruction, along with the potential dangers of teratoma formation.

An alternative PSC source are iPSCs, which are somatic cells that have been reprogrammed into a PSC state by the genetic activation of key transcription factors (i.e., Oct3/4, Sox2, Klf4, and c-Myc) (91, 92). Human iPSCs appear to resemble many pluripotent properties of ESCs in their ability to have nearly unlimited self-renewal capacity and differentiate into cells of all three germ layers, including endothelial lineage (92). In contrast to ESCs, however, iPSCs can be derived from a patient’s somatic cells, which overcomes concerns of immune rejection and the destruction of embryos. In the advent of technological advances, iPSCs can now be generated using nongenetic-based approaches, which will improve the safety of these cells for clinical use (1, 52, 80). Thus iPSCs may be the most promising patient-specific cell source for regenerative medicine.

To obviate the potential formation of teratomas, ESCs and iPSCs can be terminally differentiated into endothelial lineage before in vivo implantation. A number of robust differentiation protocols have been described that take advantage of chemical factors such as VEGF, bone morphogenetic protein-4, bFGF, and/or Wnt signaling pathways for inducing endothelial differentiation (51, 83, 108, 118).

In summary, a number of stem cells can be delivered for the treatment of CVDs, although the identity and characterization of the stem cell populations may vary depending on the phenotypic markers used for characterization. MNCs, EPCs, and MSCs constitute a readily accessible population of adult autologous therapeutic cells. However, concerns of limited plasticity in differentiation, along with reduced function and number of adult stem cells in cardiovascular patients, dampen the enthusiasm for their usage (8, 98, 104). Human iPSCs represent a new class of autologous stem cells with greater plasticity in differentiation, but the concerns of teratoma formation currently limit their translation into clinical practice.

**Mechanism of Stem Cell Participation**

To repair tissue ischemia, stem cells participate in angiogenesis by releasing angiogenic factors and/or by differentiating into vascular lineage. For example, MSCs secrete angiogenic paracrine factors such as VEGF, bFGF, and PDGF that stimulate neovessel formation (22, 45). Some investigators also reported the ability of MSCs to differentiate into endothelial lineage and/or express endothelial lineage markers (36, 49, 69, 115). We have previously shown that ECs derived from human iPSCs (iPSC-ECs) secrete angiogenic factors to similar degrees as primary ECs, including bFGF, VEGF, and angioptetin-1 (83). In addition, the iPSC-ECs can functionally incorporate into the perivascular space. EPCs and MNCs can differentiate into endothelial lineage by forming neovascularization that functionally anastomose with host circulation (3, 12). Thesevary-

### Table 1. Characterization of therapeutic stem cell types

<table>
<thead>
<tr>
<th>Type</th>
<th>Source</th>
<th>Differentiation Capacity</th>
<th>PAD Applications</th>
<th>MI Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>EPC</td>
<td>Bone marrow/peripheral blood</td>
<td>Multipotent</td>
<td>(2, 41, 47, 88, 119)</td>
<td>(43, 68)</td>
</tr>
<tr>
<td>MSC</td>
<td>Bone marrow/adipose tissue/blood</td>
<td>Multipotent</td>
<td>(25, 45, 46)</td>
<td>(56, 61, 87)</td>
</tr>
<tr>
<td>ESC</td>
<td>Inner cell mass of blastocyst</td>
<td>Pluripotent</td>
<td>(13, 35, 117)</td>
<td>(32, 50, 57, 116)</td>
</tr>
<tr>
<td>iPSC</td>
<td>Any somatic cell type</td>
<td>Pluripotent</td>
<td>(83)</td>
<td>(24, 96)</td>
</tr>
</tbody>
</table>

PAD, peripheral arterial disease; MI, myocardial infarction; EPC, endothelial progenitor cell; MSC, mesenchymal stem cell; ESC, embryonic stem cell; iPSC, induced pluripotent stem cell.
ing mechanisms enable stem cells to differentiate and/or to incorporate into vasculature or release paracrine factors to stimulate host-derived angiogenesis.

Below, we discuss the applications of these classes of stem cells in treating PAD and MI in preclinical and clinical settings.

Peripheral Arterial Disease

PAD, which affects over eight million patients in the United States, is an occlusive atherosclerotic disease caused by blockage of the arteries from cholesterol plaques (30, 31, 64). PAD limits blood supply to the lower extremities. Symptoms of PAD include intermittent claudication, which is defined as calf or buttock pain, or cramping with walking caused by inadequate blood flow to the limb (70). The most severe symptom of PAD is critical limb ischemia (CLI), defined as pain at rest due to reduced blood flow to the limb. CLI may further result in ischemic ulceration and gangrene formation. Patients with CLI have not only a high risk of amputation but a high rate of cardiovascular death, often due to complications related to coronary artery (55%) and cerebrovascular atherosclerosis (10%) (19).

Among preclinical models of PAD, the most popular and widely used one is the hindlimb ischemia model (HLI). In this experimental model, the femoral artery is ligated and excised, reducing blood flow to the lower leg by ~20% of normal blood perfusion, based on laser-Doppler spectroscopy (67). The HLI model has been widely used to evaluate the functional capacity of therapeutic stem cells (Table 2).

Preclinical Studies to Treat PAD Using Therapeutic Stem Cells

Treatment of PAD using MNCs and EPCs. Asahara and colleagues (2) first demonstrated that PB-derived CD34+ EPCs integrated with host capillary vessel walls and formed capillaries in the ischemic limbs of syngeneic mice and autologous rabbits. Shintani et al. (88) provided evidence that BM-MNCs derived from Histopaque density gradient centrifugation augmented neovascularization in a rabbit HLI model by inducing collateral vessel formation and blood perfusion. Kobayashi et al. (47) used a similar rabbit HLI model and studied the effect of Ang-I gene transfer combined with transplantation of low dose BM-MNCs isolated using Histopaque density gradient centrifugation. In this study, the authors observed notable improvement in angiographic score, an increase in capillary density, an increase in transcutaneous oxygen pressure, and a reduction in skin ulceration. The angiographic score was a quantitative analysis of collateral vessel development from angiogram films (93). It is often calculated as the ratio of grid intersections crossed by contrast-opacified arteries to the total number of grid intersections in the medial thigh area. In addition, Jeon et al. (41) studied the effect of combined angiogenic therapies in a mouse HLI model by performing BM-MNC transplantation along with EPC mobilization with granulocyte colony-stimulating factor. They reported an increase in microvessel density and more extensive expression of bFGF and VEGF when the combined therapy was performed, compared with administration of each treatment separately. Similar improvements in capillary density and blood perfusion were reported by Zhang et al. (119) when Histopaque gradient-isolated BM-MNCs were transplanted in mice with ischemic skeletal muscle.

Treatment of PAD using MSCs. As for MSCs, a number of studies have been performed to show their therapeutic effects. Kinnaird et al. (46) showed that CD44+/CD90+ MSCs were able to improve limb function, reduce autoamputation, and attenuate muscle atrophy and fibrosis in a murine HLI model. The authors (45) demonstrated that the therapeutic benefits were due to paracrine mechanisms, especially through the release of bFGF and VEGF from the cells. In a related study,

Table 2. Application of stem cells for treatment of PAD

<table>
<thead>
<tr>
<th>Disease Model</th>
<th>Cell Type</th>
<th>Injection Approach</th>
<th>Follow-up Time</th>
<th>Output Measurement</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse/rabbit HLI</td>
<td>Putative progenitor ECs</td>
<td>Tail vein injection</td>
<td>1 to 6 wk</td>
<td>Integration to capillary vessel walls, arranged into capillaries by 6 wk; incorporation of cells in capillaries and small arteries; cells localized to neovascular zones</td>
<td>(2)</td>
</tr>
<tr>
<td>Rat HLI</td>
<td>CD133+ progenitor cells</td>
<td>Injectable collagen-based matrix</td>
<td>2 wk</td>
<td>CD133+ cells retained better; cells incorporated into vascular structures; increased intramuscular arteriole and capillary density perfusion index</td>
<td>(90)</td>
</tr>
<tr>
<td>Rabbit HLI</td>
<td>BM-MNCs</td>
<td>Intramuscular injection into ischemic thigh skeletal muscle</td>
<td>4 wk</td>
<td>More detectable collateral vessels, higher capillary density, greater laser-Doppler blood perfusion</td>
<td>(88)</td>
</tr>
<tr>
<td>Mouse HLI</td>
<td>BM-MNCs or PB-MNCs</td>
<td>Intramuscular injection into ischemic skeletal muscles</td>
<td>4 wk</td>
<td>Increased blood flow ratio and increased capillary density</td>
<td>(119)</td>
</tr>
<tr>
<td>Mouse HLI</td>
<td>MSC</td>
<td>Intramuscular injection into adductor muscle at 24, 48, and 72 h post-femoral ligation</td>
<td>4 wk</td>
<td>Improve limb function, reduce autoamputation, and attenuate muscle atrophy and fibrosis</td>
<td>(45)</td>
</tr>
<tr>
<td>Rat HLI</td>
<td>MSC</td>
<td>Intramuscular injection into ischemic thigh muscle</td>
<td>3 wk</td>
<td>Higher laser-Doppler perfusion index, improvement in blood perfusion, and higher capillary density</td>
<td>(40)</td>
</tr>
<tr>
<td>Mouse HLI</td>
<td>ESC-ECs</td>
<td>Intramuscular injection into ischemic limbs</td>
<td>4 wk</td>
<td>Increased limb salvage; increased blood perfusion; increased capillary and arteriole densities</td>
<td>(13)</td>
</tr>
<tr>
<td>Mouse HLI</td>
<td>ESC-ECs and ESC-SMC</td>
<td>Intrafemoral artery</td>
<td>1 to 6 wk</td>
<td>Increased blood flow and capillary density. Cells incorporated into host circulating vessels</td>
<td>(117)</td>
</tr>
<tr>
<td>Mouse HLI</td>
<td>ESC, ESC-ECs</td>
<td>Intramuscular, intrafemoral artery, intramuscular vein injections</td>
<td>2 wk</td>
<td>ESC-ECs localized to the ischemic limb; engraftment of ESC-ECs into vasculature; improved limb perfusion and neovascularization</td>
<td>(37)</td>
</tr>
<tr>
<td>Mouse HLI</td>
<td>iPSC-ECs</td>
<td>Intramuscular injection at day 0 and day 7</td>
<td>2 wk</td>
<td>Increased blood flow and capillaries</td>
<td>(83)</td>
</tr>
</tbody>
</table>

ECs, endothelial cells; HLI, hindlimb ischemia; BM, bone marrow; PB, peripheral blood; MNCs, mononuclear cells.
the same group also provided a full spectrum of cytokine genes expressed by MSCs to examine the paracrine mechanisms. Importantly, they demonstrated that injection of cells was not required for therapeutic benefits. Instead, treatment with conditioned media from MSCs was sufficient to mediate arteriogenesis and enhance collateral flow, which provides supportive evidence of the paracrine effects of MSCs. Iwase et al. (40) reported in a rat model that delivery of adherent spindle-shaped MSCs from bone marrow produced significantly improved blood perfusion, compared with Ficoll gradient isolated BM-MNCs. The authors also compared the angiogenic potency of MSCs and BM-MNCs under an ischemic environment in vivo. MSCs appeared to have more therapeutic effect than MNCs and also differentiate into ECs and vascular smooth muscle cells by detecting vWF and smooth muscle α-actin, respectively. More recently, Han et al. (25) reported that hypoxia-preconditioned human umbilical cord-derived CD44+ CD105+ MSCs were able to induce angiogenesis and low-inflammatory immune response in immunodeficient mice with HLI, suggesting that hypoxia preconditioning may be able to boost the therapeutic effects of MSCs.

Treatment of PAD using PSC-derived ECs. ECs differentiated from human ESCs (ESC-ECs) and iPSCs (iPSC-ECs) have also been tested in HLI models. Cho et al. (13) tested the intramuscular injection of human ESC-ECs in a mouse HLI model. These cells were characteristic of primary ECs in the gene and protein expression of CD31, VE-cadherin, Tie-2, Flk-1, and vWF. When the cells were delivered to the ischemic limb, the animals demonstrated improved limb salvage and blood perfusion, along with increased capillary and arteriolar densities. Since mature vessels are composed not only of ECs but also smooth muscle cells, Yamahara et al. (117) transplanted both VE-cadherin+ ESC-ECs with smooth muscle α-actin+ ESC-derived smooth muscle cells together into the ischemic limb. When delivered together, there were reportedly synergistic effects in neovascularization and blood perfusion, compared with when only one cell type was delivered. To track the kinetics of cell survival, Huang et al. (37) used bioluminescence imaging to track VE-cadherin+ murine ESC-ECs delivered to the ischemic hindlimb by intramuscular, intrafemoral artery, or intrafemoral vein injection. The results showed that ESC-ECs delivered by all three modalities localized in ischemic limb, but the systemically delivered cells resulted in greater improvement in limb perfusion and neovascularization, compared with intramuscular delivery. Rufaiyah et al. (83) examined the therapeutic efficacy of CD31+ human iPSC-ECs in mouse HLI model. When compared with that in the saline or control fibroblast groups, intramuscular injection of human iPSC-ECs into severe combined immunodeficient mice demonstrated a significant increase in capillary density and blood perfusion for at least 28 days. These data further support the feasibility of using human iPSC-ECs in developing novel cell therapies for patients with PAD.

Myocardial Infarction

Another ischemic CVD is MI, which affects more than seven million people in the United States alone (64, 81). MI results from obstruction of the coronary arteries, leading to prolonged ischemia and death of cardiomyocytes. The massive loss of cardiomyocytes, vascular cells, and interstitial cells (in the order of 1 billion) contributes to the frequent hospitalizations and premature death of patients undergoing traditional therapies. It is widely agreed that the human myocardium has low regenerative capacity that associates with inadequate compensation for severe loss of heart muscle (44). Therefore, regenerative approaches to restore myocardium are in urgent need and have attracted a large amount of preclinical and clinical testing.

Preclinical MI models are commonly generated from occlusion of the left anterior descending coronary artery, followed by reperfusion (60). MI models can be generated from small rodents to large pigs and sheep. To repair the heart, early studies transplanted fetal, neonatal, and adult cardiomyocytes and showed stable grafts in the injured hearts (65, 77). However, because of their limited availability, researchers turned to stem cells as alternative sources to improve cardiac function (16, 20, 21, 66, 86, 101, 102). The main approaches to deliver the cells include transendocardial injection into the myocardium (58, 59), transendocardial injection using percutaneous catheters (27, 29), and intracoronary infusion using angioplasty balloons (5, 55).

Treatment of MI using MNCs. Adult stem cells have been extensively tested in the preclinical setting (Table 3). For basic research and clinical trials, the major source of adult stem cells is the bone marrow. In small and large animal models, BM-MNCs have been shown to be able to reduce infarct size and improve left ventricular function and perfusion, along with modest improvements in physiological and anatomical parameters in human patients such as peak systolic displacement, peak systolic strain, as well as left ventricular ejection fraction (LVEF) (82). Orlic et al. (68) provided strong evidence that lineage-negative (Lin−) c-kit+ bone marrow cells were able to generate de novo myocardium, occupying 68% of the infarcted portion of the ventricle 9 days after local transplantation in mice. New endothelial and smooth muscle cells were also identified in coronary vessels. Kamihata et al. (43) pioneered the transplantation of CD34+ BM-MNCs into the ischemic myocardium in swine. Regional blood flow, capillary densities were significantly increased, with improved cardiac function. The data also revealed that BM-MNCs were incorporated into more than 30% of neocapillaries and 8% of macrophages. Angiogenic ligands and cytokines, such as cardiac interleukin-1β and tumor necrosis factor-α, were identified and believed to also play a role in angiogenesis.

Treatment of MI using MSCs. As for MSCs, a number of rodent and swine models have been used to prove the ability of MSCs to engraft and differentiate in the heart. Shake et al. (87) injected autologous porcine Di-I-labeled MSCs expanded from bone marrow aspirates of swine into post-MI swine myocardium and observed successful engraftment of these cells in the scarred myocardium, as well as the expression of cardiomyocyte markers such as α-actin, tropomyosin, troponin T, myosin heavy chain, and phospholamban at 2 wk postinjection. Mangi et al. (56) genetically engineered rat MSCs by overexpressing the prosurvival gene Akt1. They found that transplantation of these cells into the ischemic rat myocardium repaired infarcted myocardium by restoring fourfold greater myocardial volume than cells transduced with reporter gene lacZ. Miyahara et al. (61) transplanted a cell sheet composed of CD29+ CD90+ MSCs onto the infarcted rat epicardium and reported that the
engrafted sheet augmented neovascularization as a result of paracrine signaling, while also reversing wall thinning and improving cardiac function.

Treatment of MI using PSC-derived ECs. For decades, ESCs have held great promise for myocardial cell therapy, despite the fact that they have raised ethical and political debates for clinical application. Li et al. (50) showed that syngeneic VE-cadherin⁺ ESC-ECs injected into the peri-infarct zone of mouse hearts reported a higher density of capillaries in the infarcted area, along with functional improvement in fractional shortening. A number of other preclinical studies also demonstrate that ESC transplantation had beneficial effects in cardiac structure and function (32, 57, 116). Recently, Chong et al. (14) successfully performed transepicardial intramuscular injection in primates using human ESC-derived cardiomyocytes from H7 or RUES2 human ESC lines. Although a preliminary study, it showed not only a large scale remuscularization but also electromechanical integration with the host tissue.

The creation of human iPSCs successfully obviates the ethical problems associated with ESC research. Gu and colleagues (24) generated iPSC-ECs from porcine iPSCs that were then transplanted into a mouse model of MI. This transplantation of CD31⁺ iPSC-ECs significantly improved fractional shortening by up to 5% and LVEF by 3.5%. A more fundamental finding of this study was achieved by using quantitative protein assays and single-cell PCR. The authors identified multiple proangiogenic and antiapoptotic factors released from iPSC-ECs that were responsible for the neovascularization and the survival of cardiomyocytes in the ischemic region. Recently, large animal models of MI were also used to test similar transplantation procedures. Templin et al. (96) evaluated the transplantation of human iPSCs (96) using in vivo molecular imaging technique to track these transgenic human iPSC lines for up to 15 wk. Neovascularization was reported in the animals with iPSC-ECs. However, one common uncertainty from the above studies is that the authors were not able to determine if the improvements in cardiac function were the result of the differentiation of stem cells into cardiomyocytes or a result of paracrine factors, such as interleukin 6 and IGF-1, secreted by the transplanted cells.

Together, these data demonstrate the feasibility and efficacy of delivering stem and progenitors to improve the function of the ischemic myocardium or limb in preclinical animal models. A number of limitations in these studies include the lack of functional immune response associated with the use of immunodeficient animal strains or immunosuppression in cases where human cells were transplanted. Furthermore, longer-term follow-up from animal studies will be required to demonstrate long-term efficacy and safety. Nevertheless, these studies provide promising results, suggesting the potential benefits of stem cell therapy for treatment of MI and PAD.

Clinical Translation of Stem Cell Therapy

Stem cell therapy for clinical treatment of PAD. It has been two decades since the first clinical trial of stem cell therapy took place to treat patients with PAD. So far, more than 50 clinical trials have been reported to use progenitor cells for the treatment of PAD with varying severity (15). The majority of clinical trials have focused on CLI patients in small phase I or II studies. The cell types that have been selected for clinical trials have focused on CLI patients in small phase I or II studies. The cell types that have been selected for clinical trials include BM-MNCs, PB-MNCs, marker-specific subsets of cells derived from bone marrow or blood, as well as MSCs. To date there is still no Food and Drug Administration-approved stem cell therapies for patients with PAD. However, we have learned a tremendous amount of knowledge about the mechanisms of angiogenesis and vascular regeneration using cell therapy. Since stem cell clinical trials in PAD patients have been described elsewhere (17, 76), below we will briefly describe several of such clinical trials.

Therapeutic angiogenesis using cell transplantation (TACT), a randomized controlled trial, was the first-reported autologous
stem cell therapy clinical trial using BM-MNCs and PB-MNCs (94). At 6-mo follow-up, patients treated with BM-MNCs showed improvement in rest pain, transcutaneous oxygen pressure, ankle brachial index (ABI), and pain-free walking distance. Similarly, Van Tongeren et al. (103) have tested delivery of CD34+ BM-MNCs in a small group of patients and revealed significant improvements in ABI, pain score, and pain-free walking distance after 12 mo. In a multicenter, double-blind, randomized start clinical trial, Intraarterial Progenitor Cell Transplantation of Bone Marrow Mononuclear Cells for Induction of Neovascularization in Patients With Peripheral Arterial Occlusive Disease (PROVASA), patients that received intraarterial delivery of BM-MNCs showed improved ulcer healing and rest pain, but no significant change in ABI or limb salvage (105). In another multicenter, randomized, double-blind trial, bone marrow aspirate concentrate (BMAC) clinical trial, improvement in amputation, pain, and quality of life were reported at 3-mo and 6-mo follow-ups (39). Together, these studies suggested that intramuscular administration of BM-MNCs results in improved ABI and lower amputation rates. In addition to BM-MNCs, PB-MNCs were also tested in a number of clinical trials through intramuscular administration in patients with PAD. In a pilot clinical trial, Huang et al. (38) reported that administration of 10^6 PB-MNC with 0.4% CD34+ cells were able to improve ABI, ulcer healing, blood perfusion, angiographic scores, and limb salvage at 3-mo follow-up (38). Similar improvements in ABIs, pain scores, and amputation rates were reported in other phase I clinical trials at 3-mo follow-up (62, 71).

MSCs were also used in multiple clinical trials due to their multipotent potential and immunosuppressive effects. Significant improvement in pain-free walking time was reported in a double-blind, randomized controlled trial comparing BM-MSCs and BM-MNCs after a 6-mo follow-up (54). Not only did treatment by BM-MSCs result in greater collateral blood vessel formation, but it also showed faster ulcer healing than the group treated with BM-MNCs. In addition to the above cell types, other cells such as allogenic MSCs, adipose-derived regenerative cells, ESCs/iPSCs, or nuclear reprogrammed stem cells are being currently explored in preclinical models and will lead to clinical trials if they show advantages to other adult stem cells.

**Stem cell therapy for clinical treatment of MI.** The treatment of myocardial injury has been increasingly strategized using stem cell approaches. Over 100 clinical trials involving stem cell therapy for the treatment of MI have been reported (33). Depending on the stage of injury, stem cell therapies have sought to prevent the progression of acute MI toward ischemic cardiomyopathy and congestive heart failure, or restore function in failing or chronically ischemic hearts. Since stem cell clinical trials in MI patients have been described elsewhere (95), below we will briefly describe several selected clinical trials.

The results of recent United States clinical trials performed by the Cardiovascular Cell Therapy Research Network indicate the lack of therapeutic efficacy of BM-MNCs for treatment of acute MI (100). Briefly, 120 patients were randomly assigned to either BM-MNC therapy or placebo at different times of introduction (i.e., 3 or 7 days after percutaneous coronary intervention). After 6 mo, MRI-based evaluation of LVEF revealed that those treated with BM-MNCs did not show significant improvement compared with the placebo group. Thus, because of the lack of promise of BM-MNCs from several clinical trials (72, 84, 99, 112), a shift in focus has been made toward other stem cell sources in the past several years.

MSCs have also been tested in clinical trials, owing to their potentially immunomodulatory property. In the Prevention of Contrast Renal Injury with Different Hydration Strategies (PO-SEIDON) trial, autologous and allogeneic human MSCs were injected in patients with left ventricular dysfunction due to ischemic cardiomyopathy. The MSCs were administered in three different doses and monitored over 1 year to assess the efficacy and safety of autologous MSC therapy based on various output measures, including a 6-min walk test, exercise peak, oxygen consumption, and LVEF. The conclusion of these trials was that MSC injection favorably affected patient functional capacity and ventricular remodeling, regardless of being allogeneic or autologous (26).

Another highly investigated cell source for the treatment of MI are CD34+ EPCs. In a randomized, double-blind, phase-II study, 167 patients with refractory angina were injected with autologous CD34+ cells versus a placebo (53). The cells were targeted to 10 sites of ischemic, viable myocardium, and the primary outcome measure was weekly angina frequency 6 mo following treatment. The results of this clinical trial demonstrated that CD34+ cell administration contributed to a reduction of angina frequency, with an improvement in exercise tolerance. Together, these clinical studies suggest that some adult stem cells may be promising candidates for treating the ischemic myocardium.

**Approaches to Enhance Stem Cell Therapeutic Potential**

Paracrine factors play an important role in improving tissue repair and functional recovery after stem cell injection. Therefore, a number of groups have developed approaches to enhance cell survival and function, along with paracrine factor delivery. Gnecchi et al. (22) showed that conditional media from hypoxic Akt-expressing MSCs significantly inhibited hypoxia-induced apoptosis and triggered vigorous contraction in adult rat cardiomyocytes in vitro. When injected into infarcted heart, the conditioned media limited the infarct size and improved ventricular function. Further characterization of the conditioned media revealed that a number of genes encoding paracrine factors were upregulated, including VEGF, FGF-2, hepatocyte growth factor, insulin-like growth factor 1 (IGF-1), and thymosin β4. Another group developed a prosurvival cocktail to boost the survival of myocardial grafts in the infarct rat heart (48). This cocktail was composed of Matrigel, a cell permeant peptide from B-cell lymphoma-extra large, cyclosporine A, a compound that opens ATP-dependent K channels, IGF-1 and ZVAD-fmk. More recently, IGF-1 and hepatocyte growth factor have shown to be able to improve LVEF and cell engraftment rate when delivered along with Sca-1+/CD31− cells in transplantation therapy (107). These studies highlight the modification of genes or extracellular microenvironment to modulate stem cell function and survival.

**Conclusion and Future Perspectives**

Stem cell therapy holds great promise to restore damaged heart and vessels. Researchers have made significant advancement in cell transplantation in preclinical and clinical settings.
However, many problems still need to be resolved. Stem cell therapy for the treatment of ischemia tissues will require robust approaches for cell characterization, uniform isolation and/or maintenance, and optimal delivery strategies. Especially in cases where multiple cell types will be replaced and restored, long-term functional integration remains a challenge for effective clinical improvement. To address this limitation, bioengineering approaches to induce ordered multicellular architecture in vascular networks may be a key factor to enhance multicellular tissue integration and function (6). Furthermore, survival of transplanted cells or grafted tissue is still a challenge, considering the immunoreactive, ischemic, and necrotic host environment. Advancements in imaging capabilities will enable the ability to better track the location and survival of stem cells. Refining the delivery modality, dose, and phenotypic identify of the cells will also be critical to further enhance the functional effect of cell therapy. In the case of PSCs, concerns of teratoma formation will need to be mitigated by the use of highly purified, terminally differentiated, stem cell derivatives. In summary, the knowledge gained from past preclinical and clinical trials provides a wealth of information for further refinement of stem cell therapy for treating CVDs. Although future studies are needed to identify the optimal stem cell treatment for ischemic cardiovascular tissues, the hope of curative stem cell therapy remains bright.

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AUTHOR CONTRIBUTIONS


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