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Concise Review: Optimized Strategies for Stem Cell-Based Therapy in Myocardial Repair: Clinical Translatability and Potential Limitation

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Key Words. Stem cell-based therapy • Optimized strategies • Secretomes • Exosomes • Ischemic heart diseases

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Received February 21, 2017; accepted for publication December 31, 2017; first published online in STEM CELLS *EXPRESS* January 13, 2018.

http://dx.doi.org/10.1002/stem.2778

Available online without subscription through the open access option.

ABSTRACT

Ischemic heart diseases (IHDs) remain major public health problems with high rates of morbidity and mortality worldwide. Despite significant advances, current therapeutic approaches are unable to rescue the extensive and irreversible loss of cardiomyocytes caused by severe ischemia. Over the past 16 years, stem cell-based therapy has been recognized as an innovative strategy for cardiac repair/regeneration and functional recovery after IHDs. Although substantial preclinical animal studies using a variety of stem/progenitor cells have shown promising results, there is a tremendous degree of skepticism in the clinical community as many stem cell trials do not confer any beneficial effects. How to accelerate stem cell-based therapy toward successful clinical application attracts considerate attention. However, many important issues need to be fully addressed. In this Review, we have described and compared the effects of different types of stem cells with their dose, delivery routes, and timing that have been routinely tested in recent preclinical and clinical findings. We have also discussed the potential mechanisms of action of stem cells, and explored the role and underlying regulatory components of stem cell-derived secretomes/exosomes in myocardial repair. Furthermore, we have critically reviewed the different strategies for optimizing both donor stem cells and the target cardiac microenvironments to enhance the engraftment and efficacy of stem cells, highlighting their clinical translatability and potential limitation.

STEM CELLS *2018; 00:000–000*

SIGNIFICANCE STATEMENT

Stem cell-based therapy has shown therapeutic superiority after ischemic heart diseases. To accelerate such therapy toward successful clinical application, many important issues need to be addressed. This review compared different types of stem cells with their dose, delivery routes and timing based on recent preclinical and clinical findings, discussed the underlying mechanisms of stem cells and particularly explored the importance of secretomes/exosomes in myocardial repair, and critically reviewed the different optimized strategies for stem cell-based therapy with their clinical translatability and potential limitation.

INTRODUCTION

Ischemic heart diseases (IHDs) resulting from coronary artery diseases and myocardial infarction (MI) are major public health problems with high rates of morbidity and mortality worldwide [1]. Severe MI leads to an extensive and irreversible loss of cardiomyocytes, followed by adverse left ventricular (LV) remodeling and cardiac dysfunction [2]. Current clinical approaches, including pharmacological, mechanical, and physical interventions, improve symptoms and quality of life of MI patients to a certain degree, however, they are insufficient to compensate the loss of myocardium and are still palliative, just delaying the progression of heart failure. Since the first use of bone marrow cells for the treatment of infarcted myocardium [3], the emergence of stem cell-based therapy has generated great hope for patients suffering from cardiac injury.

CELL RESOURCES FOR STEM CELL-BASED MYOCARDIAL REPAIR

Over the past decade, a wide variety of stem cells have been investigated for repair of the injured myocardium in substantial preclinical and clinical studies. The candidate stem cells can be broadly divided into two categories: pluripotent stem cells and adult stem cells.

Table 1 summarizes the recently published clinical trials involving stem cell-based therapy for acute MI (AMI) and chronic IHDs.

Cell Types

Pluripotent Stem Cells

Pluripotent stem cells (PSCs), including embryonic stem cells (ESCs) [32] and induced pluripotent stem cells (iPSCs) [33], possess robust abilities to self-renew and differentiate into cardiac lineages [34]. Specifically, ESC- and iPSC-derived cardiomyocytes (ESC-/iPSC-CMs) exhibit structural and functional properties similar to adult cardiomyocytes [34–36]. When transplanted into the infarcted myocardium, ESC-/iPSC-CMs progressively mature and generate grafted myocardium, leading to cardiac functional improvements [37, 38]. A recent study by Tachibana et al. found that transplantation of ESC-/iPSC-CMs exhibited ventricular functional improvements, accompanied by limited cell engraftment [39], suggesting that the paracrine activity of these cells may underlie the salutary effects. Accumulating evidence reveals that secretomes/exosomes derived from PSCs and their cardiac derivatives can activate endogenous stem cells, enhance neovascularization, inhibit cardiomyocyte apoptosis, and suppress fibrosis in animal models of MI [40–43] (Table 2).

The clinical application of human PSCs has been halted by several key concerns. For ESCs, ethical issue and safety concerns such as teratoma formation and immunological rejection severely limit their clinical translation. On the other hand, iPSCs constitute an autologous patient-specific resource that excludes any ethical or immunological concerns, however, their pluripotent nature may cause teratogenic risk [67]. To promote the clinical translation of PSC therapy in cardiac diseases, two research groups successively used nonhuman primate models of MI to assess the safety and feasibility of human ESC-CM or allogeneic iPSC-CM transplantation. They showed that these ESC-/iPSC-CMs remuscularized the infarcted primate myocardium to varying degrees, and displayed electromechanical coupling with the host myocardium [68, 69]. No evidence of immune rejection and tumor formation was observed, however, human ESC-CM transplantation caused nonfatal arrhythmias, which were rarely reported in rodent models [68]. The inconsistence of arrhythmic occurrence may be due to the differences in heart size and beating rate between primates and smaller animals. Larger hearts that require more cells to be delivered and slower rate may increase the incidence of arrhythmias [68]. The potential arrhythmic complication should be emphasized in clinical translation of PSC therapy.

Based on good manufacturing practice (GMP)-standard production and a serial of safety assessment, Menasche et al. reported the first clinical application of human ESC-derived cardiac progenitor patch in a patient with severe heart failure (Table 1). The patient showed symptomatical improvements after three months without any complications including arrhythmias, tumor formation, or immunosuppression-related adverse events [25, 70]. Although the safety and efficacy of these cells need to be further evaluated with more patients and larger randomized trials, these findings suggest a big step forward for the clinical application of human ESCs in cardiac diseases.

Adult Stem Cells

Bone Marrow Mononuclear Cells. Bone marrow mononuclear cells (BMCs) represent the most widely used adult stem cells (ASCs) in myocardial cell therapy because of their relative abundance in the bone marrow and easy isolation via density gradient centrifugation. BMCs are a mixed population of various stem/progenitor cells [67, 71]. To date, intracoronary autologous BMC therapy has yielded inconsistent results. Several early clinical trials such as BOOST and REPAIR-AMI indicated that BMC therapy improved LV ejection fraction (LVEF) after AMI [72, 73], whereas more recent trials did not detect sufficient benefits of BMCs in LVEF recovery [4, 8, 11, 12, 15, 16] (Table 1). Intracoronary infusion of unselected BMCs has shown extremely low retention in the hearts that may limit their clinical outcomes. A study in AMI patients found only $<3\%$ of unselected BMCs ($\approx$10-fold lower than that of $CD34^+$ cells) retained within the infarcted myocardium 1 hour after infusion [74]. Another study demonstrated that compared with transendocardial delivery, intracoronary cell transplantation resulted in lower retention rates and less functional improvements in patients with dilated cardiomyopathy [75]. Furthermore, the heterogeneity of BMCs may also contribute to the variable results of BMC therapy. According to the detailed analysis of BMCs from patients in the TIME, LateTIME, and FOCUS trials, a larger percentage of $CD34^+$ or $CD31^+$ BMCs correlated with greater improvements in LVEF or infarct size after cell therapy, respectively, [76, 77].

Mesenchymal Stem Cells. Mesenchymal stem cells (MSCs) are multipotent ASCs with capacity to differentiate into mesodermal lineages. They are initially described in the bone marrow, and subsequently identified in umbilical cord blood, adipose tissues, muscles, endometrium, etc. [78, 79]. Because of their immunoprivileged properties, MSCs of different origins can serve as "off-the-shelf" cell products for allogeneic transplantation [79]. As revealed in the recent trials (Table 1), both autologous and allogeneic, bone marrow- and non-bone marrow-derived (e.g., Wharton's jelly of the umbilical cord) MSC therapy conferred benefits in LVEF recovery or other efficacy endpoints for patients with AMI and chronic IHDs [7, 23, 24, 27, 29]. Various animal studies suggest that transplanted MSCs not only differentiate into cardiomyocytes and vascular cells, but also secrete a wide array of paracrine factors to mediate endogenous cardiac repair via activating resident stem cells, stimulating neovascularization, decreasing apoptosis, reducing inflammation, and preventing fibrosis. However, the cardiovascular differentiation potential of MSCs remains limited, and the benefits of MSC therapy may largely depend on the paracrine activity of these cells [67, 79, 80].

Cardiac Progenitor Cells. Various populations of resident cardiac stem/progenitor cells have been identified within adult mammalian myocardium by multiple cell markers, such as c-Kit, Sca-1, Isl-1, and Abcg2 [81–85]. Among them, c-Kit^+ cells and cardiosphere-derived cells (CDCs) have undergone clinical translation. c-Kit^+ cells are isolated from endomyocardial tissue biopsies by immunomagnetic selection, while CDCs are generated from cardiac tissue-developed multicellular cardiospheres, with uniform expression of CD105 and partial expression of c-Kit. Both c-Kit^+ cells and CDCs are clonogenic and

Table 1. Recently published clinical trials of stem cell-based therapy for acute myocardial infarction and chronic ischemic heart diseases

Clinical trial	Year	Cell type	Patient enrollment	Delivery route	Delivery timing	Cell dose	Myocardial structure and function			Other effects	Adverse events
							LV EF (%)	LV EDV/ESV	Infarct Size		
AMI											
BOOST-2 [4]	2017	Autologous BMCs and irradiated BMCs	STEMI, severely depressed LVEF	Intracoronary	8.1 ± 2.6 days post-PCI	7.0 ± 2.9 × 10^8	→	→	→	None	None
						20.6 ± 7.7 × 10^8	→	→	→		
						6.1 ± 2.6 × 10^8	→	→	→		
						20.8 ± 7.4 × 10^8	→	→	→		
REGENERATE-AMI [5]	2016	Autologous BMCs	STEMI, at least two contiguous anterior leads ≥ 0.2 mV	Intracoronary	Within 24 hours of PCI	59.8 × 10^6 ± 59.9	↗ (small non significant)	→	→	Improving myocardial salvage, and quality of life	Rare
ALLSTAR [6]	2016	Allogeneic CDCs	Anterior MI, LVEF ~42%, Infarct size ~25%	Intracoronary	/	12.5 × 10^6 25 × 10^6	→	↓ (EDV) ↘ (ESV)	→	None	None
Gao et al. [7]	2015	Allogeneic Wharton's jelly-derived MSCs	STEMI, LV regional wall-motion abnormality	Intracoronary	5 to 7 days post-PCI	6 × 10^6	↑	↓	/	Increasing myocardial viability and perfusion	None
TECAM [8]	2015	Autologous BMCs, G-CSF or both	STEMI	Intracoronary	3 to 5 days post-PCI	83 (60–117) ×10^6 in BMCs; 560 (351–915) ×10^6 in BMCs + G-CSF	→	→	→	Reducing infarct area	None
Huang et al. [9]	2015	Autologous BMCs	STEMI, LVEF < 50%	Intracoronary	Within 24 hours of PCI	4.9 ± 2.8 × 10^8	↑	↓	/	Improving myocardial perfusion	None
					3 to 7 days post-PCI		↑	↓	/		
					7 to 30 days post-PCI		→	→	/	None	
CHINA-AMI [10]	2015	Autologous N-BMCs and HP-BMCs	STEMI, WMSI > 1	Intracoronary	6 days post-PCI	100 × 10^6	→	↓ (HP-BMCs)	/	Improving myocardial perfusion and WMSI	None
SWISS-AMI [11, 12]	2013	Autologous BMCs	STEMI, LVEF < 45%	Intracoronary	5 to 7 days post-PCI	153 (119) ×10^6	→	→	→	None	None
					3 to 4 weeks post-PCI		→	→	→		
CADUCEUS [13, 14]	2012	Autologous CDCs	2–4 weeks post-MI, LVEF 25%–45%	Intracoronary	1.5 to 3 months post-MI	12.5 × 10^6 17.3 × 10^6 25 × 10^6	→	→	↓	Increasing viable heart mass, regional contractility and regional systolic wall thickening	None

Table 1. *Continued*

Clinical trial	Year	Cell type	Patient enrollment	Delivery route	Delivery timing	Cell dose	Myocardial structure and function			Other effects	Adverse events
							LV EF (%)	LV EDV/ESV	Infarct Size		
TIME [15]	2012	Autologous BMCs	STEMI, LVEF $\leq$ 45%	Intracoronary	3 or 7 days post-PCI	150 $\times$ 10^6	→	→	→	None	Rare
LateTIME [16]	2011	Autologous BMCs	AMI, LVEF $\leq$ 45%	Intracoronary	2 to 3 weeks post-PCI	150 $\times$ 10^6	→	→	→	No significant differences in wall motion	None
Chronic ischemic heart diseases											
TRIDENT [17]	2017	Allogeneic bone marrow derived-MSCs	ICM, LVEF $\leq$ 50%	Transendocardial	/	20 $\times$ 10^6	→	→	↓	Improving functional status and quality of life	Only one experienced hematoma
						100 $\times$ 10^6	↑	→	↓		None
Miyagawa et al. [18]	2017	Autologous skeletal muscle stem cell sheets	ICM and DCM, NYHA class II-III, LVEF < 35%	Epicardial attachment	More than 4 months post-PCI or CABG in ICM	Cell sheets of 3–9 $\times$ 10^8	↑ (ICM)	↓ (ICM)	/	Functional and symptomatic improvement in most ICM patients	None
							→ (DCM)	→ (DCM)	/	No improvement	
Gwizdala et al. [19]	2017	Autologous connexin-43 overexpressing muscle-derived stem/progenitor cells	Advanced HF, NYHA class II-III, LVEF $\leq$ 40%	Transendocardial	After cell culture and gene modification (36 $\pm$ 12 days)	161 $\pm$ 115 $\times 10^6$	→ (tend to improve)	→ (tend to improve)	/	Significant improvement of exercise capacity and myocardial viability	No significant ventricular arrhythmia
CHART-1 [20]	2017	Autologous cardiopoietic bone marrow-derived MSCs	IHF, LVEF $\leq$ 35% tachyarrhythmia	Intramyocardial	59.8 $\pm$ 21.6 days	>24 $\times$ 10^6	→	→	/	Better outcomes in patients with baseline LVEDV 200–370 ml	Ventricular
REGENERATE-IHD [21]	2017	Autologous BMCs, G-CSF, or both	ICM, NYHA class II-IV, impaired LVEF	Intracoronary Intramyocardial	Within 24 hours of bone marrow harvest	BMCs:/ G-CSF: 10 μg/kg/day	→ ↑	→ →	/ /	No improvement Reducing NYHA class, and improving quality of life	None
IMPACT-CABG [22]	2016	Autologous $CD133^+$ BMCs	ICM, LVEF 25%–45%, pre-CABG	Intramyocardial	During CABG	6.5 $\pm$ 3.1 $\times$ 10^6	→	→	/	No difference	None
MESAMI 1 [23]	2016	Autologous bone marrow-derived MSCs	ICM, NYHA class II-IV, LVEF $\leq$ 35%	Intramyocardial	After cell culture (17 $\pm$ 1 days)	61.5 $\pm$ 7.0 $\times$ 10^6 (40–100 $\times$ 10^6)	↑	↓ (LVESV)	/	Improving contraction, and functional status	None
DYNAMIC (review in [6])	2016	Allogeneic CDCs	ICM and DCM, NYHA class III/IVa, LVEF < 35%	Intracoronary	/	/	Still follow-up			Still follow-up	None to date
MSC-HF [24]	2015	Autologous bone marrow-derived MSCs	IHF, NYHA class II-III, LVEF $\leq$ 45%	Intramyocardial	After cell culture (46.9 $\pm$ 10.5 days)	77.5 $\pm$ 67.9 $\times 10^6$	↑	↓	/	Improving stroke volume and myocardial mass	None
Menasche et al. [25]	2015	Human ESC-derived cardiac progenitor cells	IHF, NYHA class III, LVEF = 26%	Epicardial attachment	During coronary artery bypass	A fibrin patch with 4 $\times$ 10^6	↑	↓	/	Improving functional status	None

Table 1. *Continued*

Clinical trial	Year	Cell type	Patient enrollment	Delivery route	Delivery timing	Cell dose	Myocardial structure and function			Other effects	Adverse events
							LV EF (%)	LV EDV/ESV	Infarct Size		
Perin et al. [26]	2015	Allogeneic bone marrow-derived Stro-1/Stro-3$^+$ MPCs	HF with ischemic or nonischemic pathogenesis, NYHA class II-III, LVEF $<$ 40%	Transendocardial	/	25×10^6 75×10^6 150×10^6	↗ → →	→ → ↓	/ / /	No improvement No significant improvement in functional capacity, and reducing HF-MACE	None
TAC-HFT [27]	2014	Autologous BMCs or MSCs	ICM, LVEF $<$ 50%	Transendocardial	/	/	→	→	↓ (MSCs)	Improving functional capacity (MSCs, BMCs)	None
C-CURE [28]	2013	Autologous cardiopoietic bone marrow-derived MSCs	IHF, LVEF 15%–40%	Endoventricular	1,540 (192–7,515) days post-MI	733 (605–1,168) $\times 10^6$	↑	↓	/	Improving symptoms	None
POSEIDON [29]	2012	Allogeneic and autologous bone marrow-derived MSCs	ICM, NYHA class II-III, LVEF $<$ 50%	Transendocardial	/	20×10^6 100×10^6 200×10^6	↗	↘	↓	Autologous MSCs improve functional capacity; Allogeneic MSCs reduce LVEDV	Ventricular arrhythmia in autologous group
SCIPIO [30, 31]	2011	Autologous c-Kit$^+$ CSCs	ICM, LVEF $\leq$ 40%, pre-CABG	Intracoronary	113 days post-CABG	1×10^6	↑	/	↓	Improving regional wall motion, functional status and quality of life	None

The symbols /, ↑, ↓, ↗, ↘, →, respectively, indicate not mentioned, increase, decrease, tend to increase, tend to decrease, and do not change.

Abbreviations: AMI, acute myocardial infarction; BMCs, bone marrow mononuclear cells; CABG, coronary artery bypass grafting; CDCs, cardiosphere-derived cells; CSCs, cardiac stem cells; DCM, dilated cardiomyopathy; G-CSF, granulocyte colony-stimulating factor; HF-MACE, heart failure-related major adverse cardiac events, defined as cardiac death, resuscitated ventricular fibrillation and HF rehospitalization; HP, hypoxic preconditioning; ICM, ischemic cardiomyopathy; IHF, ischemic heart failure; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; MPCs, mesenchymal precursor cells; MSCs, mesenchymal stem cells; N, normoxia; NYHA, New York Heart Association; PCI, percutaneous coronary intervention; STEMI, ST-segment elevation MI; WMSI, wall motion score index.

Table 2. Therapeutic efficacy and potential regulatory mechanisms of stem cell-derived secretomes and exosomes in cardiovascular diseases

				Myocardial structure and function				Underlying regulatory mechanisms	
Paracrine component	Cell origin	Animal model	Delivery route	LV EF (%)	LV FS (%)	LV EDV/ESV	Infarct size	Molecules	Functions
Secretomes	Mouse ESCs [44]	Mouse DIC	Intraperitoneal	↑	↑	↓	/	Undefined	Suppressing vascular apoptosis, activation and differentiation of endogenous stem cells, and enhancing neovascularization
	Mouse ESCs [40]	Mouse AMI	Intramyocardial	/	↑	↓	/	HGF, IGF-1, total antioxidants, VEGF	Suppressing apoptosis, activation and differentiation of endogenous stem cells, and enhancing neovascularization
	Human iPSC-derived MSCs [45]	Mouse DIC	Intramyocardial	/	/	/	↓	MIF, GDF-15	Cardioprotection
	Human dental pulp-derived MSCs [46]	Mouse I/R injury	Intravenous	/	↑	/	↓	HGF	Suppressing cardiomyocyte apoptosis and inflammation
	Hypoxic human adipose-derived MSCs [47]	Rat AMI	Intramyocardial	/	/	/	↓	VEGF, HGF, SDF-1	Enhancing proliferation and migration of cardiomyocytes in vitro, and reducing apoptosis in vivo
	Human amniotic membrane-derived MSCs [48]	Rat I/R injury	Intramyocardial	/	/	/	↓	Midkine, SPARC	Reducing cardiomyocyte apoptosis
								VEGF, bFGF, IGF-1, thrombopoietin, PDGF-BB, angiogenin, VEGF-D	Promoting neovascularization
	Hypoxic swine bone marrow-derived MSCs [49]	Pig AMI	Intracoronary	↑	/	↓	↓	VEGF, endothelin, epiregulin	Pro-angiogenesis
								Galectin-3, Smad-5, sFRP-1, sFRP-4	Anti-apoptosis
								TIMP-2	Anti-remodeling
	Porcine EPCs [50]	Pig I/R injury	Intracoronary	/	/	/	↓	IGF-1	Exerting anti-apoptotic, cardiotrophic and pro-angiogenic effects
	Human neonatal c-Kit$^+$ CPCs [51]	Rat AMI	Intramyocardial	↑	↑	↓	↓	HSF-1	Major regulator of the CPC secretome
								VEGFA, ANG-1	Promoting angiogenesis
								HGF	Cardioprotection, growth, survival and migration of CPCs and cardiomyocytes
								SCF, SDF-1α	Stem cell recruitment
	[a]Mouse CDCs [52]	Mouse AMI	Intramyocardial (CDCs)	→	/	→	/	Endoglin	Required for pro-angiogenic properties of the CDC secretome

Table 2. *Continued*

Paracrine component	Cell origin	Animal model	Delivery route	Myocardial structure and function				Underlying regulatory mechanisms	
				LV EF (%)	LV FS (%)	LV EDV/ESV	Infarct size	Molecules	Functions
	[a]Human CDCs from pediatric end-stage heart failure patients [53]	Rat AMI	Intramyocardial (CDCs)	↑	/	/	↓	HSF-1	Master regulator of the CDC secretome
								HSP60, HSP70	HSF-1 downstream factors
								VEGF-A, SDF-1α, PDGF-A, FGF-2, IL-6	Increasing endogenous stem cells recruitment, cardiomyocyte proliferation and angiogenesis
	CDCs from hypertensive humans and rats [54]	/	/	/	/	/	/	IL-6	Improving contractile behavior of cardiomyocytes
	[a]Human CDCs [55]	Mouse AMI	Intramyocardial (CDCs)	↑	/	↓	↓	ANG-2, bFGF, HGF, IGF-1, SDF-1, VEGF	Exerting angiogenic and antiapoptotic effects
	[a]Human CDCs [56]	Mouse AMI	Intramyocardial (CDCs)	/	/	/	/	VEGF, HGF, IGF1	Exerting cardioprotective and pro-angiogenic effects
Exosomes	Mouse ESCs [41]	Mouse AMI	Intramyocardial	↑	↑	↓	↓	miR-294	Promoting survival, cell cycle progression, and proliferation of endogenous CPCs
	Mouse iPSCs [42]	Mouse AMI	Intramyocardial	→	→	/	/	miR-21, miR-210	Suppressing cardiomyocyte apoptosis
	HIF-1α overexpressing human dental pulp-derived MSCs [57]	Mouse in vivo Matrigel plug assay	Subcutaneous	/	/	/	/	Jagged1	Inducing angiogenesis
	Akt overexpressing human umbilical cord-derived MSCs [58]	Rat AMI	Intravenous	↑	↑	↓	/	PDGF-D	Promoting angiogenesis
	GATA-4 overexpressing rat bone marrow-derived MSCs [59]	Rat AMI	Intramyocardial	↑	↑	↓	↓	miR-19a	Cardioprotection
	Human ESC-derived MSCs [60]	Mouse I/R injury	Intravenous	↑	/	↓	↓	Undefined	Restoring bioenergetics, reducing oxidative stress and activating pro-survival signaling
	Human $CD34^+$ peripheral blood mononuclear cells [61]	Mouse hind limb ischemia	Intramuscular	/	/	/	/	miR-126-3p	Promoting angiogenesis
	Human CDCs [62]	Pig AMI	Intracoronary	→	/	→	→	Undefined	Suppressing cardiomyocyte apoptosis and inflammation, preventing cardiac hypertrophy, and promoting angiogenesis and cardiomyocyte proliferation
		Pig AMI	Open-chest intramyocardial	↑	/	↓	↓		
		Pig CMI	Percutaneous intramyocardial	↑	/	↓	↓		

Table 2. *Continued*

Paracrine component	Cell origin	Animal model	Delivery route	Myocardial structure and function				Underlying regulatory mechanisms	
				LV EF (%)	LV FS (%)	LV EDV/ESV	Infarct size	Molecules	Functions
	Human pediatric c-Kit^{+} CPCs [63]	Rat I/R injury	Intramyocardial	↑	/	/	↓	miR-27a, miR-29c miR-29c, miR-96, miR-182, miR-185 miR-27a miR-138, miR-25	Regulating fibrosis Regulating cardiac hypertrophy Regulating angiogenesis Regulating apoptosis
	Mouse Sca-1^{+} CPCs [64]	/	/	/	/	/	/	miR-21	Preventing cardiomyocyte apoptosis
	Human ESC-derived cardiovascular progenitors [43]	Mouse post-infarct HF	Transcutaneous echocardiography-guided intramyocardial	↑	/	↓	↓	Undefined	Promoting cell survival, cell cycle and DNA repair, decreasing fibrosis and preventing HF
	Hypoxia-treated rat c-Kit^{+} CPCs [65]	Rat I/R injury	Intramyocardial	/	↑	/	↓	miR-17, miR-210 miR-17, miR-199a, miR-210, miR-292	Pro-angiogenesis Regulating fibrosis
	Human CPCs [66]	Rat AMI	Intramyocardial	↑	/	↓	↓	miR-210 miR-132	Inhibiting cardiomyocyte apoptosis Promoting angiogenesis

The symbols /, ↑, ↓, →, respectively, indicate not mentioned, increase, decrease and do not change.
[a]Indicated that in vivo studies were based on CDC transplantation, not CDC-derived secretome delivery.
Abbreviations: AMI, acute myocardial infarction; ANG, angiopoietin; bFGF, basic fibroblast growth factor; CDCs, cardiosphere-derived cells; CMI, convalescent myocardial infarction; CPCs, cardiac progenitor cells; CSCs, cardiac stem cells; DIC, doxorubicin-induced cardiomyopathy; EPCs, endothelial progenitor cells; ESCs, embryonic stem cells; GDF, growth differentiation factor; HF, heart failure; HGF, hepatocyte growth factor; HIF, hypoxia inducible factor; HSF, heat shock factor; HSP, heat shock protein; I/R, ischemia/reperfusion; IGF, insulin-like growth factor; IL, interleukin; iPSCs, induced pluripotent stem cells; LVEDV, left ventricular end-diastolic volume; LVEF, left ventricular ejection fraction; LVESV, left ventricular end-systolic volume; LVFS, left ventricular fractional shortening; MIF, macrophage migration inhibitory factor; miR, microRNA; MSCs, mesenchymal stem cells; PDGF, platelet-derived growth factor; SCF, stem cell factor; SDF, stromal cell-derived factor-1; sFRP, secreted frizzled related protein; SPARC, secreted protein acidic and rich in cysteine; TIMP, tissue inhibitor of matrix metalloprotease; VEGF, vascular endothelial growth factor.

multipotent, possessing the ability to differentiate into cardiomyocytes, vascular smooth muscle and endothelial cells. As highlighted in the SCIPIO and CADUCEUS trails (Table 1), intracoronary infusion of either autologous c-Kit$^+$ cells or CDCs conferred improvements in scar size and regional myocardial function in patients with IHDs, without raising any significant safety concerns such as ventricular arrhythmias and tumor formation [13, 14, 30, 31]. Currently, allogeneic human CDCs are being investigated in the ALLSTAR and DYNAMIC trials [6] (Table 1). Unexpectedly, the interim observations from ALLSTAR did not demonstrate scar size improvement after cell therapy, however, LV volume reductions in the cell-treated patients supported the biological activity of these cells (http://www.irdirect.net/prviewer/release/id/2492977). Meanwhile, allogeneic c-Kit$^+$ cell therapy for AMI is about to begin in the CAREMI trial [86].

The underlying mechanisms of cardiac progenitor cell (CPC) therapy are initially attributed to the cardiac differentiation potential of these cells [81, 82]. However, it eventually become widely accepted that paracrine mechanisms other than direct cardiac differentiation contribute to the beneficial effects of CPC therapy [51, 62, 63, 66]. Notably, CDCs outperformed commercial cell sources that may not have been optimized for regenerative potency (i.e., BMCs and MSCs) and c-Kit$^+$ cells antigenically sorted from expanded CDCs (i.e., not cultured using validated methods in SCIPIO trial) in terms of paracrine activity and functional benefits in infarcted rodent hearts [55]. However, another comparison study, based on large animal models of chronic MI, demonstrated equivalent improvements in cardiac regional function and myocyte regeneration after intracoronary infusion of allogeneic CDCs and MSCs [87].

Combined Cell Therapy

Although the optimal cell type has not been concluded yet, combined cell therapy has recently raised great interest of many investigators. Relative to single cell therapy, combined cell therapy, such as CSCs plus MSCs [88, 89], CSCs plus vascular pericyte progenitors [90] or CSC and MSC hybrids [91], has been demonstrated more effective in reducing infarct size, improving cardiac contractile function, or both in preclinical MI models. The synergistic effects are probably due to the complementary properties from different subsets of stem cells that, for example, CSCs are superior in direct cardiac differentiation and activation of endogenous stem cells, MSCs in paracrine activity and vascular pericyte progenitors in inducing angiogenesis.

Cell Dose

The optimal cell dose chosen for therapy is critical for promoting cardiac repair. However, as reviewed in Table 1, the cell dose varies in the recently published trials. The BOOST-2 trial assessed the dose and clonogenic potential of BMC therapy in LVEF recovery in AMI patients, but did not detect any improvements in LVEF or any magnetic resonance imaging secondary endpoints in patients treated with a high or low dose of clonogenic or nonclonogenic BMCs [4]. The TECAM trial compared the efficacy of BMC injection, granulocyte colony-stimulating factor (G-CSF) mobilization and the combined therapy in AMI patients. Although patients in the combined therapy group received a dose of BMCs seven times higher than that given to patients in the BMC therapy group (average 560 vs. 83 $\times$ 10^6 cells), neither group showed improvements in LVEF and LV end-systolic volume (LVESV), but both groups exhibited a small reduction in infarct area [8].

In patients with chronic ischemic cardiomyopathy, a dose-escalation study of allogeneic mesenchymal precursor cell (MPC) therapy (25, 75, or 150 $\times$ 10^6 cells) revealed that MPC injections were feasible and safe, but only a high dose of MPCs (150 $\times$ 10^6 cells) was beneficial in decreasing LVESV and LV end-diastolic volume (LVEDV) at 6 months [26]. However, the early POSEIDON trial found an inverse dose response to autologous and allogeneic MSC therapy, demonstrating that a high dose of MSCs (200 $\times$ 10^6 cells) was less effective in reducing LV volumes and increasing LVEF than a low dose of MSCs (20 $\times$ 10^6 cells) [29].

Recently, multiple-dose administrations of stem cells have been demonstrated more effective than single-dose administration. Tokita et al. and Guo et al. successively showed that three-dose infusions of c-Kit$^+$ CPCs or cardiac mesenchymal cells had cumulative beneficial effects in improving LV function in rodent models of old MI, compared with one-dose infusion [92, 93]. Reich et al. also revealed that two-dose injections of allogeneic CDCs in infarcted rat hearts led to greater improvements in cardiac function and infarct size [94]. These results indicate that although the optimal cell dose remains elusive, repeated doses of stem cells may provide therapeutic superiority in cardiac repair.

Cell Delivery Route

The major techniques to effectively deliver cells to the heart include intracoronary infusion and intramyocardial (transendocardial) injection. As reviewed in Table 1, intracoronary infusion is widely used for autologous BMC therapy in AMI patients, whereas in chronic ischemic cardiomyopathy, intramyocardial injection seems to provide better clinical outcomes. However, clinical trials comparing different delivery routes are relatively fewer. Vrtovec et al. reported the first study that transendocardial CD34$^+$ cell transplantation produced higher myocardial retention rates and greater functional improvements in patients with dilated cardiomyopathy, compared with intracoronary group [75]. The recent REGENERATE-IHD trial investigated the efficacy of G-CSF alone or in combination with intracoronary or intramyocardial injection of autologous BMCs in patients with ischemic cardiomyopathy, and found that the combination of G-CSF and intramyocardial BMC injection exerted beneficial effects on cardiac function and symptoms, while other treatment groups did not show such improvements [21].

Intramyocardial injection has certain advantages over intracoronary infusion [67, 95]. First, based on a preclinical animal study, intramyocardial injection led to higher cell retention rates within the myocardium than intracoronary delivery route (11% $\pm$ 3% vs. 2.6% $\pm$ 0.3%) [96], probably because the majority of cells were washed out during coronary infusion. Second, intramyocardial injection provides a targeted approach to deliver cells into the damaged myocardium, without requiring chemoattractive

factors that are more abundant in AMI. However, the procedure of intramyocardial injection requires 3D NOGA equipments and skilled technicians to map the endocardial surface during injection. On the other hand, intracoronary infusion is technically easier and cost-effective, however, it may cause vessel occlusion.

Cell Delivery Timing

The timing of cell delivery is an important determinant for the incorporation and efficacy of stem cells in myocardial repair. So far, most preclinical animal studies use early delivery of stem cells following MI to assess their therapeutic effects, even though several comparative studies suggested that delivery of stem cells after the acute inflammation period of MI (1-week after MI) resulted in a greater infarct reduction compared with early ($\leq$1-day) injection [97, 98]. In the clinical arena, a number of phase I/II trials assessing the timing of cell delivery in autologous BMC therapy have reported variable results (Table 1). As revealed in the TIME, LateTIME, and SWISS-AMI trials, both early (3 or 7 days, 5–7 days) and late (2–3 weeks, 3–4 weeks) delivery of BMCs following reperfusion failed to improve LV function in AMI patients [11, 12, 15, 16]. However, another comparative trial reported that AMI patients receiving BMCs either within 24 hours of reperfusion or 3–7 days after reperfusion displayed similar improvements in LV function and volumes, whereas patients receiving treatment at 7–30 days after reperfusion did not show such improvements [9]. Similarly, the REGENERATE-AMI trial demonstrated that BMC infusion within 24 hours of reperfusion in AMI patients led to a small nonsignificant improvement in LVEF [5], indicating a feasible timeframe of cell delivery in BMC therapy for AMI patients without prolonging hospitalization.

Nevertheless, late delivery of stem cells is not very ineffective for MI patients, and it may also be cell type dependent. In the CADUCEUS trial, CDC therapy at 1.5–3 months post-MI resulted in decreased scar size, increased viable myocardium and improved regional function [13, 14], probably due to the superiority of CDCs over BMCs in cardiac repair as mentioned above [55].

Potential Mechanisms of Stem Cell-Mediated Myocardial Repair

So far, three major mechanisms have been proposed to contribute to the beneficial effects of stem cell-based therapy in cardiac diseases [67, 71, 80]. First, transplanted stem cells differentiate into cardiomyocytes to replace damaged cardiac tissues. Second, transplanted stem cells form new blood vessels by differentiating into vascular cells. However, because of the low retention and poor survival of transplanted cells, the above mechanisms may not account for the global improvements in cardiac remodeling and function.

The third mechanism, widely accepted by most investigators, is that stem cells secrete high levels of paracrine factors (comprising the secretomes) that can stimulate endogenous repair mechanisms. An important component of secretomes in many cell types are extracellular vesicles, particularly exosomes. Exosomes are considered critical vehicles for intercellular delivery of bioactive cargoes, including proteins, lipids, mRNAs, and microRNAs (miRs) [99]. According to various animal studies, secretomes/exosomes derived from a variety of stem cells have been shown to improve cardiac function and attenuate adverse remodeling after delivery into the injured myocardium (Table 2). Some investigators, through comparative analysis, indicated that stem cell-derived secretomes/exosomes resembled and even outperformed stem cells in their abilities of cardiac repair [43, 44, 51]. The paracrine factors and exosomal miRs identified in these studies have been proposed to participate in promoting angiogenesis, mediating the survival of existing cardiomyocytes, supporting the recruitment, proliferation and differentiation of endogenous stem cells, improving cardiac hypertrophy, reducing inflammation, and preventing fibrosis (Table 2).

Remarkably, some exosomal miRs are involved in boosting cardiac repair. miR-17 [65] and miR-19a [59] are members of miR-17–92 cluster, which was identified as critical regulators of cardiomyocyte proliferation in hearts. Overexpression of miR-17–92 in adult cardiomyocytes protected the heart from MI-induced injury [100]. Co-administration of miR-199a [65] and miR-590 mimics was demonstrated effective in attenuating infarct size and improving cardiac function in mouse MI models [101]. miR-132 [66] was reported to activate pro-hypertrophic signaling. Injection of miR-132 inhibitor rescued cardiac hypertrophy and heart failure in mice [102].

Recently, the first translationally realistic large-animal study by Gallet et al. investigated the delivery routes and therapeutic efficacy of human CDC-derived exosomes in acute and convalescent MI [62]. They found that intramyocardial injection of CDC exosomes resulted in preserved LV function and reduced scar size, accompanied by an increase in vessel density and a decline in LV collagen content and cardiomyocyte hypertrophy. These findings may promote the clinical translation of stem cell-derived exosomes as an attractive cell-free resource for cardiac repair in future, and exosomal miRs may serve as potential therapeutic targets.

Long noncoding RNAs (lncRNAs) with unique regulatory and functional characteristics have been reported to play potential roles in cardiac pathologies [103]. For example, lncRNA Chaer was defined as an epigenetic modulator in cardiac hypertrophy via interacting with polycomb repressor complex 2 and reprogramming of cardiac hypertrophy-associated genes [104]. LncRNA Meg3 was found to regulate cardiac fibrosis after transverse aortic constriction by inducing cardiac matrix metalloproteinase-2 [105]. Nevertheless, detailed reports on lncRNAs regulating stem cell-mediated cardiovascular repair are relatively fewer. The first observation by Deng et al. revealed that genetically modified human MSCs augmented the survival and function of endogenous progenitor cells in mouse ischemic tissues by regulating lncRNA H19 [106]. The novel mechanism of stem cells in cardiovascular repair by targeting lncRNAs will become a research hotspot in the near future.

Optimized Strategies for Stem Cell-Based Therapy

Irrespective of cell type, dose, delivery routes and timing, stem cell transplantation has shown extremely low rates of cell survival and retention, which largely limit the therapeutic outcomes. First, even after direct intramyocardial injection, the majority of cells leak out of the heart or are washed away

owing to blood flow [107]. Second, post-infarction hearts undergo complex and dynamic pathological changes, displaying a harsh ischemic and inflammatory microenvironment in the acute phase of MI. Thus, the injected cells encounter a massive death because of ischemia, inflammation-related oxidative stress and detrimental cytokines, as well as anoikis (apoptosis in anchorage-dependent cells after detachment from their substrate) [37, 67]. To overcome these challenges, physical, chemical/pharmacological, and genetic approaches have been adopted to precondition and reprogram stem cells to augment their survival and/or function after transplantation. Moreover, statin treatment and tissue engineering can create a favorable cardiac microenvironment to facilitate the incorporation and effect of implanted cells during myocardial repair. A graphical overview of different strategies is given in Figure 1. The clinical translatability and potential limitation of each strategy are discussed below.

Strategies to Augment Cellular Survival and/or Function

Physical Stimulation (Hypoxic Preconditioning)

Physical stimulations, such as hypoxic preconditioning and heat shock, seem to be practical with minimal safety concerns. For the past decade, hypoxic preconditioning of stem cells by short-term exposure to low oxygen tensions has been well documented to enhance cellular survival, migration, and therapeutic efficacy in ischemic animal hearts [108–110], although oxygen concentration, preconditioning duration, and cell types are different in these studies. The underlying mechanisms primarily depend on hypoxia inducible factor (HIF)-1, which consists of oxygen-sensitive HIF-1α and constitutive HIF-1β subunits. Under hypoxic conditions, HIF-1α is stabilized and binds with HIF-1β to form a heterodimeric HIF that is subsequently translocated to the nucleus to activate downstream genes [111, 112]. In our previous studies, an adipokine leptin, whose promoter contains a hypoxia response element site driven by HIF-1 [113], displayed the highest increase in expression in hypoxic preconditioned MSCs [114]. Leptin played crucial roles in enhancing the survival and engraftment of transplanted MSCs, conferring cardioprotective and angiogenic properties and recruiting endogenous progenitor cells in autocrine and paracrine manners by binding to its receptor ObR and subsequently activating JAK/signal transducer and activator of transcription (STAT) 3/stromal cell-derived factor (SDF)-1/CXCR4 signaling [115]. Additionally, two studies showed that hypoxic conditions altered miR content in CPC-secreted exosomes and improved their post-MI repair. These exosomal miRs were respectively associated with promoting angiogenesis, reducing fibrosis, attenuating apoptosis, and improving cardiac hypertrophy [63, 65] (Table 2).

To promote the clinical translation of hypoxic preconditioned cell therapy, preclinical studies based on nonhuman primates are truly required to ensure safety and confirm possible clinical benefits [116]. Accordingly, we performed the first large sample size, long-term, nonhuman primate investigation of hypoxic preconditioned MSC therapy for the treatment of cardiac injury [117]. Our results showed that transplantation of hypoxic preconditioned MSCs led to significant improvements in cardiac function and remodeling,

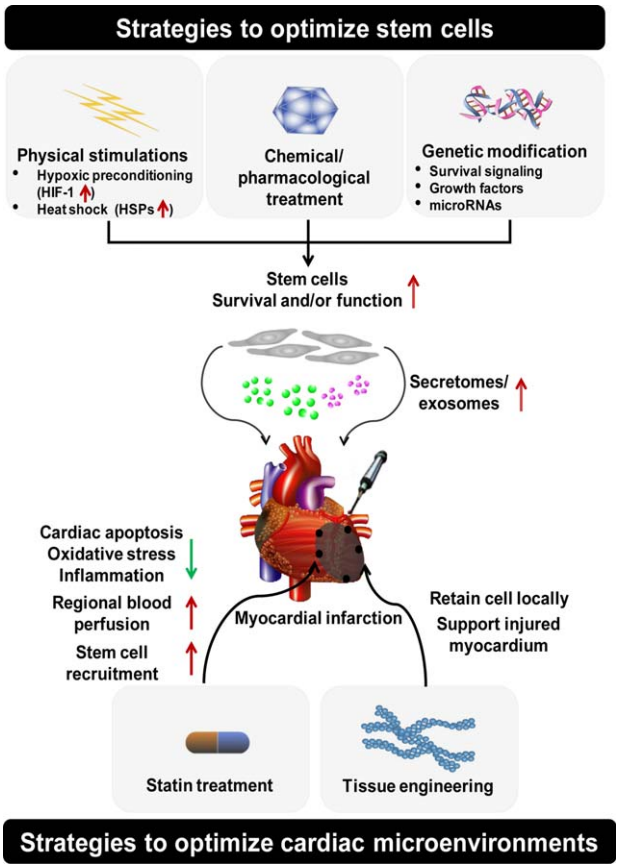

Figure 1. A graphical overview of different optimized strategies for manipulating both donor stem cells and the cardiac microenvironment in stem cell-based therapy. Abbreviations: HIF-1, hypoxia inducible factor-1; HSPs, heat shock proteins.

accompanied by an increase in cell engraftment, cardiomyocyte survival and proliferation, angiogenesis and myocardial glucose metabolism, as well as a decline in myocardial inflammation, without increasing the occurrence of arrhythmias. However, the lack of evidence of MSC differentiation and <1% engraftment of transplanted cells suggested that paracrine mechanisms, rather than remuscularization of the infarcted region, might contribute to the benefits of hypoxic preconditioned MSC therapy.

Following the encouraging results, hypoxic preconditioned cell therapy has advanced to clinical trial. The CHINA-AMI randomized controlled trial provided the first-in-man evidence that intracoronary administration of hypoxic preconditioned autologous BMCs significantly reduced LVEDV/LVESV and postponed LV remodeling in AMI patients, without increasing the occurrence of major adverse cardiovascular events [10] (Table 1). Although apparently safe and feasible, the efficacy of this strategy needs further evaluation in phase II/III trials and in larger cohorts of patients. These results serve as a possible basis for promoting hypoxic preconditioned cell therapy toward future clinical application.

Physical Stimulation (Heat Shock)

Heat shock by short-term exposure of cells to mild heat stress (42°C −43°C) is another physical approach that has been shown to enhance the survival of skeletal myoblasts, neonatal

cardiomyocytes, and hESC-derived cardiomyocytes after transplantation into hearts [118–120]. Heat shock is usually accompanied by the upregulation of heat shock proteins (HSPs) [118, 119], which have been proven as prosurvival factors. MSCs respectively engineered with HSP20, HSP70, or HSP27 were protected against oxidative stress- or hypoxia/ischemia-induced cell death, and they showed enhanced survival rates and therapeutic efficacy in infarcted rat hearts [121–123]. Additionally, Feng et al. demonstrated that heat shock significantly improved the survival of Sca-1^+ stem cells under ischemic conditions through heat shock factor (HSF) 1-mediated epigenetic repression of miR-34a expression and direct upregulation of HSP70 [124].

Heat shock can also improve the paracrine effects of stem cells. For example, heat shocked Sca-1^+ stem cells exerted cardioprotective effects on ischemic myocardium by exosomal delivery of HSF1 into cardiomyocytes and repression of miR-34a [124]. Another study reported that after heat shock, CDCs secreted high levels of cytokines such as SDF-1α, vascular endothelial growth factor (VEGF)-A, platelet-derived growth factor (PDGF)-A, interleukin(IL)-6, and fibroblast growth factor (FGF)-2, thereby restoring the injured myocardium to a greater extent [53].

Nevertheless, heat shocked cell therapy has been confined to rodent studies so far, probably due to the variable and complex nature of heat shock responses in different cells. For example, heat shock protected myoblast sheets from hypoxia-induced apoptosis but attenuated their VEGF expression, leading to a reduction in their therapeutic efficacy in heart failure [125]. Furthermore, HSPs such as HSP70 can be very immunogenic for enhanced innate and T cell response [126–128], which may induce host immune reaction after transplantation of heat shocked cells. Thus, these challenges need to be solved before heat shocked cell therapy can be considered for clinical application.

Chemical/Pharmacological Treatment

Small chemical/pharmacological molecules have certain advantages in optimizing stem cells: easy usage, efficient delivery into cells, nonimmunogenicity, and cost-effectiveness. Various chemical/pharmacological agents that activate oxygen-sensing pathways possess high potential for enhancing stem cell-based therapy. 2,4-dinitrophenol (DNP) can induce chemical hypoxia via inhibiting the electron transport chain and decreasing intracellular ATP production [129]. DNP treatment was found to activate a series of survival, angiogenic, and cardiomyogenic factors in MSCs. Transplantation of DNP treated MSCs into infarcted rodent hearts led to an increase in cell engraftment and cardiovascular differentiation, thereby improving cardiac performance and revascularization [130, 131]. Deferoxamine (DFO), an FDA-approved iron chelator, can inhibit the activity of prolyl hydroxylase that is involved in HIF-1α degradation. DFO treatment was reported to enhance the angiogenic potential of MSCs via HIF-1α-mediated secretion of paracrine factors such as VEGF and SDF-1α [132, 133]. Carvedilol, a nonselective β-blocker with antioxidant properties for superoxide scavenging, was reported to protect MSCs against oxidative stress-induced cell death [134]. Diazoxide, a highly selective mitoK_{ATP} channel opener, was shown to enhance the survival and therapeutic effects of MSCs for the repair of infarcted myocardium via NF-kappaB-dependent miR-146a expression by targeting Fas, a tumor necrosis factor (TNF) receptor superfamily member [135].

Additionally, various pharmacological agonists targeting cardiovascular system have emerged in myocardial cell therapy. Angiotensin type 2 receptor (AT2R), a major component of renin-angiotensin (RAS) system, plays an important role in cardiac repair after MI [136]. According to our previous studies, preconditioning of BMCs with an AT2R agonist CGP42112A exerted cardioprotective effects via the activation of ERK/endothelial nitric oxide synthase (eNOS)/nitric oxide (NO) signaling. Transplantation of AT2R-stimulated BMCs improved overall cardiac performance and reduced infarct size in rat infarcted hearts by increasing the survival and mobilization as well as anti-inflammatory, cardioprotective, and angiogenic properties of transplanted cells [137, 138].

Chemical reprogramming of fibroblasts into functionally induced cardiomyocytes holds great promise for cardiac regeneration. Numerous chemical compounds have been tested, such as the transforming growth factor-β inhibitor SB431542, the glycogen synthase kinase-3 inhibitor CHIR99021, the Wnt inhibitor XAV939, the PDGF pathway inhibitors SU16F and JNJ10198409, as well as Forskolin and Parnate [139–141]. Fu et al. and Cao et al. successively reported the generation of cardiomyocyte-like cells from mouse and human fibroblasts using different chemical cocktails. These chemically induced cardiomyocyte-like cells exhibited contractile properties and cardiac-specific transcriptomes [139, 140]. When transplanted into infarcted rodent hearts, chemically treated human fibroblasts further matured into cardiomyocytes and partially remuscularized the infarcted area [140]. The chemical approach of cardiac-lineage reprogramming may provide important implication for optimizing stem cells and enhancing their cardiogenic potency.

Most chemical/pharmacological reagents used in these studies are not FDA-approved drugs. Their clinical application would be restricted by human safety issues related to cardiotoxicity, hepatotoxicity, neurotoxicity, and teratogenicity [142]. To advance the clinical application of chemical/pharmacological treated cell therapy, cardiovascular drugs with similar effects and proven safety profiles should be explored to substitute for these compounds to optimize stem cells. For example, the phosphodiesterase-5 inhibitors including tadalafil, have been approved for clinical use in patients with pulmonary arterial hypertension [143]. Recently, tadalafil was reported to promote MSC survival in ischemic hearts for cardiac repair via miR-21-dependent suppression of Fas [144].

Genetic Modification

Ex vivo genetic modification of stem cells is a powerful strategy to increase cell survival, paracrine factor secretion and reparative capacity in the damaged myocardium. Overexpression of prosurvival signaling molecules (e.g., Akt, Bcl-2, and Bcl-xL) [145–147] or knockdown of apoptotic factors (e.g., caspase-8) [148] in MSCs have been proven to augment cell survival and efficacy in ischemic rat hearts. Pim-1 kinase is a downstream effector of Akt. Transplantation of Pim-1 overexpressing CSCs showed increased cell engraftment and differentiation, as well as enhanced myocyte formation and neovascularization, resulting in augmented cardiac function

and reduced infarct size in small and clinically relevant large animal models of MI [149, 150].

In addition to antiapoptotic factors, numerous genes encoding cytokines, chemokines or growth factors, such as angiopoietin-1 (Ang-1), VEGF, insulin-like growth factor 1 (IGF-1), SDF-1α, or hepatocyte growth factor (HGF), have been genetically engineered into stem/progenitor cells to enhance their ability to persistently express and secrete these factors. This can not only augment cellular survival, retention, and differentiation, but also promote endogenous cardiac repair through paracrine mechanisms [151–155].

miRs have been proposed as important regulators in stem cell-mediated cardiac repair [156]. The therapeutic efficacy of stem cells in damaged myocardium can be markedly enhanced by manipulating them with specific miRs. According to our recent studies, miR-211, which was activated by STAT3, improved MSC migration by targeting STAT5A and regulating MAPK signaling. Intravenous delivery of miR-211 overexpressing MSCs led to a significant increase in cell retention, vessel density, and viable myocardium in the peri-infarct area [157]. Furthermore, transplantation of stem/progenitor cells respectively modified with miR-1, miR-23a, miR-375, miR-133a, let-7b, miR-377, or miR-495 was shown to attenuate infarct size and improve ventricular function in ischemic rodent hearts. Mechanistically, manipulation of these miRs variously increased the survival, engraftment, and differentiation of transplanted cells or enhanced their cardioprotective and proangiogenic activities [158–164].

Meanwhile, genetic modification can impact the therapeutic efficacy of stem cell-released exosomes (Table 2). For example, exosomes secreted from GATA-4 overexpressing MSCs exerted cardioprotective effects on ischemic myocardium by delivering miR-19a into cardiomyocytes, reducing PTEN and activating Akt/ERK signaling [59]. Akt overexpressing MSC-derived exosomes promoted angiogenesis in ischemic myocardium by delivering high levels of PDGF-D [58].

Despite encouraging preclinical results, the clinical application of genetically modified stem cell therapy has been impeded by several challenges. First, the commonly used gene vectors, including retro-/lentiviral vectors, can result in random integrations that may activate nearby proto-oncogenes by insertional mutagenesis. Moreover, nontargeted gene transfer using viral vectors often causes the risk of transgene inactivation. The genotoxicity may alter cellular functions particularly in stem cells (e.g., tumorigenicity, immunogenicity, and differentiation potential) [165–167]. Second, the constitutive and unregulated expression of transgenes may induce tumorigenesis and other detrimental effects [168, 169]. Therefore, exploring safe and stable genetic modification systems, such as site-specific integration techniques using nonviral vectors [170] or the combined utility of adeno-associated virus and clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated nuclease 9 (Cas9) system [171], is likely required before genetically modified stem cell therapy progresses to clinical settings. Recently, the first-in-human transendocardial delivery of genetically modified stem/progenitor cells using nonviral vectors for the treatment of advanced heart failure was demonstrated to be safe, feasible, and effective [19] (Table 1), indicating a breakthrough for the clinical application of genetically modified stem cell therapy in cardiac diseases.

Strategies to Optimize Cardiac Microenvironments

Statin Treatment

Statins, HMG-CoA reductase inhibitors, are one of the most heavily prescribed drugs that have pleiotropic effects on the cardiovascular system [172]. Based on various preclinical animal studies, statin (e.g., atorvastatin, simvastatin, or rosuvastatin) treatment could modulate the post-infarct milieu by inhibiting cardiac cell apoptosis, oxidative stress, and inflammation, as well as increasing regional blood perfusion, thereby facilitating the survival, engraftment, and cardiovascular differentiation of implanted MSCs. Statin/MSC combined therapy after MI exhibited greater improvements in cardiac function and remodeling than those from either statin treatment or MSC transplantation alone. The underlying mechanisms involved the activation of eNOS or JAK2/STAT3 or the inhibition of RhoA/ROCK/ERK in the infarcted myocardium [173–179]. Additionally, statin administration could enhance the mobilization and homing of stem cells into the ischemic myocardium via upregulating cardiac SDF-1α and activating CXCR4 coupling [180]. The SDF-1α/CXCR4 axis-driven stem/progenitor cell recruitment can also be achieved by fucoidan (sulfated polysaccharide) [181], tadalafil [144], parathyroid hormone [182], and erythropoietin [183] treatment, respectively. Despite promising results, the combined therapy for IHDs has not been reported in human studies yet, and two clinical trials evaluating the therapeutic efficacy of BMCs/MSCs with atorvastatin in MI patients are still ongoing (NCT00979758 and NCT03047772).

Recently, several in vitro studies revealed that statin treatment impaired the biological characteristics of stem cells by inhibiting cell proliferation or increasing cell senescence and apoptosis [184–186]. The adverse cytostatic effect of statins may compromise the activation, proliferation, differentiation, and recruitment of endogenous stem cells to the damaged myocardium, which are usually involved in cardiac repair process. Furthermore, statin treatment can cause several adverse effects including myopathy, rhabdomyolysis, liver damage, and type 2 diabetes [187]. Therefore, the dosage, treatment duration, and potential side effects of statins need to be evaluated further before this strategy can be translated to clinical settings.

Tissue Engineering

The injured myocardium lacks the specific architecture, vascularity, and metabolism of normal cardiac tissues. Tissue engineering, via engineering stem cells on scaffolds, has been demonstrated to be an ideal strategy with synergistic effects, which not only allows the grafted cells to be retained longer in the infarcted hearts, but also provides a supportive environment to the damaged myocardium [188]. So far, a variety of autologous and allogeneic stem cells have been used. The scaffolds to seed cells have varied from hydrogels to 3D patches with natural or synthetic sources. In the AUGMENT-HF trial, injectable hydrogels were demonstrated to modify the shape of the dilated LV and improve the clinical outcomes of patients with advanced heart failure [189, 190]. Accumulating evidence suggests that hydrogels and/or bioactive agents can act as injectable delivery vehicles for stem cells to enhance the survival, retention and efficacy of these cells in the infarcted hearts. For example, transplantation of hydrogel-

encapsulated ESCs or MSCs showed a better cardiac remodeling and functional improvement than that due to regular cell transplantation or hydrogel injection alone [191, 192]. Hydrogels combined with antioxidant nanoparticles were reported to effectively scavenge reactive oxygen species in the MI area and protect delivered MSCs from oxidative damage, thereby improving their survival and therapeutic effects [193].

Three-dimensional (3D) patch-based systems are being widely studied for cardiac repair. Using a porcine MI model, Ye et al. demonstrated that, compared with regular cell transplantation, injection of human iPSC-derived cardiovascular cells through an IGF-1-containing 3D patch resulted in a twofold increase in the engraftment rate of transplanted cells, leading to significant improvements in myocardial wall stress, metabolism, and contractile performance [194]. The cytokine-loaded patch not only formed a physical barrier to retain the cells locally, but also enabled prolonged cytokine release to promote cell survival.

In combination with extracellular matrix bioinks, 3D printing technology can produce precisely controlled 3D tissues by mimicking the outer shape and inner architecture of native tissues [195]. Several groups reported that 3D-printed scaffolds could improve cell-cell interactions, as well as cellular survival and differentiation after being seeded with different cell types, such as CPCs and MSCs [196, 197]. Gao et al. demonstrated that 3D-printed scaffolds promoted the maturation, calcium signaling and functional electrophysiological integration of seeded human iPSC-derived cardiovascular cells to generate beating cardiac patches [198]. After transplanted into the infarcted myocardium, these cell patches enhanced cardiac function and prevented adverse remodeling, accompanied by a relatively long-term and high rate of cell engraftment, as well as enhanced cardiomyogenesis and angiogenesis [196–198].

Given the promising outcomes, significant efforts are underway to translate cardiac patches to clinical trials. As mentioned above, human ESC-derived cardiac patch is a good example of a cell and tissue engineered construct that has been translated to the clinical setting [25, 70]. Furthermore, the manufacturing process and quality control of biomaterial products for clinical application must comply with GMP standards.

Conclusion and Future Perspectives

Since 2001, stem cell-based therapy has become a remarkably potential strategy for cardiac repair/regeneration and functional recovery after AMI and other chronic IHDs. Despite encouraging results in substantial preclinical animal studies, the therapeutic effects of stem cells remain controversial in the clinical community as many trials do not confer sufficient benefits for patients suffering from cardiac injury. To promote cell therapy toward successful clinical application, many key issues (e.g., the optimal cell type, dose, delivery route and timing, precise mechanisms of action, long-term cell engraftment) need to be fully addressed with more molecular, translational and clinical studies [199].

With respect to the cell type, PSCs (e.g., ESCs and iPSCs) and ASCs (e.g., MSCs and CPCs) have different superiority and mechanisms in repairing the injured hearts. Direct head-to-head comparative studies with detail design are truly required to define the optimal cell type. Combined cell therapy that complements properties from different types of stem cells may exert synergistic effects in cardiac repair. The optimal cell dose, delivery route, and timing are important determinants for increased cell engraftment and enhanced therapeutic efficacy. Although numerous studies are accumulating with variable results, it is likely that intramyocardial delivery and repeated dose administration can produce higher cell retention and greater therapeutic outcomes.

It is widely accepted that paracrine mechanisms rather than de novo cardiomyocyte or blood vessel formation may be the major mechanisms underlying the beneficial effects of stem cell-based therapy. Stem cell-derived secretomes/exosomes have shown therapeutic superiority as an attractive cell-free resource. The underlying regulatory components (e.g., protein, miRs, lncRNAs) are being identified and gradually become potential targets in boosting cardiac repair. Encouraging results from the first translationally large animal study provide a possible basis for promoting stem cell-derived exosomes toward clinical application in future.

Due to hostile microenvironment of the injured myocardium, poor survival and low engraftment of transplanted stem cells severely limit the therapeutic potential of cell therapy. Various optimized strategies including physical, chemical, pharmacological, genetic, biomaterial approaches are being investigated for manipulating both donor stem cells and the target cardiac microenvironment to enhance the engraftment and efficacy of stem cells. Several strategies have already progressed to phase I clinical trials, such as hypoxic preconditioning, genetic modification, and tissue engineering. However, in many cases, larger randomized clinical trials and more clinically relevant large animal studies are truly required to overcome limitations and accelerate the progress of optimized strategies toward FDA-approved clinical applications.

Acknowledgments

We thank Dr. Keith A. Webster (Vascular Biology Institute, Miller School of Medicine, University of Miami) for embellishing the manuscript. This work was supported by the National Basic Research Program of China (973 Program, 2014CB965100 and 2014CB965103), grants from National Natural Science Foundation of China (81770484, 31371498, 81320108003, 81622006, 81670261, 81370247, and 31101052), Science and Technology Department of Zhejiang Province Public Welfare Project (2014C33190), Fundamental Research Funds for the Central Universities (2016XZZX002–03 and 2016FZA7005).

Author Contributions

R.W., X.H., and J.W.: wrote the manuscript, summarized the tables, and organized the graphical overview, final approval of manuscript.

Disclosure of Potential Conflicts of Interest

The authors indicated no potential conflicts of interest.

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