Chapter 7

The Proangiogenic Potential of Mesenchymal Stem Cells and Their Therapeutic Applications

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Abstract

Mesenchymal stem cells (MSCs) can be isolated from many tissue types and following *in vitro* culture expansion, large numbers of patient-specific or allogenic cells can be produced for clinical applications. MSCs exhibit anti-inflammatory and immunomodulatory properties and are identified as lacking major histocompatibility complex (MHC) class II molecules. Cellular-based approaches using MSCs to enhance new blood vessel formation have shown promise in preclinical models and preliminary clinical trials. Transplantation of MSCs *in vivo* has significantly enhanced the formation of new blood vessels and promoted the healing of chronic wounds. The proangiogenic potential of MSCs can be further enhanced through gene delivery such as vascular endothelial growth factor (VEGF) or endothelial nitric oxide synthase (eNOS) providing long-term therapeutic expression. In this chapter, we review recent advances on the isolation and characterization of MSCs and *in vivo* applications for promoting angiogenesis. Enhancement of angiogenesis is also required for improved healing in myocardial infarction and cerebral ischemia, and the use of MSCs in these areas will also be reviewed. Furthermore, the combination of MSCs with biomaterials has greatly improved their survival and potency with improved vascularization of tissue-engineered constructs and integration within the host. In summary, this chapter provides an overview of both the basic science supporting the proangiogenic properties of MSCs and their translational use.

Keywords: mesenchymal stem cell, angiogenesis, clinical trials, myocardial infarction, wound healing

1. Stem cells

Stem cells can be broadly described as a group of undifferentiated cells capable of self-renewal (cell division without differentiation) and can subsequently differentiate into specialized cell

types [1, 2]. Stem cell division can be described as symmetric and asymmetric [1]. Symmetric cell division yields two daughter cells showing the same characteristics of the parent stem cell and has the potential to differentiate into other lineage cell types [1]. On the other hand, asymmetric cell division yields differentiated cells through the development of lineage-specific intermediate progenitor cells [3]. Progenitor cells are generated as an intermediate state before the stem cell is converted into the fully differentiated cell type [2] and are regarded as being committed to differentiating along a particular cellular developmental pathway. There are two types of resident stem cells, which are categorized as embryonic stem cells (ESCs) or somatic/adult stem cells. ESCs are referred to as pluripotent, an ability to differentiate into all the cell types in the body, whereas adult stem cells are multipotent and demonstrate a restricted ability to differentiate into multiple lineages.

1.1. Embryonic stem cells

Embryonic stem cells (ESC) are a class of unspecialized cells derived from the inner cell mass of a blastocyst, which is an early stage of the embryo containing 200–250 cells [4, 5]. ESCs are pluripotent stem cells, which can differentiate into any cell type represented within three germ layers (mesoderm, ectoderm, and endoderm) [6]. In response to various stimuli during development, ESCs can be differentiated into specialized cells, which have specific roles in the body [7, 8]. There are two key features, which characterize ESCs, pluripotency (the ability to differentiate into all three germ layers, ectoderm, endoderm, and mesoderm) and self-renewal (the ability to go through numerous cycles of cell division while maintaining the undifferentiated state) whereby they are maintained as pure populations of undifferentiated cells in culture for extended periods of time, retaining a normal karyotype unlike tumor cell lines [6]. Over the past two decades, ESCs have been used as a model system for studying the basic processes in mammalian development and cellular differentiation events [9]. ESCs have also provided a valuable platform for regenerative medicine and tissue engineering for the development of future treatments of human diseases. Furthermore, ESCs have been also used as a reference *in vitro* model for understanding key molecular mechanisms, which control cell fate and organogenesis [10].

2. Induced pluripotent stem cells (iPS cells)

To find an alternative pluripotent cell type to ESCs, in 2006, the Japanese scientists Shinya Yamanaka and Kazutoshi Takahashi demonstrated the groundbreaking discovery of induced pluripotent stem cells (iPSCs). iPS cells are artificially created embryonic-like stem cells generated by over expressing four transcription factors in somatic cells such as fibroblasts [11]. These iPS cells exhibited similar features to ESCs. Since iPS cells are artificially created cells, they do not have ethical and immunological problems associated with ESCs. Therefore, iPS cells show potential in cell biological research, including their application in cell therapy, drug screening, and disease modeling.

2.1. Generation of iPS cells

Differentiated cells can be reprogrammed into a pluripotent state by the transfer of nuclear contents into oocytes [12], and the fusion of somatic cells with embryonic stem (ES) cells can

also result in reprogramming to a pluripotent state [13]. These studies revealed that oocytes and ES cells contain factors, which may be responsible for the conversion of somatic cells to a pluripotency state. In 2006, Yamanaka and Takahashi demonstrated that, mouse embryonic or adult fibroblasts can be reprogrammed back to an embryonic-like state by the overexpression of four transcription factors, OCT4, SOX2, KLF4, and cMYC [10, 11]. They named these ES-like reprogrammed cells as induced pluripotent stem cells (iPSCs). In 2007, the same investigators demonstrated the generation of iPSCs from human fibroblasts [14]. Yu and colleagues have also reported the generation of human iPSCs from fibroblasts with a slightly different combination of transcription factors, in which KLF4 and cMYC were replaced with NANOG and LIN28 [15]. Both of these iPS cells exhibited similar features to ES cells including morphology, proliferation, ESC-specific gene expression profiles, and teratoma formation. This method of cellular reprogramming has been shown to be universal and can be applied to a variety of cell types such as B-cells [16], liver cells [17], and umbilical cord blood mononuclear cells [18]. Moreover, iPS cells have been generated from different species such as monkey [19], rat [20], and horse [21].

2.2. Limitations of iPS cells in clinical applications

Even though iPS cells have provided a solution for many of the obstacles raised with ESCs, iPS cells also have inherent disadvantages in terms of clinical applications, which include teratoma formation [22] and the use of oncogene cMYC as a reprogramming factor, which can lead to tumorigenesis [23].

A second issue associated with the therapeutic application of iPS cells is their immunogenicity. Transplanted iPS cells have been considered to be immune tolerant by the recipient. However, induction of T-cell-dependent immune response in recipients has been demonstrated [24].

3. Adult stem cells

Adult stem cells or somatic stem cells are multipotent stem cells, which can be found in specific cellular niches of organs and tissues. Adult stem cells are essential for maintaining the health of organs throughout a life time [25]. Somatic stem cells were first identified about 40 years ago with the discovery of hematopoietic stem cells and bone marrow stem cells (mesenchymal stem cells) [26]. Adult stem cells can be found in many tissues such as brain [27], liver [28], heart [29], lung [30], and adipose [31]. Adult stem cells are multipotent; they can self-renew and differentiate to all the cell types in their tissue environment and as well as other lineages such as cardiomyocytes [32], neurons [33], and endothelial cells [34].

The use of adult stem cells in cell therapy applications is currently limited due to several factors:

1. Limited differentiation potential [35].
2. The results obtained in animal models may not be directly translated to humans [35].
3. Loss of proliferative capacity under standard culture conditions as well as the method for the delivery of adult stem cells to the patient may impact on their ability to survive post-transplantation [35, 36].

3.1. Mesenchymal stem cells

Mesenchymal stem cells (MSCs) or mesenchymal stromal cells were discovered by Friedenstein et al. in 1968 [26]. He observed many different types of cells in bone marrow cultures some of which were adherent to tissue culture plastic, showed fibroblastic morphology and formed colonies. These cells were named as colony forming unit fibroblasts (CFU-F) [37] and were found to differentiate into bone, adipose, cartilage, and muscle tissue. Caplan coined the term "mesenchymal stem cells" (MSCs) [38] and MSCs obtained from human bone marrow aspirates were characterized [39]. The BM aspirate was first separated by density gradient separation and plated on tissue culture plastic and the attached cells were counted based on their colony formation ability. Approximately, 0.001–0.01% cells of total cells were found to be MSCs and expressed CD29, CD90, CD71, and CD106 surface markers and were negative for CD45, CD14, and CD34. Importantly, they found that these cells could undergo 40 population doublings *in vitro* over 10 weeks.

MSCs have now been isolated from many other tissue sources such as adipose tissue, umbilical cord blood, placenta, and even from dental pulp. Increasing research interest is in finding stem cells from different organs and focusing on strategies to repair the same organs with autologous stem cells. Interestingly, cell isolated from a variety of different tissues have shown different CD marker expression profile, cellular phenotype, and population doublings. While no definitive single surface marker for MSCs had been described so far, an internationally accepted set of criteria has been established by the International Society for Cellular Therapy (ISCT) [40]. Accordingly, ISCT outlined that MSCs should be positive for CD73, CD90, and CD105, negative for CD19, CD34, CD45, CD11b, and HLA-DR. In addition, they should attach to the plastic tissue culture plates and demonstrate an ability to differentiate to adipocytes, chondrocytes, and osteoblasts *in vitro*.

For cell therapy applications, MSCs are remarkable since they show antiapoptotic and immunomodulatory features providing them with nonimmunogenic properties. MSCs release a variety of cytokines. Therefore, when MSCs were injected to the damaged tissues (kidney) in animal models, they could reduce the apoptotic rate of the surrounding cells, which was mediated by the secretion of several growth factors like VEGF, FGF2, and TGF-β from MSCs cultured in hypoxic conditions [41–43]. More specifically, these studies have shown that infusion of MSCs is important for revascularization, which contributes to the recovery from acute kidney injury in mouse models through the secretion of growth factors. Furthermore, proliferation of T-cells was inhibited when co-cultured with MSCs *in vitro* [44–46]. In addition to T-cells, the activity of other immune responsive cells such as natural killer cells, B-cells, and immature dendritic cells have also been modulated by MSCs [47–49]. In particular, MSCs can inhibit B-cell proliferation by inhibiting the G0/G1 phase through the release of paracrine factors that affect B-cell differentiation and IgM, IgG, and IgA production. The immunomodulatory effects of MSCs on T-cells and NK cells have also been shown to be driven by cytokines such as TGF-β, PGE2, and IL10 [49–53].

Thus, MSCs are important candidates for cellular-based therapies as they feature the following characteristics.

1. A repertoire of defined surface markers and an ability to produce relatively homogenous cultures.
2. Ease of *in vitro* expansion resulting in high cell concentration without significant loss of properties.

3. Ability to differentiate into a variety of different cell types.
4. Possess immunosuppressive attributes, which contribute to their possible use in allogeneic grafting [47–49].

3.2. Clinical trials using mesenchymal stem cells

In the literature, there are many studies investigating the regenerative capacities of MSCs in different disease models generated by employing different nonhuman animal species. Cardiac regeneration, liver regeneration, kidney regeneration, autoimmune diseases, graft versus host disease (GvHD), neurological diseases, pulmonary diseases, osteogenic diseases, and cartilage repair are the most widely studied conditions. Moreover, MSCs are also being investigated extensively by clinical trials, mostly in United States, Europe, and East Asia, with trials investigating MSC use in neurological, liver, bone, heart diseases, GvHD, and some autoimmune diseases such as diabetes. In the following section, the clinical application of MSCs will be discussed and a particular attention will be given to their role in heart disease.

4. Mesenchymal stem cells in vascular repair

The formation of new vessels is the cornerstone of successful cardiac repair. There are three mechanisms of postnatal neovascularization: (1) angiogenesis, (2) arteriogenesis, and (3) postnatal vasculogenesis [54] with progenitor cells migrating from the bone marrow and to site of sites of tissue damage resulting in the generation of new capillaries. Whether the formation of new capillary networks and vessel integration into neighboring tissue is associated with direct differentiation of MSCs to endothelial cells is still unknown or the importance of secreted factors [55, 56]. MSCs have been shown to exist in perivascular niches with similarities to pericytes, which may account for their ability to promote vascularization [57]. Expression of MSC markers has also been detected on the surface perivascular cells without *in vitro* culture, which may point to a very localized depot of progenitors in vessels [58]. *In vitro*, MSCs express α-smooth muscle actin and β-actin filaments [59], whereas *in vivo* studies have shown that MSCs express an endothelial phenotype that can enhance microvascular density [60]. However, contrary evidence has shown that the number of vessels harboring progenitor/adult stem cells is low and that the secretion of proangiogenic factors may be the dominant mechanism associated with vasculogenesis [61] and neoangiogenesis [62]. Interesting work by Chen and colleagues have shown significant increases in the levels of VEGF and basic fibroblast growth factor (bFGF) in MSC-treated rats, which resulted in angiogenesis following intravenous injection 24 h after middle cerebral artery occlusion (MCAO). They further showed significant increases in newly formed capillaries at the boundary of the ischemic lesion in rats treated with MSCs compared with rats treated with phosphate buffered saline (PBS) [62]. Further evidence of MSC-supported neovessel formation, comes from Markel and colleagues [63] who showed that MSCs under-expressing VEGF have significantly less cardio reparative capabilities. In this work, female adult rats were subjected to ischemia-reperfusion injury and following injury, VEGF knockout MSCs or normal MSCs were infused into the coronary circulation. Following MSC treatment, it was observed that VEGF knockout MSCs

significantly impaired myocardial function while normal MSCs showed improvement highlighting the importance of VEGF as a paracrine factor associated with MSCs.

4.1. Neovessel formation

The process of neovessel formation is an important event during embryonic development and also in adult tissues following injury such as ischemic infarction. Neovessels from the neighboring normal tissues are needed to form the vessel network and restore blood supply to the damaged tissues. Both ECs and SMCs are essential for the formation of blood vessels; however, the detailed mechanism of SMC migration and differentiation is not fully understood.

Until recently, it was accepted that vessels in adult ischemic tissues could only grow by angiogenic mechanisms, in which the sprouting of mature ECs from pre-existing vessels was likely in response to angiogenic factors. However, recent studies have revealed that endothelial progenitor cells (EPCs) circulate postnatally in peripheral blood. These may be recruited from the bone marrow and incorporate into sites of active neovascularization in ischemic hind limbs, ischemic myocardium, injured corneas, and tumor vasculature [64]. This process is termed postnatal vasculogenesis [65]. EPCs participate in vasculogenesis by the differentiation into endothelial cells (ECs) and thereby promote angiogenesis through the production of angiogenic growth factors [66]. Accumulating evidence has shown that EPCs have a therapeutic potential for vascular repair through promoting the reendothelialization of damaged vessel walls and the neovascularization of ischemic tissues [67, 68].

Bone marrow–derived mesenchymal stem cells (BMSCs) and alternatively named multipotent adult progenitor cells (MAPCs) can be induced to differentiate into endothelial-like cells *in vitro* and subsequently promote neoangiogenesis *in vivo* [69, 70]. The bioactivity of secreted molecules from BMSCs has been shown to increase collateral remodeling and perfusion in ischemic tissues in animal models, again highlighting the importance of paracrine mechanisms following local delivery [71, 72].

Recently, it has been shown that adult BMSCs, under appropriate *in vitro* environmental cues, can be induced to undergo vasculogenic differentiation culminating in microvessel morphogenesis. When rat BMSCs were seeded onto a three-dimensional (3D) tubular scaffold, the maturation and co-differentiation into endothelial and SMC lineages, which led to successful microvessel formation was observed [73]. A separate study showed that locally delivered, activated cardiac progenitor cells (CPCs) could generate coronary vasculature by dividing and differentiating into both ECs and SMCs, restoring blood supply to ischemic myocardium [74].

4.2. Mesenchymal stem cells in cardiac repair

Ischemic heart disease is associated with the highest mortality rate among all diseases (http://www.who.int). There is an urgent need for alternative cell-based therapies to treat cardiovascular diseases. Broadly, ischemic heart diseases are characterized by a shortage of blood supply to different regions of the heart, resulting in these regions undergoing necrosis and apoptosis. With a limited endogenous regeneration available to the mammalian heart, heart transplantation is often the only therapeutic option currently available.

Cell therapy to regenerate damaged cardiac tissue is an exciting alternative to heart transplantation. In 1995, Wakitani et al. reported the generation of cardiomyocytes *in vitro* from rat bone marrow-derived MSCs (rBMSCs) [75]. Following this, several studies reported the successful differentiation of MSCs into cardiomyocytes [76, 77]. Both of these studies demonstrated the *in vitro* generation of beating cardiomyocytes from rat bone marrow MSCs.

Many *in vivo* studies have since been performed to investigate the efficacy of MSCs in cardiovascular regeneration. In 2002, Shake et al. demonstrated that swine bone marrow–derived MSCs could be differentiated into functional cardiomyocytes when injected into the infarcted swine myocardium [78]. On the other hand, when MSCs were injected intracardially in a canine model, the MSCs were differentiated into smooth muscle cells and endothelial cells [79] and further studies showed that, when MSCs were injected into a rat myocardial infarct, there was a significant reduction in the damaged area [80]. Moreover, genetic modification of MSCs to overexpress Akt, exerted a beneficial effect [81], suggesting that genetic modification of MSCs would provide a better platform for cardiovascular repair. It is also possible that Akt may activate mammalian target of rapamycin complex 1 (mTORC1) and forkhead box o3 (Foxo3a), which are acted downstream of Akt to promote cardiomyocyte reprogramming [82].

4.3. Direct MSC stimulation of endogenous repair

MSC transplantation to the heart has been shown by multiple groups to stimulate proliferation and differentiation of endogenous cardiac stem cells [83–85]. Neomyogenesis can be promoted by two related mechanisms through the stimulation of endogenous cardiac stem cells (c-kit + and other lineages such as cardiac fibroblasts) and enhancement of myocyte cell cycling [83]. To demonstrate this, GFP+ allogeneic MSCs were injected into infarcted swine hearts and allowed to form chimeric clusters of immature MSCs and endogenous c-kit+ cardiac stem cells. These clusters exhibited cell-cell interactions mediated by connexin-43 gap junction formation and N-cadherin mechanical connections. Importantly, the endogenous c-kit+ cell population was increased by 20-fold in MSC-treated animals relative to controls; furthermore, the c-kit+ cells showed a high capacity for myocyte lineage commitment [83]. It has been demonstrated that, when MSCs were co-cultured with rat ventricular myocytes, MSCs became actin-positive and formed gap junctions with the native myocytes [86]. Furthermore, an improvement in myocardial wall thickening in pigs with hibernating myocardium, which is a pathology when some segments of the myocardium exhibit abnormalities of contractile function, was induced upon MSC injection [85] compared with controls. This same study also found a fourfold increase in c-kit+ and CD133+ populations that co-expressed Gata4 and Nkx2.5 at 3 days through to 2 weeks in animals receiving MSCs. In a preclinical study, the combination of human MSCs and c-kit+ cardiac stem cells showed enhanced cardiac regeneration [87].

5. Preclinical trials of MSCs for cardiac repair in animal models

Toma et al. showed that human MSCs were differentiated to a cardiac fate when injected into murine hearts [88]. In this study, MSCs labeled with lacZ were injected into the left ventricle of

the adult mice, and after 1 week post injection, the lacZ-labeled MSCs morphologically resembled the surrounding host cardiomyocytes; furthermore, they expressed cardiac-specific genes such as α-actinin and cardiac troponin T. It has been shown that MSCs can modulate host immune responses when allogeneic porcine MSCs were injected (2×10^8 cells) intramyocardially into 3-day-old immune-competent porcine-infarcted hearts, this resulted in long-term engraftment and a significant decrease in scar tissue without an inflammatory response [89]. MSCs have also been tested in numerous cardiovascular settings. In a separate study, where porcine MSCs were injected endomyocardially of one of three MSC doses (2.4×10^7, 2.4×10^8, 4.4×10^8 cells) into the porcine heart 5 days after infarction, an improvement in ejection fraction (EF) and a reduction in scar formation were seen in MSC-treated animals [90]. The effect of MSC dosage was examined in ovine models of MI where different doses of ovine BM-derived MSCs (2.5×10^7, 3.75×10^7, 5×10^7 cells) were directly injected into sheep hearts 1 h post MI [91], and improvements in end-diastolic volume were only seen in animals receiving the two lower doses, although the EF increased regardless of the cell dosage [91] suggesting that there may be a therapeutic threshold relating to the total number of cells that can be injected and a beneficial therapeutic outcome. In a study with a different species (canine), chronic myocardial ischemia was produced by the implantation of an ameroid constrictor in the proximal left anterior descending coronary artery (LAD) and diagonal branch ligation, followed by the injection of allogeneic canine MSCs (1×10^8 cells) into the heart resulted in increased EF, vascular density, and a decrease in scar tissue [79]. Furthermore, it has been reported that the region specific administration of allogeneic porcine MSCs (2×10^8 cells) to the border and to infarct zones of porcine myocardium 3 days after MI also reduced scar size by 50% [89] with improvements in EF, left ventricular end-diastolic pressure, relaxation time, and systolic compliance in the treated animals. Furthermore, in a model of acute myocarditis in rats myocardial inflammation was attenuated when autologous rat MSCs (3×10^6 cells) were injected into 10-weeks-old animals [92], together with the increased capillary density in MSC-treated animals.

6. Human clinical trials of MSC-based therapies for cardiac repair

6.1. Acute myocardial infarction

In a phase I randomized study, 53 patients received different doses of allogeneic human MSCs (0.5, 1.6, and 5.0×10^6 hMSCs/kg) 7–10 days post MI [93]. The MSCs were injected intravenously. Six months after infusion, clinical data showed fewer arrhythmic events, and an improved EF. Following the success of this pilot study, a phase II trial was established to investigate whether allogeneic MSCs were as safe and effective as autologous MSCs in patients with left ventricular (LV) dysfunction due to ischemic cardiomyopathy [94]. Upon intravenous infusion of allogeneic MSCs (2×10^7 cells) within 7 days of an acute MI, resulted in reduced cardiac hypertrophy, stress-induced ventricular arrhythmia, heart failure, LV end-diastolic volumes, and increased EF. Interestingly, allogeneic MSCs did not stimulate significant donor-specific alloimmune reactions. In a separate study, Chen and colleagues have injected autologous MSCs (1×10^{11} cells) intracoronarily in patients with subacute MI and observed decreased perfusion defects, improved left ventricular ejection fraction, and left ventricular remodeling 3 months after therapy [95]. Other clinical benefits attributed to MSCs include decreased perfusion defects and improved left ventricular ejection fraction and left ventricular remodeling

when MSCs were administered to patients with subacute MI [96]. In addition to bone marrow MSCs, adipose-derived MSCs have also been used to treat acute MI. A trial with 14 patients, which tested the safety of intracoronary injection of freshly isolated adipose-derived MSCs after myocardial infarction [97] demonstrated improved cardiac function, accompanied with a significant improvement in perfusion defect and a 50% reduction in myocardial scar formation.

There are a reported 41 clinical trials in which MSCs have been applied in relation to cardiac injury and repair between 2010 and 2015 [98]. There is also an ongoing clinical trial using adipose-derived MSCs, in patients with chronic myocardial ischemia [71] where they used culture-expanded adipose tissue-derived MSCs. This study has been designed to investigate the safety and efficacy of intramyocardial delivery of VEGF-A165-stimulated autologous adipose tissue-derived MSCs to improve myocardial perfusion and exercise capacity [99]. **Table 1** summarizes completed and ongoing clinical trials.

6.2. Phase III clinical trials

There are six ongoing phase III clinical trials using MSCs. Of note, one of these studies [100] applied autologous MSCs treated *ex-vivo* with cardiogenic growth factors (TGF-β, BMP4, FGF2, cardiotrophin, and α thrombin) to enhance their commitment to the cardiopoietic lineage and investigators reported significant improvements in EF and end-systolic volume compared with controls. Other phase III studies are currently underway, in which one in United States is treating 600 patients with chronic heart failure (https://clinicaltrials.gov/ct2/show/NCT02032004) all the phase III clinical trials currently undergoing are also listed in **Table 1**.

Clinical trial ID	Disease	Phase	No. of patients/ status	MSC source	Country
NCT01076920	Chronic ischemic cardiomyopathy	I, II	10/completed	Autologous	France
NCT01219452	Idiopathic dilated cardiomyopathy	Phase I, II	Unknown	Unknown	China
NCT01392105	Acute myocardial infarction	Phase II, III	80/completed	Autologous	South Korea
NCT01394432	Acute myocardial infarction	Phase III	50/recruiting	Autologous	Russia
NCT01392625	Dilated cardiomyopathy	Phase I, II	36/active, not recruiting	Autologous and allogenic	United States
NCT01449032	Myocardial ischemia (MyStromalCell Trial)	Phase II	60/completed	Unknown	Denmark
NCT01291329	Acute myocardial infarction (AMI)	Phase II	160/completed	Autologous	China
NCT01753440	Coronary artery disease and ischemic cardiomyopathy	Phase II, III	30/unknown status	Allogenic	Greece
NCT01759212	end-stage heart failure undergoing left ventricular assist device implantation	Phase II, III	5/unknown status	Allogenic	Greece

Clinical trial ID	Disease	Phase	No. of patients/ status	MSC source	Country
NCT01739777	Cardiopathy in dilated stage, of different etiology	Phase I, II	30/completed	Allogenic	Chile
NCT01720888	Ischemic dilated cardiomyopathy	Phase II	80/active, not recruiting	Autologous	Malaysia
NCT01957826	Idiopathic dilated cardiomyopathy	Phase I, II	70/recruiting	Autologous	Spain
NCT01709279	Ischemic heart failure	Unknown	6/recruiting by invitation	Autologous	Japan
NCT01557543	Revascularization for coronary artery disease with depressed left ventricular function	Phase I	24/active, not recruiting	Autologous	United States
NCT01652209	Acute myocardial infarction	Phase III	135/recruiting	Autologous	South Korea
NCT01610440	Duchenne muscular dystrophy	Phase I, II	15/unknown status	Unknown	China
NCT01946048	Ischemic cardiomyopathy	Phase I	10/unknown status	Allogenic	China
NCT02013674	Chronic ischemic left ventricular dysfunction secondary to myocardial infarction	Phase II	30/active, not recruiting	Allogenic	United States
NCT01913886	Ischemic cardiomyopathy	Phase I, II	10/unknown status	Autologous	Brazil
NCT01781390	Myocardial infarction	Phase II	105/active, not recruiting	Allogenic	Australia, Belgium Denmark,New Zealand
NCT01770613	Myocardial infarction	Phase II	50/active, not recruiting	Allogenic	United States
NCT02398604	Hypoplastic left heart syndrome	Phase I	30/recruiting	Allogenic	United States
NCT02097641	Acute respiratory distress syndrome	Phase II	60/recruiting	Allogenic	United States
NCT02323477	Myocardial infarction	Phase I, II	79/recruiting	Allogenic	Turkey
NCT02387723	Ischemic heart disease and heart failure	Phase I	10/completed	Allogenic	Denmark
NCT02032004	Chronic heart failure due to left ventricular systolic dysfunction of either ischemic or nonischemic etiology	Phase III	600/recruiting	Allogenic	United States Canada
NCT02501811	Ischemic heart failure	Phase II	144/recruiting	Autologous	United States

Clinical trial ID	Disease	Phase	No. of patients/ status	MSC source	Country
NCT02472002	Coronary graft disease in heart transplant patients	Phase I, II	14/recruiting	Autologous	France
NCT02439541	Ischemic cardiomyopathy	Phase I, II	40/recruiting	Unknown	China
NCT02408432	Recent onset anthracycline-associated cardiomyopathy	Phase I	45/recruiting	Allogenic	United States
NCT02509156	Anthracycline-induced cardiomyopathy	Phase I	36/recruiting	Allogenic	United States
NCT02460770	A pilot study to investigate bone marrow-derived mesenchymal stem cells (MSC) administration from left ventricular assist device	Phase I	4/recruiting	Autologous	France
NCT02467387	Nonischemic heart failure	Phase II	23/active, not recruiting	Allogenic	United States
NCT02503280	Chronic ischemic left ventricular dysfunction and heart failure secondary to myocardial infarction	Phase I, II	55/Active, not recruiting	Allogenic	United States
NCT02568956	Ischemic heart disease	Phase I, II	64/active, not recruiting	Autologous	Unknown
NCT02368587	Ischemic cardiomyopathy	Phase II	160/active, not recruiting	Unknown	Unknown
NCT02462330	Chronic ischemic cardiomyopathy and left ventricular dysfunction	Phase II	90/recruiting	Autologous	France
NCT02635464	Chronic ischemic cardiomyopathy	Phase I, II	45/recruiting	Allogenic	China
NCT02504437	Ischemic heart disease	Phase I, II	200/active, not recruiting	Allogenic	Unknown

Table 1. Clinical trials of MSCs for cardiovascular repair.

7. Direct reprogramming of adult stem cells

With increasing use of MSCs in clinical trials, improving the ability of MSCs to become cells of interest has been the main focus of reprogramming. Genetic modification is one approach to convert an adult cell from one developmental lineage to another and this is

mainly achieved by overexpression of lineage-restricted transcription factors and various gene transfer methods have been used.

7.1. Gene delivery of reprogramming factors via retroviral vectors

Retroviral vectors are commonly used as gene delivery systems since they are well characterized and they have a high transduction efficiency. For gene delivery approaches, replication of defective viral vectors is used. In these vectors, coding regions for the genes necessary for additional rounds of virion replication and packaging are deleted. Viruses generated from replication-defective vectors can infect their target cells and deliver genes of interest, but avoid triggering the lytic pathway, which would result in cell lysis and death. Replication-defective retroviral vectors can usually package inserts of up to 10 kb. The major disadvantage of the retrovirus-mediated gene delivery approach is the requirement for cells to be actively dividing to allow transduction by the viral vectors. Thus, slowly dividing or nondividing cells such as neurons are difficult to transduce efficiently with retroviruses. Stable integration of retroviral DNA into the host genome provides a platform for the persistent expression of transgenes; however, this may lead to insertional mutagenesis. Proviral integration could occur within a transcriptional active region of the host genome, which could result in dysregulation of gene expression. In a landmark study by Idea et al., using a Moloney murine leukemia virus (MMLV) retrovirus-mediated gene delivery approach, demonstrated that mouse cardiac and dermal fibroblasts could be reprogrammed into cardiac muscle cells using three cardiac-specific transcription factors, Gata4, Mef2c, and Tbx5 [101]. In this expression vector, expressions of the transgenes were driven by the 5′MMLV long terminal repeat (LTR) promoter, which can be silenced by methylation [11]. This method has been used by several groups and the efficiency of reprogramming has been enhanced by using alternative transcription factors or small molecules (summarized in **Tables 2** and **3**). The reprogramming efficiency of the retrovirus-mediated gene delivery approach is partially dependent on the stoichiometry of the delivered transcription factors [102]. It was reported that a higher reprogramming efficiency than that achieved in the original GMT experiment when the stoichiometry of the transcription factors is altered [102]. In this investigation, six different polycistronic lentiviral vectors were constructed to cover all possible combinations of G, M, T with identical internal 2A sequences. Using this approach, it was shown that the splicing order of G, M, T resulted in distinct G, M, and T protein expression levels, when using a polycistronic vector that resulted in higher protein level of Mef2c with lower levels of Gata4 and Tbx5 (MGT vector), which significantly enhanced reprogramming efficiency compared to separate G, M, T transduction as evident by cardiac-specific gene expression such as cTnT. In addition, the MGT vector resulted in more than a 10-fold increase in the number of mature beating cardiomyocytes. On the other hand, addition of an extra transcription factor Hand2 has also resulted in enhanced reprogramming efficiency [103]. In addition, combinations of small molecules such as SB431542, CHIR99021, 6-bromoindirubin-3′-oxime (BIO), and lithium chloride (LiCl) to replace transcription factors have also been reported to induce cardiac reprogramming [104]. Of note, CHIR99021 is a GSK3 inhibitor, which can up-regulate canonical Wnt signaling increased cardiac reprogramming efficiency.

Species	Cell types	Reprogramming factors	Delivery method	References
Mouse	Embryonic fibroblasts	Gata4, Mef2c, Tbx5, Hand2, Nkx2.5	Lentivirus	[109]
Mouse	embryonic fibroblasts	Gata4, Mef2c, Tbx5, miR133	Retrovirus/lentivirus miRNA transfection	[110]
Mouse	Embryonic fibroblasts	Mef2c, Tbx5, Myocd	Lentivirus	[111]
Mouse	Embryonic fibroblasts	Gata4, Tbx5, Mef2c, Myocd, Srf, Mesp1, Smarcd3	Lentivirus	[112]
Mouse	Embryonic and dermal tail tip fibroblast	Gata4, Mef2c, Tbx5, Hand2, Nkx2.5, TGFB inhibitor	Lentivirus	[113]
Mouse	Embryonic cardiac and dermal tail tip fibroblasts	Oct4, Sox2, Klf4	Retrovirus	[114]
Mouse	Neonatal cardiac fibroblasts	miR1, miR133, miR208, miR499, JAK inhibitor I	Plasmid	[115]
Mouse	Postnatal cardiac and dermal tail tip Fibroblast Fibroblasts	Gata4, Mef2c, Tbx5	Retrovirus	[101]
Mouse	Adult cardiac and dermal tail tip fibroblasts	Gata4, Mef2c, Tbx5, Hand2	Retrovirus	[103]
Mouse	$Sca1^{+}$ side population CSCs	Gata4, Mef2c, Tbx5, Myocd	Lentivirus	[116]
Human	Neonatal derma, cardiac and ESC derived fibroblasts	GATA4, MEF2C, TBX5, ESSRG, MESP1	Retrovirus	[117]
Human	Adult dermal and cardiac and fibroblasts	GATA4, MEF2C, TBX5, HAND2, miR1, miR133	Retrovirus	[118]
Human	ADSCs	Gata4, Tbx5, Baf60c	Lentivirus	[32]
Human	ADSCs	GATA4, MEF2C, TBX5, ESRRG, MESP1, MYOCD, ZFPM2	Retrovirus	[119]
Human	ADSCs	Gata4, Mef2c, Tbx5	PEI method	[120]
Human	Fibroblasts	Small molecules	Supplemented with medium	[104]
Mouse	Embryonic fibroblast	MyoD transactivation domain fused Mef2c, Gata4, Tbx5, Hand2	Retrovirus	[121]
Mouse	Dermal tail tip, embryonic and cardiac fibroblasts	Akt1, Gata4, Mef2c, Tbx5, Hand2	Retrovirus	[82]
Mouse	Embryonic fibroblasts	ROCK inhibitor, TGF-β inhibitor, Gata4, Hand2, Mef2c, Tbx5	Retrovirus, and AAV	[122]

Table 2. *In vitro* cardiac reprogramming.

Species	Reprogramming factors	Vector	Delivery method	References
Mouse	Gata4, Mef2c, Tbx5	Retrovirus	Intramyocardial injection	[123]
Mouse	Gata4, Mef2c, Tbx5, Hand2	Retrovirus	Intramyocardial injection	[103]
Mouse	Gata4, Mef2c, Tbx5, Thymosin β4	Retrovirus	Intramyocardial injection	[124]
Mouse	miR1, miR133, miR208, miR499	Lentivirus	Intramyocardial injection	[125]
Mouse	Gata4, Mef2c, Tbx5	Retrovirus	Intramyocardial injection	[126]
Rat	Gata4, Mef2c, Tbx5, Vegf (121, 165, 189)	Lentivirus/adenovirus	Intramyocardial injection	[127]

Table 3. *In vivo* cardiac reprogramming.

8. Angiogenic properties of MSCs combined with biomaterials

Application of MSCs together with a biomaterial to improve vascularization of damaged tissue as in the case of myocardial infarction or to enhance wound healing is an attractive approach to maintain cell viability and localization. MSCs have been incorporated into a wide range of biomaterials including collagen-based hydrogels and cell sheet techniques. Angiogenesis is one component of successful wound healing, which includes wound closure, reducing inflammation, skin regeneration, and remodeling of the extracellular matrix (ECM). The proangiogenic properties within a wound environment include possible direct differentiation into endothelial cells and secretion of proangiogenic molecules. Murine MSCs seeded in a pullulan-collagen hydrogel enhanced healing time in a mouse excisional wound together with increased survival of transplanted cells and secretion VEGF [105]. In an alternative wound model, rats subjected to severe burns and treated with human umbilical cord MSCs showed increased healing accompanied with reduced expression of proinflammatory cytokines IL-1, IL-6, and TNF-alpha [106]. Efficient cell seeding of biomaterial constructs is important for clinical translation and it has been demonstrated that capillary-based uptake of adipose-derived stem cells into a pullulan-collagen hydrogel could be performed rapidly and these cell laden gels demonstrated increased *in vivo* wound healing and secretion of proangiogenic factors. Comparison of the *in vitro* angiogenic capabilities of a range of adult stem cells has shown that bone marrow–derived MSCs were superior to adipose-derived MSCs in terms of tubule formation and VEGF secretion and interestingly placental chorionic villi-derived MSCs also showed promise. The ability of MSCs to show *in vitro* endothelial-like characteristics is strongly dependent on culture conditions and underlying substrate, and the majority of studies only show endothelial-like trans-differentiation in the presence of low serum endothelial media and the use of a matrigel-based extracellular matrix [107]. While nitric oxide has been shown to be an important modulator of the vasculogenic potential of MSCs [61] and nonviral ectopic expression of eNOS promotes endothelial transdifferentiation [108], eNOS, or NO does not appear to be expressed or produced in nondifferentiated MSCs. Adipose-derived MSCs engineered to express eNOS and seeded onto a decellularised human saphenous vein and implanted as an aortal bridge showed viability for up to 2 months in a rabbit model.

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9. Conclusions

In conclusion, MSCs have been well documented to have both proangiogenic and myogenic properties and a significant number of clinical trials represent the current efforts to translate this therapeutic potential. Gene modification of MSCs represents a promising strategy with both viral and nonviral vectors to reprogram cells toward endothelial and cardiac lineages and improve the capability of transplanted MSCs to promote neovessel formation and repair of damaged myocardium.

Abbreviations

CFU	colony forming unit
EPCs	endothelial progenitor cells
ESCs	embryonic stem cells
hPL	human plasma lysate
iPSC	induced pluripotent stem cells
MSCs	mesenchymal stem cells

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