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REVIEW

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Two complementary strategies to improve cell engraftment in mesenchymal stem cell-based therapy: Increasing transplanted cell resistance and increasing tissue receptivity

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ABSTRACT

Over the past 2 decades, therapies based on mesenchymal stem cells (MSC) have been tested to treat several types of diseases in clinical studies, due to their potential for tissue repair and regeneration. Currently, MSC-based therapy is considered a biologically safe procedure, with the therapeutic results being very promising. However, the benefits of these therapies are not stable in the long term, and the final outcomes manifest with high inter-patient variability. The major cause of these therapeutic limitations results from the poor engraftment of the transplanted cells. Researchers have developed separate strategies to improve MSC engraftment. One strategy aims at increasing the survival of the transplanted MSCs in the recipient tissue, rendering them more resistant to the hostile microenvironment (cell-preconditioning). Another strategy aims at making the damaged tissue more receptive to the transplanted cells, favoring their interactions (tissue-preconditioning). In this review, we summarize several approaches using these strategies, providing an integral and updated view of the recent developments in MSC-based therapies. In addition, we propose that the combined use of these different conditioning strategies could accelerate the process to translate experimental evidences from pre-clinic studies to the daily clinical practice.

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Introduction

Mesenchymal stromal cells, also referred to as mesenchymal stem cells (MSCs), were first discovered in 1974.[1] MSCs are a heterogeneous group of cells mainly obtained from bone marrow (BM-MSC), adipose tissue (ADSC) and umbilical cord blood (UCB-MSC). Since the 90s, MSCs have been investigated and used in cell-based therapies for several different human diseases. This extensive research has contributed identification of 3 main properties of MSCs offering real potential in regenerative medicine: 1–they are multipotent cells with the capacity to be differentiated *in vitro* and *in vivo* into diverse cells types including adipocytes, osteoblasts, hepatocytes, myoblasts and neuron-like cells; 2–they can be recruited to the damaged tissue by following chemotactic signals; and 3–they produce and secrete several factors into the extracellular space (cytokines, chemokines, growth factors, exosomes, microvesicles, miRNA) that mediate their regenerative and immunomodulatory effects in the transplanted recipient.[2]

The number of registered clinical trials using MSCs worldwide is increasing exponentially, and so is the promise of their use in daily clinical practice.[3] At present, 588 MSC-based clinical trials, either complete or ongoing, appear in the database of the US National Institute of Health, targeting different types of human diseases. These include: central nervous system diseases (12.5%), heart and blood diseases (10.5%), bone and cartilage diseases (10.5%), autoimmune diseases (8.5%) and liver diseases (5.4%).[4] Beneficial effects of MSC-based therapies have been demonstrated in many diseases; however, the degree of benefits in the outcomes is highly variable among individuals and not perdurable over time. In most cases, limitations in the clinical outcomes have been attributable to the poor cell engraftment of MSCs in the target tissue.[2]

It is currently believed that even if a low count of transplanted cells are able to persist in the target tissue, they are able to mediate beneficial effects. This is explained by the repair mechanism by which MSCs exert

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their actions. In recent years, it has been believed that MSCs mediate tissue repair via a transdifferentiation process, whereby they graft, differentiate and become an integral part of the target tissue mediating regeneration. However, there has been a paradigm shift in this hypothesis, and it is currently accepted that after homing into the target tissue, MSCs produce several humoral factors including cytokines and extracellular vesicles (which themselves contain many signaling peptides and receptors as well as mRNA and miRNA). The humoral factors secreted by MSCs improve the function of the surrounding tissue and have actions in distant tissues as well, which ameliorate organ function without requiring transdifferenciation of the MSCs.[5] This paracrine hypothesis may contribute to explain why, even if the transplanted MSCs reside in non-target tissues after grafting, they are still able to exert beneficial effects. Based on these premises, current efforts have been made in order to enhance the survival of transplanted MSCs as a priority for the treatment efficacy. If a small percentage of transplanted viable cells are able to persist in target tissues producing such beneficial effects, then increasing MSC engraftment and survival seems critical for ameliorating the clinical efficacy of MSC-based therapies.

From the clinical point of view, some strategies have been proposed to improve cell survival and consequently, therapeutic efficacy. For instance, determination of the optimal time point for cell delivery;[6] optimization of methods for cell isolation and *ex vivo* expansion;[7] and evaluation of different delivery routes for MSC administration (local *vs.* systemic).[8] From the pre-clinical point of view, important advances in understanding the interactions between the target tissue and the transplanted MSCs have been accomplished in the last decade, and these mainly relate to the mechanisms by which MSCs are able to integrate in the receptor tissue.

The gradual loss of organ function in almost all diseases is caused by the death of specialized cells in the tissue. This result corresponds to a variety of different malign episodes, among which the most frequent are ischemia/reperfusion, autoimmune responses or exposure to cytotoxic drugs including chemotherapeutic agents. In this context, an initial inflammatory response in the damaged tissue is characterized by increased oxidative stress, a remodelling of the extracellular matrix (ECM), and an increase in the release of chemokines to the blood flow. Several earlier described chemokines promote the migration of leucocytes to the inflammation site. One of the best studied is CXCL12 or stromal cell-derived factor-1 (SDF-1), which is also involved in the homing of transplanted MSC. Homing is the capacity of infused cells to migrate to the injury tissue; the migration process is classified in 3 stages: 1-chemotaxis/traffic, 2-rolling and transendothelial migration and finally and 3-integration into the parenchyma.[9] MSC express different receptors or transmembrane ligands that are involved in each stage. Regarding the first stage: MSC express a broad range of chemokine receptors, including CXCR4 (receptor for SDF-1), which has a relevant role in MSC homing.[10] In addition, MSC can express receptors for cytokines (IL-6, PDGF, TGF-β1, TNF-α) and several growth factor receptors (IGF-1R and VEGFR), with their ligands being released in large quantities by damaged tissue during the inflammatory process.[11] In the next stage, MSCs interact with the endothelium through P-selectins expressed on endothelial cells, promoting MSC recruitment.[12] Different surface proteins of MSCs are also involved in this interaction, mainly from the integrin family. Some of them are very late antigen-4 (VLA-4), vascular cell adhesion molecule-1 (VCAM-1) and intercellular cell adhesion molecules (ICAM), and they are required for the transendothelial migration phase.[12] When MSC are anchored to the endothelial cells, they express proteolytic enzymes such as metalloproteinases (MMPs) in order to pass through the basal membrane and get into the parenchymal space.[13] The third step needed to achieve MSC integration into the target tissue requires the correct interaction between MSCs and the ECM, and these are critical to allow cell survival and finally, the permanency of MSC engraftment.

The lack of cell adhesion due to inappropriate MSC-ECM interaction induces an apoptotic process known as anoikis (Greek word meaning "homeless"). ECM stimulates cell survival thorough integrin receptors activating intracellular signaling cascades such as the PI3K/Akt and the MEK/ERK pathways.[14] Anoikis is responsible, at least in part, for the low percentage of MSC engraftment of "unprepared" transplanted cells. Other signals that decrease their survival derive from the hostile microenvironment of the target tissue, where a highly inflammatory and cytotoxic process is taking place, which aims at removing anything unnecessary in the affected area.

In this review, we focus on describing different preconditioning strategies used to promote MSC resistance to adverse microenvironments. We also cover the conditioning methodologies used to render the target tissue more receptive to transplanted MSC (Fig. 1). Only the articles that demonstrated an enhanced cell engraftment by strategies conditioning MSCs or the target tissues were included in this review. Articles that reported a clinical benefit of the cell therapy without considering the transplanted cell engraftment were excluded. The experimental details of the described strategies were summarized and presented in Table 1 (A and B). We propose that the combination of both

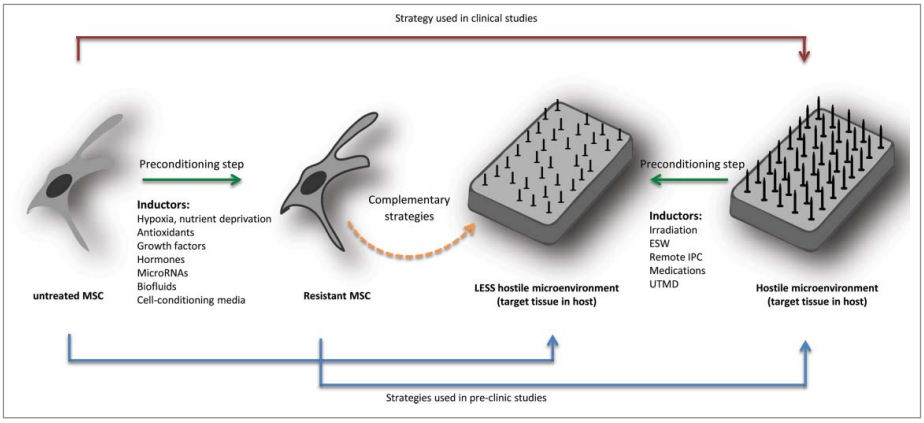

Figure 1. Summary of MSC based strategies to improve cell engraftment. The red arrow represents the strategy used in clinical trials where untreated MSCs have been transplanted into the target tissue in the host (hostile microenvironment symbolized as a bed of nails). Blue arrows represent the strategies used in pre-clinic studies where MSCs or target tissue received a preconditioning procedure to promote cell engraftment. The orange dotted arrow represents the combination of both strategies suggested in this review. Abbreviations: ESW, Extracorporeal shock waves; IPC, ischemic postconditioning; UTMD, ultrasound-target microbubble destruction.

approaches can be used to increase cell engraftment and consequently, to achieve long-term benefits of MSC therapies.

Induction of MSC resistance to the hostile microenvironment

Ex vivo MSC culture has been normally carried out under atmospheric oxygen tension (21% O_2). In 2007, Fehrer and colleagues demonstrated for the first time, that BM-MSC (in their *in vivo* niche) proliferated with 1-7% oxygen.[15] This discovery provided new insights for MSC cultivation protocols. In fact, many studies have reported that manipulation of oxygen tension impacts during *in vitro* MSC cultivation, has an effect on their properties in differentiation capacity, proliferation rate and secretome profiles.[16]

Since transplanted MSC will undergo hypoxia inside the inflamed tissue, the hypoxia stimulation, previous to transplant, has been investigated as a training strategy termed preconditioning, in order to improve the *in situ* cell survival. Pre-treatment of BM-MSC with hypoxia (1%) for 24 hrs. increased fourfold its engraftment in comparison to untreated cells in an animal model of idiopathic pulmonary fibrosis,[17] improving pulmonary function and reducing tissue collagen content in the transplanted tissue. Authors suggested that the enhancement of survival rate of engrafted BM-MSCs is partially due to the up-regulation of hepatocyte growth factor (Hgf). Wang and colleagues reported that the intracavernous administration of hypoxia-preconditioned ADSC (HP-ADSC) was more efficient at reverting diabetic erectile dysfunction (measured by intracavernosal pressure) compared to administration of untreated ADSC. A week after cell transplantation, the number of HP-ADSC in the tissue was 50% higher compared to untreated ADSC, probably due to the high gene expression of SDF-1 and CXCR4 inducted in ADSC by hypoxic preconditioning.[18] The beneficial effects of hypoxic preconditioning on BM-MSC were also proved in an acute myocardial infarction murine model. Engraftment of pre-treated BM-MSC was 2.5 higher than untreated BM-MSC after one day of its intravenous administration, reducing myocardial infarct size and decreasing cardiac damage. Authors also suggest that high expression of CXCR4 in pre-treated BM-MSC promoted cell survival in myocardial ischemic tissue.[19] Similar benefits of MSC preconditioning have been observed in the homing and retention of human adipose tissue-derived MSCs (hASCs) after *in vivo* transplantation. Preconditioning hASCs increased their expression of CXCR4, which combined with exogenous SDF-1α delivery, allowed for further homing/retention of ASCs after transplantation.[20] Importantly these results highlight that, in addition to MSC preconditioning, increasing, the chemokine attraction profile of the target tissue contributes to MSC retention. Regarding clinical trials that explore the use of hypoxic preconditioning to enhance the stem cell therapeutic potential for myocardial repair, in 2015 Hu and colleagues provided the first-in-man evidence that intracoronary administration of preconditioned-bone marrow mononuclear cells, following acute myocardial infarction, improves cardiac functional parameters without any adverse effect.[21]

Antioxidants have also been used to protect MSCs from the hostile microenvironment in the target tissue after transplantation. Xu and colleagues investigated whether high-density lipoprotein (HDL) could protect

Table 1. Strategies to improve cell engraftment. (A) Promoting MSC resistance to a hostile microenvironment. (B) Increasing tissue receptivity to MSCs.

A

MSC-resistance inductor	Disease animal model	MSC source / delivery route and time of administration	Effect on MSC (mechanisms proposed)	Effect on transplant recipient (induction vs. control)	Grade of cell engraftment (Induction vs. control) Cell tracking method	References
Hypoxia (1%, 24hs)	Idiopathic pulmonary fibrosis (IPF) induced by bleomycin (mice)	BM-MSC / intratracheal instillation, 3 days after IPF induction	↑Hgf, Vegf, Ho-1 ↑Hif-1α, bcl-2,	↓collagen deposition ↓inflammatory cytokines ↑pulmonary function	↑4 fold, at 4 days / β-Galactosidase staining	[17]
Hypoxia (1%, 24hs)	Erectile dysfunction in DMT1 induced by streptozotocin (rat)	ADSC / intracavernous injection, 8 weeks after DMT1 induction	↑ Hif-1α, bFgf, Vegf, Ang-1 ↑Sdf-1α, Cxr4	↓collagen deposition ↑intracavernosal pressure	↑1.5 fold, at 1 week / DiI fluorescent dye	[18]
Hypoxia (1%, 24hs)	Myocardial infarction (mice)	BM-MSC /intravenous injection 1 day after AMI induction	↑Cxr4	↓infarct size ↑cardiac function	↑2.5 fold, at 1 day after MI / GFP transduction	[19]
HDL(20–200 μg/ml, 24hs)	Myocardial infarction (rat)	BM-MSC /intramyocardial injection after 10 min of LAD ligation	Activation of PI3K/Akt signaling pathway	↑cardiac function	↑3 fold at 4 days after MI / GFP transduction	[22]
Curcumin(10 μM, 24hs)	Myocardial ischemia-reperfusion injury (rat)	ADSC / intramyocardial injections after 1 week of LAD ligation	Activation of PTEN/Akt/p53 survival signaling pathway	↓infarct size ↑cardiac function ↑neovascularization	↑2 fold at 7 days after MI / DiI fluorescent dye	[24]
Trimetazidine(10 μM, 6hs)	Myocardial ischemia-reperfusion injury (rat)	BM-MSC / ADSC / intramyocardial injections after reperfusion of LAD ligation	↑ Hif-1α, bcl-2	↓infarct size ↑cardiac function ↑neovascularization	↑2 fold at 3 days after MI / CM-DiI fluorescent dye	[26]
Atorvastatin(1 μM, 24hs)	Myocardial infarction (rat)	BM-MSC / intravenous injection 1 day after MI induction	↑Cxr4	↓collagen deposition ↓infarct size ↓inflammatory cytokines ↑cardiac function	↑1.7 fold at 3 days after MI / CM-DiI fluorescent dye	[28]
Melatonin(5 μM, 24 hs)	Myocardial infarction (rat)	ADSC / intramyocardial injection after LAD ligation	Activation of SRT1 and bcl-2 survival signaling pathway	↓infarct size ↓collagen deposition ↑cardiac function ↑neovascularization	↑2.5 fold at 14 days after MI / BLI	[29]
miRNA-133a (transient transfection, 50 nM)	Myocardial ischemia-reperfusion injury (rat)	BM-MSC / intramyocardial injection after LAD ligation	Inhibition of Apaf-1 / Caspase 9 / Caspase 3 apoptosis signaling pathway	↓infarct size ↓collagen deposition ↑cardiac function	↑2 fold at 7 days after MI / Iron oxide and Prussian blue staining	[30]
PRPCR (0.1–20%, 1–7 days)	Wound skin (rat)	BM-MSC / into wound margins after surgery	Activation of PI3K/Akt/NF-$\kappa\beta$ signaling pathway	↑% of wound closure	↑1.2 fold during wound closure process / GFP transduction	[32]

(*Continued on next page*)

Table 1. (*Continued*)

B						
Tissue receptivity inductor	Disease animal model	MSC source / delivery route and time of administration	Effect on transplanted recipient	Mechanisms of cell engraftment	Grade of cell engraftment (Induction vs. control) /Cell tracking method	References
Irradiation(X-ray, 15 Gy)	Hepatic fibrosis induced by thioacetamide (rat)	BM-MSC / intravenous (portal vein) after irradiation	↓collagen deposition ↓inflammation status ↑liver function	↓Tgf-β1, α-SMA and collagen I	↑1.5 fold at 3 weeks post-transplantation / sry gene analysis	[36]
UTMD	Diabetic nephropathy induced by streptozotocin (rat)	BM-MSC / intravenous (tail vein) after microbubble injection	↓inflammation status ↑renal function	↑renal interstitial capillary and VCAM-1 expression	↑ 2 fold at 3 day post-transplantation / GFP transduction	[37]
ESW (0.04 mJ/mm^2 at 1000 impulses)	Chronic spinal injury (rat)	BM-MSC / intravenous (tail vein) / 24hs post ESW	↑locomotor activity	↑Sdf-1α and Cxcr4 in tissue	↑ 1.3 fold at 4 weeks post-transplantation / PKH6 red fluorescence dye	[39]
IPC(3 cycles, 30 s reperfusion and occlusion)	Lung ischemia-reperfusion injury (rat)	BM-MSC / intravenous injection after lung reperfusion	↓inflammation status ↑lung function	↓oxidative stress damaged ↑Sdf-1α and Vegf ↑Antioxidant enzymes	↑3 fold at 1 day post-transplantation / GFP transduction	[42]
Remote IPC(4 cycles of 5 min reperfusion and occlusion)	Myocardial ischemia-reperfusion injury (rat)	BM-MSC / intravenous injection after 8 days of ischemia induction by LAD ligation	↑cardiac function	↑Sdf-1α	↑2 fold at 1 month post-transplantation / sry gene analysis	[43]
Remote IPC (3 cycles, 30 s reperfusion and occlusion)	Myocardial ischemia-reperfusion injury (rat)	BM-MSC / intramyocardial injection after ischemia induction by LAD ligation	↓collagen deposition ↑cardiac function	↑Sdf-1α and Vegf ↑Antioxidant enzymes	↑2 fold at 3 weeks post-transplantation / sry gene analysis	[44]
Rosuvastatin(20mg/Kg/day)	Myocardial infarction (mice)	ADSC / intramyocardial injection immediately after LAD ligation	↓collagen deposition ↑cardiac function	Activation of PI3K/Akt and MEK/ERK signaling pathway in ADSC	↑ 1.5 fold at 3 weeks post-transplantation / GFP-transduction and BLI	[46]
bFGF (2 mg)	Myocardial infarction (canine)	BM-MSC / retrograde coronary venous infusion after 1 week of MI	↓collagen deposition ↓infarct size ↑cardiac function	↑Neovascularization ↑MSC in situ differentiation	↑2 fold at 1 month post-transplantation / GFP-transduction	[47]

BM-MSC against oxidative stress-induced apoptosis. This study is based on the fact that HDL reduces the risk associated with cardiovascular ischemic disease, protecting endothelial cells from apoptosis induced by intracellular oxidative stress.[22] After exposure of BM-MSC to HDL (HDL-BMMSC), cells were administrated in the myocardium of an animal model of post-myocardial infarction recovery. Cardiac function improvement and cell engraftment were significantly higher in the HDL-BMMSC based therapy than untreated BM-MSC based therapy. *In vitro* studies suggested that HDL activated the antiapoptotic PI3K/Akt signaling pathway.[22] Curcumin is also known for its antioxidant properties (ROS-scavenging activity).[23] Liu and colleagues demonstrated in an *in vitro* study that curcumin exposure activated the pro-survival signaling pathway PTEN/Akt/p53 in ADSC. Indeed, cell therapy with curcumin-preconditioned ADSC (C-ADSC) was more efficient in attenuating myocardial damage compared with untreated ADSC in a mouse model of ischemia-reperfusion injury. Importantly, this effect correlated with increased cell engraftment of transplanted ADSC.[24]

Trimetazidine (TMZ) is used to attenuate the consequence of myocardial ischemic-reperfusion injury in the clinical practice because TMZ increases cell tolerance to ischemia by maintaining cellular homeostasis.[25] Preconditioning of BM-MSC with TMZ for 6 h induced the expression of Hif-1α. Preconditioned cells exhibited a better engraftment capacity and therapeutic performance, increasing myocardial function and neovascularization after administration in an animal model of cardiac ischemia-reperfusion injury.[26]

Statins are a group antilipidemic compounds that inhibit the enzyme HMG-Coa reductase, which is involved in the production of cholesterol. However, statins have additional pleiotropic effects post-ischemia-reperfusion, including improvement of endothelial dysfunction, antioxidant properties and inhibition of inflammatory responses. For this reason, using statins is a common treatment in the clinical practices for the prevention of tissue injury after a heart or brain infarction.[27] Li and colleagues demonstrated that Atorvastatin (AT) preconditioning of BM-MSC up-regulated CXCR4 expression, increasing cell survival and cardiac performance in an animal model of acute myocardial infarction.[28] Similarly; melatonin, a neurohormone with anti-inflammatory and antioxidant properties, has been proved to exhibit cytoprotective effects against ischemic injury in the liver, kidneys, brain and the heart. Han and colleagues demonstrated in a pre-clinic study that the pre-treatment of ADSC with melatonin could facilitate ADSC based therapy for myocardial infarction, possibly through promoting survival of ADSC via SIRT1 signaling.[29]

Dua and colleagues demonstrated that epigenetic reprograming of MSCs by microRNAs previous to transplant rendered them more resistant to the hostile microenvironment. BM-MSC were transfected with a double-stranded miR-133a, which is abundantly expressed in heart and is down-regulated in patients after myocardial infarction. Apaf-1, a pro-apoptotic factor involved in intrinsic apoptosis is a target of miR-133a. BM-MSCs transfected with miR-133a administered in an animal model of myocardial infarction led to a significant increase in cell engraftment, cardiac function, and decreased fibrosis compared to control BM-MSC.[30]

Because the processes of homing and engraftment depend on multiple variables, and their mechanisms are not fully understood, some researchers have tested a "multiple stimulation/reprograming" strategy to increase MSC engraft capacity. Platelet rich plasma (PRP) has been used in human applications since the 1970s for its healing properties attributed to secreted proteins.[31] Peng and colleagues described that PRP-preconditioning of BM-MSC induced PI3K/Akt/NF-$\kappa\beta$ signaling, enhancing cell survival and regenerative function in a wound healing murine model.[32]

Cell-conditioned media has been used *in vitro* to investigate the stimuli (coming from the cellular microenvironment) that the transplanted cells receive inside the target tissue.[33] Smith and colleagues reported a cell culture-based approach to enhance hADSC engraftment in brain tumors by pre-exposing ADSC to glioma-conditioned media and extracellular matrix proteins (fibronectin and laminin). This method contributes to educate the MSCs in order to enhance their homing capacity and to specifically direct them to localized tumors.[34]

Screening technology is a new approach to identify new molecules that could render MSCs more resistant to a hostile microenvironment. Recently, a novel method for screening small molecules that enhance homing of systemically administrated cells was developed. Levy and colleagues screened 9000 signal transduction modulators to identify hits that increase MSC surface expression of homing ligands that bind to intercellular adhesion molecule 1 (ICAM-1). They identified a kinase inhibitor called Ro-31-8425. Preconditioned-MSCs with Ro-31-8425 exhibited increased homing into inflamed sites, and displayed improved anti-inflammatory properties in lipopolysaccharide-induced inflamed mouse ears.[35] The examples presented here clearly point to the beneficial consequences of modulating MSCs prior to transplantation, either via hypoxic preconditioning, drug treatment,

ROS attenuation, immunomodulation, chemotactic transformation and genetic programming (Fig. 1).

Induction of target tissue receptivity for transplanted MSC

The approaches used to prepare the damaged tissue to promote the engraftment of transplanted cells are summarized in Table 1B. Shao and colleagues reported that regional hepatic irradiation with X-rays before BM-MSC transplantation, ameliorated thioacetamide-induced liver fibrosis in rats. Hepatic irradiation promoted homing of BM-MSC, reducing the inflammatory status and increasing liver function.[36] The ultrasound-target microbubble destruction (UTMD) technique used to increase wall vessel permeability, favors the extravasation of cells into the parenchymal space. Zhang and colleagues showed that UTMD increased renal protection after intravenous administration of BM-MSC, using an animal model of diabetic nephropathy. Authors suggested that this protective effect is mediated by an enhancement of homing (via increasing capillary permeability) and retention of BM-MSC (mediated by upregulation of VCAM-1) into the kidneys.[37] Extracorporeal shock wave (ESW) therapy is a non-invasive treatment for chronic tendinopathies. Although the biological mechanism of this therapy is not clear, it appears to promote neovascularization and the removal of damaged ECM components.[38] Lee and colleagues described a beneficial effect on BM-MSC engraftment after ESW treatment in a chronic spinal cord injury rat model, presumably by up-regulation of SDF-1 and CXCR4 expression.[39] Regarding clinical studies, in the CELL-WAVE randomized clinical trial, a positive, albeit modest improvement was observed in left ventricular ejection fraction at 4 months after intracoronary infusion of bone marrow-derived mononuclear cells (BMC). Patients with post-infarction chronic heart failure received ESW 24 hrs before BMC transplantation.[40]

Procedures to attenuate heart failure resulting from myocardial infarction have been investigated in animals and humans in the past decades. The most successful procedure to date, called post-conditioning, is based on the application of repeated vascular occlusion for brief periods at the onset of reperfusion after an ischemic event. Recent studies demonstrated that ischemic post-conditioning (IPC) reduces reperfusion injury, described as cellular death induced by oxidative stress, increased inflammation levels and extracellular matrix remodeling.[41] Chen and colleagues combine, for the first time, ICP with stem cell based therapy to prevent tissue damage after an ischemic episode. Using an animal model of pulmonary ischemia reperfusion injury, they reported intravenous administration of BM-MSC after IPC enhances pulmonary function and cell engraftment in the lungs.[42] Jiang and colleagues have also shown that remote conditioning (an alternative to conditioning where ischemia is applied to a distant tissue) enhanced cell retention and cardiac function in the myocardium after BM-MSC transplantation in a myocardial ischemia-reperfusion animal model, suggesting that SDF-1 is a key molecule in the cell engraftment mechanism.[43] In 2016, the same research group demonstrated that these beneficial effects were mainly attributed to the hospitable microenvironment for engrafted cells.[44]

As we mentioned above, statin administration attenuates the effects of an ischemic episode in the heart or the brain, mainly reducing the inflammation in the injured tissue. Yang and colleagues reported that AT, a member of the statin family, decreased the hostility of the cardiac microenvironment and facilitated survival of MSC administration in a post-infarct *in vivo* model.[45] Moreover, Zhang and colleagues reported that the combined therapy of Rosuvastatin and MSCs has a synergistic effect on improving myocardial function after infarction, improving the survival of engrafted ADSC, at least in part, through the PI3K/Akt and MEK/ERK 1/2 signaling pathways.[46]

The proliferative growth factor of b-fibroblasts (bFGF) promotes cell survival, migration and the differentiation capacity of BM-MSC *in vitro*, and these abilities may improve BM-MSC engraftment to target tissue. Wang and colleagues observed that the co-administration of bFGF with BM-MSC (by retrograde coronary venous infusion) in an animal model of myocardial infarction, enhanced BM-MSC survival and differentiation, recovering cardiac function and preventing adverse remodelling.[47] A summary or the reported findings in MSC-based therapy in pre-clinic and clinical studies, in addition to our suggested strategy are summarized in Figure 1.

Future perspective and conclusions

The first milestone to overcome during the development of MSC therapy has been to guarantee the biosafety of their use in clinical trials.[48] At present, consensus in the scientific community agrees that MSC therapy is safe when its isolation, *ex vivo* expansion, and administration are made following Good Manufacturing Practice (GMP) guidelines. The number of clinical studies evaluating the regenerative effect of MSCs in multiple diseases is growing fast and therapeutic results are increasingly positive. However, concurrently, scientists have observed that the long-term benefit of MSC therapy is restricted by the poor engraftment of transplanted cells. At present,

the second milestone aims to achieve a stable MSC-regenerative effect in the transplant host. The discovery of new mechanisms of cell homing and engraftment will present a stronger possibility to improve the interactions between transplanted cells and tissue cells, for instance, modulating the expression of adhesion and migration molecules in MSCs.[49] On the other hand, in order to prevent the difficulties of cell engraftment, researchers have also investigated the use of biomaterial to mimic the natural niche of MSCs with encouraging results, particularly in bone and cartilage regeneration in order to draw MSC-host tissue interactions.[50,51]

Strategies of MSC preconditioning or damaged tissue preconditioning have been successful in pre-clinic studies for different diseases, increasing the cell engraftment, the gain of tissue function and consequently, the efficacy of cell therapy. The successful use of statins for MSC preconditioning or for damaged tissue preconditioning to increase the MSC therapy efficacy in the treatment of myocardial infarction consequences after reperfusion suggests that even more powerful results of combined strategies may be achieved. Therefore, we propose that using both complementary strategies will enable acceleration of the process to translate the experimental evidence from the pre-clinic studies to the daily clinical practice, reaching the next milestone (Fig. 1). In addition, we call for more research in this area, in particular, we advocate research that includes various modalities of combining the strategies to produce excellent clinical results.

Abbreviations

α-SMA	α smooth muscle actin
Akt	serine/threonine-specific protein kinase
ADSC	adipose derived mesenchymal stem cells
ATR2	angiotensin type 2 receptor
bFGF	β-fibroblast growth factor
Bcl-2	B-cell lymphoma 2
BLI	in vivo bioluminescent imaging
BM-MSC	bone marrow mesenchymal stem cells
Cxcr4	CxC chemokine receptor type 4
DMT1	Diabetic mellitus type 1
ESW	Extracorporeal shock waves
GFP	green fluorescent protein
Gy	gray
HDL	High density lipoprotein
Hgf	hepatocyte growth factor
Ho-1	heme oxigenase-1
Hif-1α	hypoxia-inducible factor-1 α
IPC	ischemic postconditioning
LAD	left anterior descending coronary artery
PI3K	phosphoinositide 3-kinase
PRPCR	platelet rich plasma clot releasate
PTEN	phosphatase and tensin homolog
ERK	extracellular signal-regulated kinase
MEK	tyrosine/threonine kinase
MI	myocardial infarction
Sdf-1α	stromal cell-derived factor-1alpha
SRT1	silent mating type information regulation 2 homolog 1 or NAD-dependent deacetylase sirtuin-1
Tgf-β1	transforming growth factor β
VCAM-1	vascular cell adhesion protein-1
Vegf	vascular endothelial growth factor
sry	sex determining region Y
UTMD	ultrasound-target microbubble destruction

Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

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