Can the outcomes of mesenchymal stem cell-based therapy for myocardial infarction be improved? Providing weapons and armour to cells

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Abstract

Use of mesenchymal stem cell (MSC) transplantation after myocardial infarction (MI) has been found to have infarct-limiting effects in numerous experimental and clinical studies. However, recent meta-analyses of randomized clinical trials on MSC-based MI therapy have highlighted the need for improving its efficacy. There are two principal approaches for increasing therapeutic effect of MSCs: (i) preventing massive MSC death in ischaemic tissue and (ii) increasing production of cardioreparative growth factors and cytokines with transplanted MSCs. In this review, we aim to integrate our current understanding of genetic approaches that are used for modification of MSCs to enable their improved survival, engraftment, integration, proliferation and differentiation in the ischaemic heart. Genetic modification of MSCs resulting in increased secretion of paracrine factors has also been discussed. In addition, data on MSC preconditioning with physical, chemical and pharmacological factors prior to transplantation are summarized. MSC seeding on three-dimensional polymeric scaffolds facilitates formation of both intercellular connections and contacts between cells and the extracellular matrix, thereby enhancing cell viability and function. Use of genetic and non-genetic approaches to modify MSC function holds great promise for regenerative therapy of myocardial ischaemic injury.

1 | INTRODUCTION

Myocardial infarction (MI) and post-infarct chronic heart failure (CHF) continue to be a major health burden worldwide.¹ In the absence of reperfusion, ongoing myocardial ischaemia leads to progressive necrosis of cardiac myocytes and therefore to diminished contractile performance of the heart, which culminates in CHF.² Current pharmacological treatments can only slow or, at best, prevent CHF progression but cannot restore functional myocardial tissue.^{3–5} Thus, the unmet clinical need for myocardial repair after irreversible ischaemic injury requires the development of new treatment approaches.

One of the options available is cell therapy based on delivery of living autologous or allogeneic stem cells into the injured heart.⁶ Currently, the following main types of stem cells have been used for cell therapy of MI: embryonic stem cells (ESCs), hematopoietic CD34+ stem cells (HSCs), induced pluripotent stem cells (iPSCs) and

mesenchymal stem cells (MSCs). Preclinical studies demonstrated that ESC-derived exosomes can augment function in infarcted hearts⁷; however, clinical use of ESCs is still discouraged due to various limitations, such as increased risk of tumour formation, high rejection rates and ethical concerns.^{8–10} Tissue sources of HSCs and MSCs include bone marrow, peripheral blood and umbilical cord blood, which are more readily available for processing and clinical applications. HSC studies demonstrate the lack of transdifferentiation into cardiac cells in ischaemic tissue.¹¹ In addition, it is known that DNA repair system is deficient in HSCs, thereby leading to accelerated cell ageing followed by mutations and gonadal dysfunction.¹² It is suggested that the efficacy of HSCs is explained by their ability to migrate directly to sites of injury.¹³ The use of iPSCs and MSCs raises less ethical concerns in comparison to ESCs and HSCs. Nuclear reprogramming of iPSCs can result in cardiogenic differentiation and formation of contracting cardiomyocyte sheet.¹⁴ Nonetheless, detailed studies of iPSCs indicate

that delivery of iPSC-derived cardiomyocytes may not be entirely safe because of increased risk of tumorigenicity and both genetic and epigenetic abnormalities.¹⁵ Among the different types of stem and progenitor cells, MSCs have been most intensively studied in terms of MI therapy.¹⁶ MSCs are defined as multipotent stem cells that are of mesodermal origin and also have the following properties: they can differentiate into adipose, bone and cartilage cells; readily adhere to plastic surfaces; express CD105, CD73 and CD90; and do not express CD34, CD45, CD14, CD11b, CD79, CD19 or human leucocyte antigen-antigen D related.¹⁷ Although the antiremodelling effect of MSC transplantation after MI has been demonstrated in numerous experimental and clinical studies (for review, see^{18,19} and references therein), there is no coherent interpretation of the mechanisms involved.²⁰

At present, several lines of evidence explain improved left ventricular (LV) function after treatment with MSCs (Figure 1). First, it is suggested that transplanted MSCs release numerous cytokines and paracrine factors that promote angiogenesis,²¹ activate cytoprotective pathways in reversibly injured cardiomyocytes,^{22,23} prevent fibrosis and LV dilation²⁴ and stimulate proliferation/differentiation of resident cardiac stem cells.^{25,26} Second, MSCs can differentiate into endothelial and smooth muscle cells, thereby directly contributing to angio- and arteriogenesis.^{27,28} Third, it cannot be ruled out that transplanted MSCs can differentiate into cardiac myocytes.^{29,30} The relative contribution of each mechanism to the overall antiremodelling effect is currently unknown.³¹

Recent meta-analyses of randomized clinical trials on MSC-based therapy of MI and CHF have demonstrated good safety, but have also highlighted the need for improving efficacy because the increase in LV systolic function is only 3–10%.^{32–34} One of the main factors limiting the effectiveness of stem cell-based therapy of MI is the low survival rate of engrafted cells. In 2006, Freyman et al.³⁵ showed that only ~3% of administered MSCs remained viable at 14 days after

treatment in a porcine model of MI, and injection of labelled MSCs in the LV cavity in mice showed the presence of only 0.44% of the cells in the heart at 4 days after treatment.²⁸ Several mechanisms may account for these extremely high cell death rates.³⁶ A certain proportion of cells can die during preparation of the cell suspension because of impaired mechanisms of integrin-mediated survival.³⁷ Loss of intercellular communication and disruption of the contacts between cells and the extracellular matrix can stimulate proapoptotic signalling, resulting in a specific form of cell death termed anoikis.³⁸ Programmed cell death mechanisms could also be activated in transplanted MSCs secondary to reactive oxygen species (ROS)-mediated inhibition of MSCs anchoring to matrix proteins of the recipient heart.^{39,40} After transplantation into the infarct and/or peri-infarct area, MSCs are subjected to different potent stressors, including hypoxia, acidosis, nutrient deficiency, ROS, inflammatory mediators and cytotoxic agents (Figure 2).⁴¹ Post-infarct inflammation is associated with massive infiltration of necrotic tissue with neutrophils, monocytes and macrophages.⁴² These inflammatory cells produce additional amounts of ROS and proinflammatory cytokines, which can cause necrosis and apoptosis of transplanted MSCs.⁴³ MSCs, in comparison with other types of stem cells, can better withstand the inflammatory and immune injury in the recipient heart owing to their immunosuppressive and immunomodulatory properties.^{44,45}

It follows, therefore, that successful clinical application of MSCs for cardiac repair after MI requires additional research effort aimed at increasing the survival of transplanted MSCs. Other goals of MSC modification using genetic targeting as well as physical and chemical conditioning include (i) reduction of rejection rates, (ii) stimulation of proliferation/differentiation and (iii) augmentation of paracrine effects.⁴⁶ This review highlights the latest progress in terms of MSC modification and provides focus areas for the development of next-generation cell products for MI treatment.

FIGURE 1 Putative mechanisms underlying the cardioreparative effects of mesenchymal stem cells (MSCs) after myocardial infarction. Several possible mechanisms, including differentiation in smooth muscle cells and endothelial cells, secretion of paracrine factors and differentiation in cardiomyocytes, can explain the improved left ventricular function noted after treatment with MSCs

FIGURE 2 Microenvironmental factors affecting mesenchymal stem cell (MSCs) in the recipient myocardial tissue. After transplantation, MSCs are subjected to hypoxia, acidosis, nutrient deficiency, oxidative stress, inflammatory mediators and cytotoxic agents

2 | GENETIC MODIFICATION OF MSCS

2.1 | Increased MSC survival and proliferation

The fate of the transplanted MSCs depends on the balance between hypoxia-induced proapoptotic signalling, primarily mediated through Toll-like receptor 4 (TLR4) and G protein-coupled receptors, and pro-survival pathways, including the phosphoinositide 3-kinase (PI3K), protein kinase B (Akt) and extracellular signal-regulated kinase 1/2 (ERK1/2) pathways. The results of the studies on genetic modification of MSCs aiming towards increased survival and proliferation are summarized in Table 1. It is quite difficult, however, to make direct comparison of the results obtained in different studies because of the varying techniques of cell viability assessment: exposure of the cells in vitro to hydrogen peroxide,⁴⁷ hypoxic stress in vitro⁴⁸ and apoptosis rate in the peri-infarct area.⁴⁹

The first successful attempt at retroviral Akt1 gene transduction in MSCs was performed by Mangi et al.⁵⁰ in 2003. Intramyocardial transplantation of Akt-overexpressing MSCs in rats provided greater functional benefits and infarct size reduction than non-transduced cells did. Similar results were later obtained after intracoronary administration of Akt-transduced MSCs in a porcine model of myocardial ischaemia-reperfusion.⁴⁷ Similar to genetic approaches enhancing prosurvival signalling, targeted deletion of the proapoptotic TLR4 was found to result in decreased hypoxia-induced apoptosis of mouse MSCs,⁴⁸ increased production of angiogenic factors and increased cardioprotective effects.⁵¹ Significant improvement in MSC survival was noted in several studies after transfection of MSCs with the haem oxygenase-1 (HO-1) gene.⁵² In addition, transplantation of HO-1-expressing MSCs in the ischaemic heart resulted in decreased LV remodelling and increased cardiac function. HO-1 or heat shock protein (HSP) 32 catalyses the conversion of haem to carbon monoxide, biliverdin and free

iron; this enzyme plays a crucial role in cytoprotection and is involved in the cardiac ischaemic preconditioning response. Overexpression of other heat shock proteins such as HSP27 and HSP20 has been also shown to increase MSC survival, reduce apoptosis and improve the LV ejection fraction.^{49,53}

Overexpression of the transcriptional factor GATA-4 in rat MSCs resulted in increased production of angiogenic factors, increased assembly of human umbilical vein endothelial cells into capillary-like tubes after treatment with GATA-4-MSC-conditioned medium, and decreased myocardial scar size in an in vivo model of MI.⁵⁴

Intramyocardial administration of MSCs transfected with vectors encoding antiapoptotic proteins such as Bcl-2,⁵⁵ Bcl-xL,⁵⁶ Connexin 43⁵⁷ and survivin⁵⁸ has been found to result in moderate improvement of the LV ejection fraction in rodents because of increased MSC survival, increased secretion of vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF) and platelet-derived growth factor (PDGF), as well as improved neovascularization. Genetic modifications targeting apoptotic genes might increase the risk of tumour development, and they should, therefore, be tested more rigorously in long-term experiments. Hypoxia-inducible factor 1- α (HIF-1 α) is another important prosurvival transcription factor that regulates a battery of genes responsible for increased resistance to hypoxia. Hyperexpression of HIF-1 α in MSCs along with their subsequent intracardiac injection in infarcted rats results in improved cell survival, increased cell adhesion, decreased myocardial fibrosis and increased cardiac contractile function.⁵⁹

Tissue kallikrein (TK) is a protease that generates the cardioprotective vasodilator peptides bradykinin and kallidin. Adenoviral transduction of MSCs with the TK gene decreases the hypoxia-induced apoptosis rate and reduces caspase-3 activity in the cells.⁶⁰ In in vivo experiments, transplantation of TK-hyperexpressing MSCs in infarcted rats resulted in decreased inflammation, improved cardiac function

TABLE 1 Gene overexpression/knockout studies investigating survival and/or proliferation of MSCs. i.m., intramyocardial administration; i.c., intracoronary infusion; N/A, not applicable

Gene (protein)	Gene function/animal species/model	Route of administration	Main results	Ref.
Akt1 (Akt)	Overexpression/rat/MI	i.m.	↑ Cardiac function, ↑ myocardial salvage	50
Akt1 (Akt)	Overexpression/pig/MI	i.c.	↑ LV ejection fraction, ↓ infarcted area, ↑ resistance to apoptosis	47
TLR4 (TLR4)	Knockout/mouse/MSC culture	N/A	↓ Hypoxia-induced apoptosis	48
TLR4 (TLR4)	Knockout/mouse/MSC culture and myocardial ischaemia in the isolated rat heart	i.c.	↑ Angiogenic factor production, ↑ cardioprotection	51
HMOX1 (HO-1)	Overexpression/mouse/MSC culture and MI	i.m.	↓ Apoptosis, ↑ MSC survival, ↓ LV remodelling, ↑ LV function	52
HSPB1 (HSP27)	Overexpression/rat/ MSC culture and MI	i.m.	↑ MSC survival, ↓ apoptosis, ↑ cardiac function	49
HSPB6 (HSP20)	Overexpression/rat/ MSC culture and MI	i.m.	↓ ROS-induced apoptosis, ↑ secretion of VEGF, FGF, IGF-1, ↓ fibrosis, ↑ angiogenesis, ↑ LV ejection fraction	53
GATA4 (GATA-4)	Overexpression/rat/MSC culture and MI	i.m.	↑ Expression of angiogenic factors, ↑ MSC survival, ↑ in vitro angiogenesis, ↓ infarct size, ↑ cardiac function	54
BCL2 (Bcl-2)	Overexpression/rat/ MSC culture and MI	i.m.	↓ Apoptosis, ↑ VEGF secretion, ↑ MSC survival in vivo, ↓ infarct size, ↑ cardiac function	55
BCL2L1 (Bcl-xL)	Overexpression/rat/ MSC culture and MI	i.m.	↓ In vitro and in vivo apoptosis, ↑ secretion of VEGF, IGF, PDGF, ↑ angiogenesis, ↑ cardiac fraction	56
GJA1 (Connexin43)	Overexpression/rat/MSC culture and MI	i.m.	↑ Tolerance to hypoxia, ↑ MSC survival in vivo, ↓ infarct size, ↑ cardiac function	57
BIRC5 (Survivin)	Overexpression/rat/MSC culture and MI	i.m.	↑ Secretion of VEGF, ↑ MSC survival in vivo, ↑ angiogenesis, ↑ cardiac fraction, ↓ infarct size	58
HIF1A (HIF-1α)	Overexpression/rat/MSC culture and MI	i.m.	↑ Cell adhesion and migration, ↑ expression of paracrine factors, ↑ cardiac fraction, ↑ angiogenesis	59
KLK1 (Tissue kallikrein)	Overexpression/rat/MSC culture and MI	i.m.	↓ Apoptosis in vitro, ↑ cardiac function, ↓ infarct size, ↓ inflammation in vivo	60
MDK (Midkine)	Overexpression/rat/MSC culture and MI	i.m.	↓ Apoptosis, ↑ expression of VEGF, TGF-β, IGF-1, SDF-1α in vitro, ↑ cardiac function in vivo	61
MIR1-1 (MiR-1)	Overexpression/mouse/MI	i.m.	↑ MSC survival in vivo, ↑ cardiac function	62
MIR133A1 (MiR-133a)	Overexpression/rat/MSC culture and MI	i.m.	↑ MSC survival in vitro, ↑ cardiac function, ↓ fibrosis	63
MIR210 (MiR-210)	Overexpression/human/MSC culture	N/A	↑ MSC survival, ↑ ERK and Akt activity	65
MIR23A (MiR-23a)	Overexpression/rat/MSC culture and MI	i.m.	↓ MSC apoptosis in vitro, ↑ LV function, ↓ infarct size in vivo	64
MIRLET7B (MiR Let-7b)	Overexpression/rat/MSC culture and MI	i.m.	↑ Expression of p-MEK, p-ERK, Bcl-2 in vitro, ↓ expression of caspase-3, ↑ cardiac function, ↓ infarct size, ↑ angiogenesis in vivo	67
MIR34A (MiR-34)	Overexpression/mouse/MI	i.m.	↓ LV function, ↑ fibrosis, ↓ vessel density	12

and decreased scar size. Similar effects were observed in rats treated with midkine-transduced MSCs.⁶¹

MicroRNAs can specifically recognize homologous transcripts and inhibit their translation through several mechanisms. Therefore, genetic modification of MSCs resulting in increased expression of certain microRNAs may elicit multiple effects. For example, injection of MSCs hyperexpressing microRNA-1 in the peri-infarct area in mice is associated with increased in vivo MSC survival and improved cardiac function.⁶² Similar data were obtained in vitro and in vivo on

using MSCs overexpressing microRNA-133a⁶³ and microRNA-23a.⁶⁴ microRNA-210 overexpression led to increased in vitro survival of human MSCs, both in hypoxic⁶⁵ and ROS-enriched environments.⁶⁶

Transduction of MSCs with the microRNA let-7b results in increased expression of phospho-mitogen-activated protein kinase, phospho-ERK and Bcl-2 in vitro.⁶⁷ Injection of let-7b-overexpressing MSCs in the peri-infarct area is associated with decreased caspase-3 expression, improved contractile performance and smaller scar size relative to that in control rats. Some microRNAs may have a negative

impact on post-MI recovery via inactivation of growth factor transcripts. For example, microRNA-34 is known to inactivate or knock down the mRNA for stem cell factor, which stimulates MSC differentiation in endothelial cells with the formation of new capillaries. Thus, intramyocardial transplantation of microRNA-34-overexpressing bone marrow-derived MSCs in mice was found to result in increased infarct size, decreased LV function and decreased capillary density.¹²

2.2 | Stimulation of MSC differentiation and integration in the host tissue

The cells in ischaemic myocardial tissue produce increased amounts of various chemotactic factors and chemokines. The processes of MSC homing and integration into the recipient cardiac tissue are governed by interaction between several key chemokines/corresponding receptors such as chemokine (C-X-C motif) ligand 1 (CXCL1)/CCR1,⁶⁸ stromal cell-derived factor-1 (SDF-1)/CXCR4⁶⁹ and hepatocyte growth factor (HGF)/c-met.⁷⁰ Several studies have shown improved MSC integration in myocardial tissue and differentiation due to overexpression of chemokines and other regulatory proteins (Table 2). In particular, CCR1 overexpression in mouse MSCs resulted in increased migration and survival, as well as decreased infarct size and cardiac function, in an MI model.⁶⁸ Likewise, CXCR4,^{71,72} SDF-1⁷³ and HGF^{70,74} hyperexpression were found to improve homing of MSCs in the peri-infarct area, attenuate LV remodelling and improve contractility. In addition, intramyocardial injection of HGF-MSC in infarcted pigs resulted in decreased density of sympathetic nerve endings,

better electrical stability of the heart and decreased arrhythmias.⁷⁵ Integrin-linked kinase (ILK) directly interacts with integrin molecules, thereby playing an indispensable role in integrin-mediated cell adhesion and signalling. ILK overexpression was found to be associated with increased proliferation of MSCs, decreased in vitro and in vivo apoptosis, and improved LV function in a porcine model of MI^{76,77} and also prevented anoikis in cultured human MSCs.^{76,77} Hyperexpression of the cytoprotective enzyme glycogen synthase kinase-3 β (GSK-3 β) and angiogenic factor angiogenin also resulted in amelioration of cardiac function and increased angiogenesis in rodents.^{78,79} Increase in the engraftment rate and cardiomyogenic differentiation was noted after transplantation of human myocardin-overexpressing MSCs into immunodeficient mice with MI.⁸⁰ In that study, although the MSCs had not acquired the cardiomyocyte phenotype, the expression of at least one of the three cardiac markers analysed was detected in all cases, which was associated with moderate increase in LV function.

2.3 | Stimulation of paracrine factor production

To date, MSC secretome analysis has identified >200 unique proteins.^{81,82} Many MSC-derived paracrine factors can promote myocardial regeneration after MI through prevention of ongoing cell death, stimulation of angiogenesis and activation of cardiac stem cells.³⁰ In addition, MSC-specific growth factors and cytokines can modulate processes such as post-infarct inflammation, fibrosis, myocardial contractility and metabolism.^{22,30} The most important components of the MSC secretome implicated in myocardial regeneration include VEGF,

TABLE 2 Main studies showing improved MSC differentiation and integration in myocardial tissue due to genetic modification of MSCs. i.m., intramyocardial administration; i.c., intracoronary infusion; i.v., intravenously; N/A, not applicable

Gene (protein)	Gene function/animal species/model	Route of administration	Main results	Ref.
CCR1 (CCR1)	Overexpression/mouse/MSC culture and MI	i.m.	↑ Migration, ↓ in vitro apoptosis, ↓ infarct size, ↑ angiogenesis, ↑ cardiac function	68
CXCL12 (SDF-1)	Overexpression/rat/MSC culture and MI	i.m.	↓ In vitro apoptosis, ↑ homing and viability, ↑ HGF expression, ↑ cardiac function	73
CXCR4 (CXCR4)	Overexpression/rat/MI	i.v.	↑ Migration, ↑ angiogenesis, ↑ cardiac function	71
CXCR4 (CXCR4)	Overexpression/rat/MI	i.v.	↑ Homing, ↑ cardiac function, ↓ fibrosis	72
HGF (HGF)	Overexpression/rat/MI	i.m.	↑ Angiogenesis, ↓ scar size, ↑ cardiac function	70
HGF (HGF)	Overexpression/pig/MI	i.c.	↑ Perfusion, ↑ LV ejection fraction, ↓ apoptosis, dose dependency	74
HGF (HGF)	Overexpression/pig/MI	i.v.	↑ Angiogenesis, ↓ apoptosis, ↓ sympathetic nerve endings, ↓ arrhythmia	75
ILK (ILK)	Overexpression/human/MSC culture	N/A	↓ Anoikis	76
ILK (ILK)	Overexpression/pig/MSC culture and MI	i.m.	↑ Proliferation, ↓ in vitro and in vivo apoptosis, ↓ LV remodelling, ↑ cardiac function	77
GSK3B (GSK-3 β)	Overexpression/mouse/MI	i.m.	↑ Differentiation, ↑ angiogenesis, ↑ VEGF expression, ↓ LV remodelling, ↑ cardiac function	78
ANG (Angiogenin)	Overexpression/rat/MSC culture and MI	i.m.	↓ In vitro apoptosis, ↑ angiogenesis, ↑ cardiac function	79
MYOCD (Myocardin)	Overexpression/human/mouse MI	i.m.	↑ Engraftment, ↑ cardiomyogenic differentiation, ↑ cardiac function	80

HGF, PDGF, SDF-1, fibroblast growth factor β (FGF β), transforming growth factor β (TGF β), angiopoietin 1 (Ang-1), granulocyte colony-stimulating factor (G-CSF) and interleukin-8 (IL-8).⁸³ Given the pivotal role played by paracrine factors in MSC-mediated cardiac regeneration, numerous studies have used transduction of MSCs with vectors expressing growth factors and cytokines (Table 3). In particular, intramyocardial transplantation of Ang-1^{79,84}, HGF^{85,86}, and IGF-1⁸⁷-overexpressing MSCs after MI was found to result in improved cardiac function, decreased infarct size and increased angiogenesis. Notably, most of these genetic modifications resulted in the activation of PI3K-Akt signalling both in the transplanted MSCs and in the recipient heart, underscoring the functional significance of this pathway in the protective effects of MSCs.⁸⁸ Several studies showed that transplantation of VEGF-overexpressing MSCs resulted in better vascularization of the infarcted area of the heart, lesser infarct size and improved contractility in comparison with the findings for controls treated with intact MSCs.^{89–91} Transduction of MSCs with the prostaglandin I₂ synthase (PGIS) gene with subsequent transplantation into the peri-infarct area in mice resulted in reduced oxidative stress, increased systolic LV function, and reduced remodelling, relative to those noted in the group treated with intact MSCs.⁹² The beneficial effects of PGIS overexpression in MSCs might be explained by increased production of prostacyclin, an eicosanoid with well-documented vasodilatory and cytoprotective properties.

Although MSCs improve myocardial contractility and contribute to regeneration of endothelial cells in injured arteries, native MSCs can induce neointimal proliferation through direct or indirect effects on vascular smooth muscle cells (VSMCs). Receptor activity-modifying protein 1 (RAMP1) is one of the main determinants of ligand specificity of calcitonin gene-related peptide (CGRP), which is strongly involved in the regulation of proliferation and apoptosis. The ability of exogenous RAMP1 to potentiate the antiproliferative effect of CGRP in VSMCs has been used by Shi et al.⁹³ as the rationale for transducing MSCs with the hRAMP1 gene and for their testing in rabbit models of carotid artery injury and MI. Intravenous administration of

hRAMP1-overexpressing MSCs was found to be associated with improved cardiac function, decreased infarct size, diminished area of the neointima and decreased intima/media ratio vs those obtained with native MSCs. Genetic hyperexpression of paracrine factors in MSCs can have pleiotropic effects, which can be exemplified by the fact that intramyocardial transplantation of SDF-1 α -hyperexpressing MSCs significantly increases myocardial HGF expression.⁷³

Altered expression profiles of certain paracrine factors in MSCs can arise from non-genetic causes such as the age of the donor animal. Liang et al.⁹⁴ found higher expression of pigment epithelium-derived growth factor (PEDF) in MSC culture obtained from 18-month-old mice than that from 8-week-old animals. Unsurprisingly, MSCs obtained from old animals demonstrated lesser therapeutic benefits in the MI model than did the same dose of MSCs obtained from young mice. However, increased PEDF production in young donor-derived MSCs abolished the benefit observed, whereas suppression of PEDF production in old donor-derived MSCs strongly enhanced their protective effect. Therefore, manipulation of PEDF expression may be an interesting tool for improving the efficacy of MSCs, especially in MSCs obtained from adult and old donors.

From the data considered so far, it follows that genetic modification of MSCs resulting in hyperexpression of antiapoptotic/prosurvival proteins, transcription factors, chemokines/chemokine receptors, cytokines and growth factors can substantially increase the cardioreparative effects of MSC-based therapy of myocardial ischaemic injury.

3 | MSC PRECONDITIONING WITH PHYSICAL AND CHEMICAL ENVIRONMENTAL FACTORS

3.1 | Anoxia and severe hypoxia

While bone marrow-derived MSCs are typically cultured under normoxic conditions (21% O₂), their *in vivo* microenvironment is characterized by significantly lower oxygen tension. Moreover, myocardial tissue

TABLE 3 Gene overexpression studies investigating modulation of secretion of key paracrine factors by MSCs. i.m., intramyocardial administration; N/A, not applicable

Gene (protein)	Gene function/animal species/model	Route of delivery	Main results	Ref.
Ang1 (Ang1)	Overexpression/rat/MI	i.m.	↑ Vascular density, ↑ cardiac function, ↓ infarcted area	84
Ang1 (Ang1)	Overexpression/rat/ MSC culture and MI	i.m.	↑ Tolerance to hypoxia (in vitro), ↑ vascular density, ↑ cardiac function	79
Hgf (HGF)	Overexpression/rat/MI	i.m.	↑ Vascular density, ↓ collagen content, ↑ cardiac function	85
Hgf (HGF)	Overexpression/rat/MI	i.m.	↓ Apoptosis, ↓ infarct scar size, ↓ interstitial fibrosis, ↑ vascular density, ↑ cardiac function	86
Igf-1 (IGF-1)	Overexpression/rat/ MSC culture and MI	i.m.	↑ MSC survival, ↓ infarcted area, ↑ vascular density	87
Vegf (VEGF)	Overexpression/rat/ MSC culture and MI	i.m.	↑ MSC survival (in vitro), ↑ vascular density, ↓ LV remodelling	89
Vegf (VEGF)	Overexpression/rat/ MSC culture and MI	i.m.	↑ Vascular density, ↑ cardiac function	91
PGIS (PGIS)	Overexpression/mouse/MI	i.m.	↑ Cardiac function, ↓ fibrosis	92
hRAMP1	Overexpression/rabbit/MI and carotid artery injury	i.v.	↑ Cardiac function, ↓ infarct size, ↓ neointima, ↓ proliferation of vascular smooth muscle cells	93

oxygen tension in the peri-infarct area, which is a typical site for MSC administration in preclinical models, usually does not exceed 1%.⁹⁵ As such, several research groups have used extended periods of anoxia/severe hypoxia (1–3%) to precondition MSCs prior to their transplantation.^{96–98} In general, transplantation of hypoxia-preconditioned MSCs results in lesser infarct size and greater capillary density than that obtained with MSCs cultured under normoxic conditions (Table 4). The mechanisms underlying this phenomenon include hypoxia-mediated activation of the PI3K-Akt signalling pathway⁹⁹ and increased expression of angiogenic and antiapoptotic factors, such as HIF-1 α , angiopoietin 1, VEGF/VEGF receptors, erythropoietin, Bcl-2 and Bcl-xL.⁹⁷

3.2 | Hyperoxia and hydrogen peroxide

Interestingly, hyperoxia (100% O₂) can also precondition MSCs. Exposure of MSCs to excessive oxygen results in increased cell viability, possibly because of decreased caspase expression and increased expression of Akt1 κ B and Bcl-2.¹⁰⁰ Oxidative stress preconditioning can also be simulated by exposure of MSCs to hydrogen peroxide; compared with the findings for intact MSCs, dramatically higher interleukin-6 (IL-6) expression associated with increased MSC migration and proliferation, improved vascularization of the peri-infarct area and decreased myocardial fibrosis was noted after transplantation of these conditioned MSCs.¹⁰¹

3.3 | Hydrogen sulphide

Similar results have been obtained after priming of MSCs with the gasotransmitter molecule hydrogen sulphide, which increases MSC survival rates and Akt, Erk1/2 and GSK-3 β expressions.¹⁰²

4 | PHARMACOLOGICAL CONDITIONING OF MSCS

The main studies on MSC conditioning with pharmacological agents are summarized in Table 5.

4.1 | Angiotensin receptor blockers

Intramyocardial transplantation of angiotensin receptor blocker-pretreated human MSCs was found to result in better cardiomyogenic differentiation and LV function than those obtained with non-treated MSCs in vivo in nude rats after MI.¹⁰³ In another study, incubation of MSCs with valsartan suppressed their adipogenic differentiation; however, the effect on cardiomyogenic differentiation was not investigated.¹⁰⁴

4.2 | Trimetazidine

The metabolic cytoprotective agent trimetazidine prevents hypoxia- and serum deprivation-induced apoptosis of MSCs in vitro via activation of the Akt pathway.¹⁰⁵ In vivo studies on infarcted rats demonstrated that trimetazidine-preconditioned MSCs were more effective in improving cardiac function and decreasing infarct size than non-preconditioned cells.^{106,107} The underlying mechanisms may include decreased expression of proinflammatory cytokines, activation of the Akt pathway and increased expression of Bcl-2.

4.3 | Nicorandil and diazoxide

The adenosine triphosphate-sensitive potassium channel openers nicorandil and diazoxide were found to decrease MSC apoptosis in vitro and in vivo, respectively, after transplantation.^{108,109} In addition, intramyocardial delivery of diazoxide-treated MSCs was associated with smaller infarct size and better cardiac function than those noted for non-treated MSCs in a rat model of MI.¹⁰⁸

4.4 | Lipopolysaccharide

Intramyocardial transplantation of lipopolysaccharide-primed vs native MSCs resulted in increased MSC survival, enhanced LV function and decreased fibrosis, possibly because of increased VEGF expression and PI3K/Akt pathway activation.¹¹⁰

TABLE 4 Main studies on MSC preconditioning with physical and chemical environmental factors. i.m., intramyocardial administration; i.v., intravenous infusion; N/A, not applicable

Factor	Animal species/model	Route of delivery	Main results	Ref.
Anoxia	Rat/MI	i.m.	↑ Cardiac function, ↓ infarct size, ↑ arteriole density	96
Hypoxia (0.5% O ₂)	Mouse/MSC culture and MI	i.m.	↑ Expression of pro-survival and pro-angiogenic factors in vitro, ↑ angiogenesis in vivo	97
Anoxia	Rat/diabetic cardiomyopathy	i.m.	↑ Cardiac function, ↑ capillary density, ↓ myocardial fibrosis	98
Hypoxia (1–3% O ₂)	Human/MSC culture	N/A	↑ PI3K-Akt signalling pathway, ↑ expression of cMet, HGF	99
Hyperoxia (100% O ₂)	Rat/MSC culture	N/A	↓ Expression of caspases 1, 3, 6, 7, 9, ↑ expression of Akt1, NF- κ B, Bcl-2, ↓ apoptosis	100
Hydrogen peroxide	Mouse/MI	i.v.	↑ Expression of IL-6 →, ↑ migration and proliferation of endothelial cells, ↑ neovascularization	101
Hydrogen sulphide	Rat/MI	i.m.	↑ MSC survival, ↑ cardiac function, ↓ infarct size	102

4.5 | Melatonin

In MSC culture, addition of melatonin contributes to increased MSC survival rates, decreased ROS generation and a decreased Bax/Bcl-2 ratio.¹¹¹ These data fit well with the findings of Han et al.,¹¹² which showed that melatonin pretreatment causes decrease in MSC apoptosis, oxidative stress, and inflammation and improvement in cardiac function after MI.

4.6 | Other substances

Amelioration of cardiac function in rodent models of MI was also noted after transplantation of MSCs treated with bioactive substances, such as angiotensin II,¹¹³ salvianolic acid,¹¹⁴ oxytocin,¹¹⁵ pioglitazone,¹¹⁶ neuropeptide Y¹¹⁷ and atorvastatin.^{118–120}

5 | MSC CONDITIONING WITH CYTOKINES, GROWTH FACTORS AND OTHER PROTEINS, AS WELL AS COCULTURE AND FUSION OF MSCS WITH OTHER CELLS

Pretransplantation “shaping” of the MSC phenotype by means of their *in vitro* exposure to various protein ligands has been explored in numerous studies (Table 6). For example, incubation of rat MSCs with insulin-like growth factor-1 (IGF-1) was found to lead to increased CXCR4 expression, increased MSC survival, apoptosis inhibition and stimulation of anti-inflammatory activity of MSCs.^{121,122} In *in vivo* settings, decreased post-infarct LV remodelling and improved function were noted after intravenous or intramyocardial administration of IGF-1-treated MSCs, in comparison with the findings for intact MSCs.^{121,122} Likewise, incubation of MSCs with SDF-1 results in increased cell viability and proliferation *in vitro* and improved post-MI LV function *in vivo*.¹²³ Decrease in the serum deprivation-induced MSC apoptosis rate together with increase in MSC survival under hypoxic conditions were observed after incubation of rat MSCs with HSP90.¹²⁴ Hahn et al.¹²⁵ treated rat bone marrow-derived MSCs with a cocktail of growth factors, including IGF-1, FGF-2 and bone morphogenetic protein-2 (BMP-2). This kind of priming not only increased MSC survival and differentiation *in vitro* but also decreased infarct size in the *in vivo* MI model; it also improved LV function and enhanced gap junction formation of transplanted MSCs, which, however, was not associated with increased arrhythmia incidence.

In a somewhat analogous approach, Peng et al.¹²⁶ used platelet-rich plasma clot releasate (PRCR) as a natural source of various growth factors for stimulation of MSC survival and functionality. Decreased H₂O₂-induced apoptosis rates, increased VEGF and PDGF expression, and activation of PI3K/Akt/NF- κ B signalling were noted for PRCR-primed MSCs. A combination of two proinflammatory cytokines, namely interleukin-1 β (IL-1 β) and tumour necrosis factor- α (TNF- α), was found to cause increase in vascular cell adhesion molecule 1 (VCAM-1) expression in MSCs and in adhesion intensity.¹²⁷ These *in vitro* findings were corroborated by decreased infarct size

and improved LV function after intramyocardial transplantation of IL-1 β /TNF- α -treated MSCs *in vivo* after MI. Greater cardiomyogenic differentiation of MSCs was observed after their incubation with autologous serum in the presence of TGF- β 1 vs the findings for standard culture medium with or without foetal bovine serum.¹²⁸ Cardiogenic differentiation was confirmed by an increased number of beating areas and expression of cardiac-specific genes such as GATA4 and cardiac troponin T.

Another promising approach to *ex vivo* adaptation of MSCs to the potentially hostile microenvironment of the ischaemic heart is their seeding on polyacrylamide gels containing extracellular matrix proteins isolated from decellularized healthy and infarcted rat hearts harvested at different time points after coronary artery ligation.¹²⁹ This study showed that extracellular matrix proteins stimulated the expression of several proangiogenic, prosurvival, antifibrotic and immunomodulatory growth factors in MSCs. The remodelled matrix stimulated the expression of certain cardiac markers (eg, GATA4) in MSCs; however, this was associated with limited expression of other markers such as Nkx2.5. Molecular signals driving cardiogenic MSC differentiation could also be released during coculture of MSCs with neonatal rat cardiac myocytes.¹³⁰ In this study, the number of MSCs expressing cardiac markers (α -sarcomeric actin, troponin I, myocyte-specific enhancer factor 2C) was directly correlated with the amount of neonatal myocytes in coculture.

An extremely new approach to enhancing the functionality of stem cells before transplantation is the formation of cell hybrids produced by fusion of MSCs and cardiac progenitor cells (CardioChimeras).¹³¹ In a murine model of MI, intramyocardial transplantation of CardioChimeras enabled greater reduction in infarct size and improvement of LV function relative to the findings obtained with combinatorial and individual cell population-treated groups.

6 | TISSUE ENGINEERING AND MULTICELLULAR PREPARATIONS OF MSCS FOR CARDIAC REPAIR

The routine procedure for MSC preparation and intramyocardial administration is characterized by several drawbacks. First, MSC detachment from the collagen surface with trypsin/ethylenediaminetetraacetic acid can cause partial degradation of the extracellular matrix and loss of intercellular contacts, thereby predisposing the cells to anoikis. Second, intramyocardial injection of cell suspensions may itself contribute to MSC injury and disintegration, followed by leakage of the cell preparation from the delivery channel. Third, since MSCs are usually injected in several discrete sites of the peri-infarct area, the procedure commonly results in inhomogeneous distribution of cells.

To overcome these problems, many research groups focused on the development of three-dimensional polymeric scaffolds for MSCs. Seeding of MSCs on these supporting biomaterials facilitates the formation of both intercellular connections and contacts between the cells and extracellular matrix, thereby enhancing cell viability and function. A discussion of *ex vivo* studies describing the effects of different

TABLE 5 Main studies on MSC conditioning with pharmacological agents. i.m., intramyocardial administration; i.v., intravenous infusion; N/A, not applicable

Pharmacological agent	Animal species/model	Route of delivery	Main results	Mechanism(s) of action	Ref.
Candesartan	Nude rat/MSC culture and MI	i.m.	↑ Cardiac function	↑ Cardiogenic differentiation	103
Valsartan	MSC culture	N/A	↓ Adipogenic differentiation	—	104
Trimetazidine	MSC culture	N/A	↓ Apoptosis	Activation of Akt pathway	105
Trimetazidine	Rat/MI	i.m.	↓ Inflammation, ↓ apoptosis, ↑ cardiac function, ↓ infarct size	↓ Inflammatory cytokines, ↓ Bax, ↑ Bcl-2	106
Trimetazidine	Rat/MSC culture and MI	i.m.	↑ Cellular viability, ↑ metabolic activity in vitro, ↑ cardiac function in vivo	Activation of Akt pathway, ↑ Bcl-2 expression	107
Diazoxide	Rat/MI	i.m.	↑ MSC survival, ↓ infarct size, ↑ cardiac function	Opening of mitochondrial ATP-sensitive potassium channel →, ↓ apoptosis	108
Nicorandil	MSC culture	N/A	↑ MSC survival	Opening of mitochondrial ATP-sensitive potassium channel → ↓ apoptosis	109
Lipopolysaccharide	Rat/MI	i.m.	↑ MSC survival, ↑ cardiac function, ↓ fibrosis, ↑ vascular density	↑ Expression of VEGF, activation of PI3K/Akt pathway	110
Melatonin	Mice/MSC culture and MI	i.m.	↑ MSC survival, ↑ cardiac function, ↓ inflammation, ↓ apoptosis, ↓ oxidative stress	↑ SIRT1 signalling, ↑ expression of Bcl2	112
Melatonin	MSC culture	N/A	↑ MSC survival	↓ ROS, ↓ ratio of Bax/Bcl-2	111
Angiotensin II	Rat/MI	i.m.	↑ Cardiac function, ↓ fibrosis, ↓ infarct size	↑ VEGF, ↑ gap junction formation	113
Salvianolic acid B	Rat/MI	i.m.	↑ Cardiac function, ↓ fibrosis, ↓ infarct size	↓ Apoptosis, ↑ angiogenesis	114
Neuropeptide Y	Rat/MSC culture and MI	i.m.	↑ Cardiac function, ↓ fibrosis, ↓ remodelling, ↑ angiogenesis	↑ Expression of FGF-2, SDF-1 α , cycline A2, ↑ cardiogenic differentiation	117
Atorvastatin	Rabbit/MI	i.m.	↑ Cardiac function, ↓ myocardial remodelling	↓ Cell apoptosis, ↑ cardiogenic differentiation	118
Atorvastatin	Rat/MI	i.m.	↑ Cardiac function, ↓ infarct size	↓ RhoA/ROCK signalling pathway	119
Atorvastatin	Swine/MI	i.m.	↑ Cardiac function, ↓ infarct size	↑ Nitric oxide synthase	120
Oxytocin	Rat/MI	i.m.	↑ Cardiac function	↑ Connexin 43, cTnI, α -sarcomeric actin	115
Pioglitazone	Rat/MSC culture and MI	i.m.	↑ Cardiac function	↑ Cardiogenic differentiation	116

biomaterials on MSC differentiation/function is beyond the scope of this chapter, but the interested reader is referred to several recent reviews.^{8,132} Here, we have focused on studies related to the transplantation of various ex vivo engineered multicellular MSC preparations in animal models of MI. On the basis of the available literature, the following classification of engineered multicellular MSC preparations might be proposed: (i) MSC monolayers, (ii) MSC spheroids, (iii) hydrogel scaffolds (natural/biological, synthetic and composite) and (iv) encapsulated MSCs.

6.1 | MSC monolayers

The use of MSC monolayers for cardiac repair after MI was pioneered by Miyahara et al.¹³³ in 2006. Seeding of rat adipose tissue-derived MSCs on a thermosensitive dish with subsequent transient cooling of confluent culture down to 20°C resulted in spontaneous detachment

of the cell sheet from the substrate. Application of the cell sheet onto the epicardium at 4 weeks after coronary ligation in rats resulted in reversal of scar thinning, improved LV function and increased animal survival. The cardioprotective effects of methylcellulose hydrogel-grown MSC sheet fragments after their intramyocardial injection were superior in comparison with those obtained with regular MSC treatments in rat¹³⁴ and porcine¹³⁵ models of MI. Placement of the MSC sheet on the surface of the heart at 4 weeks after MI in rats resulted not only in improved LV function but also in increased myocardial IGF-1, SDF-1 α , HIF-1 α and VCAM-1 expression.¹³⁶

6.2 | MSC spheroids

MSC spheroids are another form of multicellular MSC associations lacking any foreign material. Lee et al.¹³⁷ described spheroid 3D bullets consisting of human umbilical cord blood-derived MSCs, which

TABLE 6 Main studies on MSC conditioning with cytokines, growth factors, cytoprotective proteins and co-culture. i.m., intramyocardial administration; i.v., intravenous infusion; N/A, not applicable

Factor	Animal species/model	Route of delivery	Main results	Ref.
IGF-1	Rat/MI	i.v.	↑ CXCR4 expression, ↑ MSC survival, ↑ capillary density, ↓ LV remodelling, ↑ LV function	121
IGF-1	Rat/MI	i.m.	↑ MSC survival, ↓ inflammation, ↓ apoptosis, ↑ LV function	122
SDF-1	Rat/MSC culture and MI	i.m.	↑ Cell viability and ↑ proliferation in vitro, ↓ infarct size, ↓ fibrosis, ↑ LV function in vivo	123
IGF-1, FGF-2, BMP-2	Rat/MSC culture and MI	i.m.	↑ MSC survival and differentiation in vitro, ↓ infarct size, ↑ LV function, ↑ gap junction formation in vivo	125
HSP90	Rat/MSC culture	N/A	↓ Apoptosis, ↑ MSC survival	124
IL-1 β , TNF- α	Rat/MI	i.m.	↑ Expression of VCAM-1 and ↑ adhesion in vitro, ↓ infarct size and ↑ cardiac function in vivo	127
Autologous serum plus TGF- β 1	Rat/MSC culture	N/A	↑ MSC proliferation, ↑ cardiac genes expression	128
Extracellular matrix	Rat/MSC culture	N/A	↑ Cardiac differentiation, ↑ expression of HGF, SDF1	129
Co-culture with neonatal cardiomyocytes	Rat/MSC culture	N/A	↑ Expression of cardiac markers	130
Platelet-rich plasma clot releasate	Rat/MSC culture and skin wound	Injection in wound margins	↓ Apoptosis, ↑ expression of VEGF, PDGF, activation of PDGFR- α /PI3K/AKT/NF- κ B signalling in vitro, ↓ wound size and ↑ epithelization in vivo	126
Hybrid cells produced by fusion of MSCs and cardiac progenitor cells (CardioChimeras)	Mouse/MI	i.m.	↑ Persistence and engraftment, ↓ infarct size, ↑ cardiac function, ↑ capillary density	131

enabled significant preservation of myocardial contractility and prevention of LV dilatation when injected into the myocardium at 3 days after MI in rats. Rat adipose tissue-derived MSCs showed a 20-fold increase in expression of cardiac markers in vitro when assembled in chitosan membrane-grown MSC spheroids.¹³⁸

6.3 | Hydrogel scaffolds

In the past 10 years, considerable information has been accumulated on the use of biological and synthetic polymeric scaffolds for improving the cardioreparative properties of MSCs. In 2008, Wei et al.¹³⁹ showed that local application of a cardiac patch consisting of multi-layered MSCs in a sliced biological scaffold resulted in improved LV function and increased myocardial expression of angiogenic factors in infarcted rats. Suturing of an MSC-containing collagen scaffold to the epicardium of the infarcted rat heart resulted in improved perfusion, decreased infarct size, increased contractility and reduced adverse remodelling.¹⁴⁰ Placement of an MSC-colonized hyaluronan-based scaffold in the small pouch in the ventricular wall of the heterotopically transplanted infarcted rat heart was found to result in increased vascularization and diminished fibrosis of the graft.¹⁴¹ Transfer of the extracellular matrix scaffold from cardiac fibroblast culture with human ESC-derived MSCs to the epicardial surface of the infarcted mouse heart induced intensive migration of MSCs to the myocardium.¹⁴²

Many different types of synthetic MSC-containing scaffolds were found to provide better functional improvement than free MSCs in infarcted hearts: for example, elastic biodegradable

poly(lactide-co- ϵ -caprolactone) scaffold,¹⁴³ silanized hydroxypropyl methylcellulose hydrogel,¹⁴⁴ polysaccharide-based scaffold,¹⁴⁵ radio-frequency plasma surface-functionalized electrospun poly(ϵ -caprolactone) fibres,¹⁴⁶ porous polyethylenimine blended with poly(D,L-lactic-co-glycolic acid) microparticles¹⁴⁷ and poly(ethylene glycol)-b-polycaprolactone-(dodecanedioic acid)-polycaprolactone-poly(ethylene glycol)/ α -cyclodextrin hydrogel.¹⁴⁸ Examples of MSC scaffolds consisting of several components include the type I collagen-glycosaminoglycan scaffold,¹⁴⁹ alginate/chitosan scaffolds forming opposite-charge polyelectrolyte complexes¹⁵⁰ and the poly(ϵ -caprolactone)/gelatin nanofiber patch.¹⁵¹ When these MSC preparations were sutured to the epicardium after coronary ligation, they caused increased migration of MSCs to the infarct region, improved LV function and reduced scar size as compared with the findings for native MSCs.

6.4 | Encapsulated MSCs

Multicellular MSC preparations are also produced after encapsulation of MSCs into semipermeable polymer coatings. While the coating material is easily permeable to gases, nutrients and signalling molecules, it effectively prevents MSC injury by separating the cells from antibodies and immune cells. Encapsulated viable cells retain the ability to secrete growth factors and cytokines but cannot integrate into the host tissue and differentiate; the latter property of encapsulated cells increases the safety of cell-based therapy in terms of tumour transformation and adverse immune responses to grafted cells. In the study by

Yu et al.,¹⁵² human MSCs were encapsulated in arginylglycylaspartic acid-modified alginate microspheres and injected into the rat heart at 1 week after MI. This was associated with decreased ventricular remodelling and infarct area and enhanced formation of arterioles. Genetically modified glucagon-like peptide-1-overexpressing MSCs were encapsulated in alginate capsules, followed by intracoronary administration in pigs with MI, which resulted in robust LV function improvement, reduced inflammation and decreased epicardial infarct size.¹⁵³ Levit et al.¹⁵⁴ developed an original technique of attachment of alginate-encapsulated MSCs to the heart surface with a hydrogel patch. In comparison with the findings for empty capsules and free MSCs, use of encapsulated MSCs enabled the greatest reduction in scar size and increase in peri-infarct microvasculature density.

Thus, the use of polymeric scaffolds for MSCs holds great promise for regenerative therapy of myocardial ischaemic injury. The rapidly evolving field of three-dimensional, biodegradable, biomimetic scaffolds offers solutions to problems such as poor survival and engraftment of MSCs in the infarcted heart, low differentiation potential of transplanted MSCs, and inhomogeneous distribution of the cells in the damaged tissue.

7 | CONCLUSION AND PERSPECTIVES

Over the last decade, research has made significant strides in identifying different ways of MSC modification, ultimately aiming to prevent massive MSC death in the recipient tissue as well as to boost the cardioreparative effects of MSCs. The main approaches used to modify MSCs are summarized in Figure 3. According to the technique employed, all approaches to MSC modification can be classified into two categories: genetic and non-genetic approaches. Depending on the function of the target gene, genetic approaches can either increase MSC survival and proliferation, stimulate MSC differentiation and integration in the host tissue, or enhance the production of paracrine

factors by MSCs. Non-genetic approaches for MSC priming mainly include various forms of preconditioning, such as preconditioning with physical factors, drugs, or cytokines and growth factors. MSCs are sometimes preconditioned by exposure to cocktails of bioactive molecules or by coculture with other cells. An extremely new approach to MSC modification is direct fusion with cardiac stem cells, leading to the production of hybrids or chimeras endowed with the properties of both stem cell types. Tissue engineering and multicellular preparations of MSCs preserve intercellular communication but may simultaneously reduce the effects of migration and integration.

The results of preclinical studies on MCS effectiveness in MI are quite variable, with some studies showing significant functional and/or structural improvement and others demonstrating only marginal protection. There are several important sources of variability, including species-related issues, model of MI (eg, permanent coronary ligation vs ischaemia-reperfusion), time point of cell transplantation, duration of the entire experiment, the dose of the cells and the route of their administration (intravenous, intramyocardial or intracoronary).

It is still an open question as to which approaches to MSC modification are more useful for clinical applications. At present, pharmacological MSC preconditioning and tissue engineering approaches seem to be most suitable for translation to clinical arena because of rigorous preclinical evidence for therapeutic potential and lack of safety concerns. In addition, recent large-scale preclinical study of hypoxia-preconditioned MSC in infarcted non-human primates provided strong evidence for enhanced effectiveness of preconditioned cells, thereby increasing the translatability of this approach.¹⁵⁵ Detailed analysis of long-term outcomes and careful monitoring of potential side-effects will be required before clinical trials on the effectiveness of genetically modified MSCs can be contemplated. It is anticipated that the most promising approaches for MSC modification could be developed using a combination of different approaches, for example, MSC preconditioning with certain cytokines and subsequent encapsulation in biodegradable three-dimensional scaffolds. Future studies will provide

FIGURE 3 Summary of the main approaches used for mesenchymal stem cell (MSC) modification for cardiac repair after infarction. MSCs can be modified using either genetic or non-genetic manipulations. Non-genetic approaches include MSC preconditioning with physical or chemical factors. MCS-containing epicardial patches, capsules and three-dimensional scaffolds represent multicellular MSC preparations

information required to produce MSCs with predefined properties, thus paving the way for personalized cell-based therapy of MI.

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