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ABOUT COVER

AIMS AND SCOPE

The primary aim of *World Journal of Stem Cells* (*WJSC*, *World J Stem Cells*) is to provide scholars and readers from various fields of stem cells with a platform to publish high-quality basic and clinical research articles and communicate their research findings online. *WJSC* publishes articles reporting research results obtained in the field of stem cell biology and regenerative medicine, related to the wide range of stem cells including embryonic stem cells, germline stem cells, tissue-specific stem cells, adult stem cells, mesenchymal stromal cells, induced pluripotent stem cells, embryonal carcinoma stem cells, hemangioblasts, lymphoid progenitor cells, *etc*.

INDEXING/ABSTRACTING

The *WJSC* is now abstracted and indexed in Science Citation Index Expanded (SCIE, also known as SciSearch®), Journal Citation Reports/Science Edition, PubMed, PubMed Central, Scopus, Biological Abstracts, BIOSIS Previews, Reference Citation Analysis, China Science and Technology Journal Database, and Superstar Journals Database. The 2024 Edition of Journal Citation Reports® cites the 2023 journal impact factor (JIF) for *WJSC* as 3.6; JIF without journal self cites: 3.5; 5-year JIF: 4.2; JIF Rank: 16/31 in cell and tissue engineering; JIF Quartile: Q3; and 5-year JIF Quartile: Q3; JIF Rank: 105/205 in cell biology; JIF Quartile: Q3; and 5-year JIF Quartile: Q2. The *WJSC*'s CiteScore for 2023 is 7.8 and Scopus CiteScore rank 2023: Histology is 11/62; Genetics is 78/347; Genetics (clinical) is 19/99; Molecular Biology is 131/410; Cell Biology is 104/285.

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EDITORIAL

Priming mesenchymal stem cells to develop "super stem cells"

Khawaja Husnain Haider

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Abstract

The stem cell pre-treatment approaches at cellular and sub-cellular levels encompass physical manipulation of stem cells to growth factor treatment, genetic manipulation, and chemical and pharmacological treatment, each strategy having advantages and limitations. Most of these pre-treatment protocols are non-combinative. This editorial is a continuum of Li *et al*'s published article and Wan *et al*'s editorial focusing on the significance of pre-treatment strategies to enhance their stemness, immunoregulatory, and immunosuppressive properties. They have elaborated on the intricacies of the combinative pre-treatment protocol using pro-inflammatory cytokines and hypoxia. Applying a well-defined multi-pronged combinatorial strategy of mesenchymal stem cells (MSCs), pre-treatment based on the mechanistic understanding is expected to develop "Super MSCs", which will create a transformative shift in MSC-based therapies in clinical settings, potentially revolutionizing the field. Once optimized, the standardized protocols may be used with slight modifications to pre-treat different stem cells to develop "super stem cells" with augmented stemness, functionality, and reparability for diverse clinical applications with better outcomes.

Key Words: Cell survival; Cell therapy; Preconditioning; Pre-treatment; Stem cells; Super stem cells

Core Tip: Stem cell pre-treatment is a promising approach for accentuating their stemness and reparability and improving their resistance to apoptosis in the harsh microenvironment of the injured tissue. The current stem cell pre-treatment protocols allow for enhancing their therapeutic efficacy to surmount the limitations of mesenchymal stem cells (MSCs), *i.e.,* mobility, survival, engraftment, and paracrine activity. Besides a novel sub-cellular preconditioning strategy, the combinative preconditioning approach exposing the cells to a proinflammatory milieu under hypoxia improves MSCs' immunoregulatory potential. Refining a combinatorial pre-treatment protocol using a well-defined mechanistic understanding may allow the exploitation of the full benefits of the pre-treated "super MSCs" for enhanced stemness, functionality, and reparability, including immunosuppressive and immunomodulatory properties.

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INTRODUCTION

The restorative and reparative potential of stem cell-based therapy renders cell-based therapy superior to the existing treatment approaches, which merely provide symptomatic relief and, at best, slow down the disease process. Incidentally, from among the embryonic, extraembryonic, and adult tissue-derived stem cells, mesenchymal stem cells (MSCs) have progressed to the advanced phases of clinical assessment after extensive characterization in experimental and translational settings. The extent of interest in MSCs can be gauged from the astonishing 91636 and 1631 searches retrieved from PubMed and Clinicaltrials.gov, respectively, after using the term "mesenchymal stem cell" as of 22nd March 2024 during the writing up of the editorial.

Mesodermal in origin, MSCs are non-hematopoietic, multipotent, and constitute a heterogeneous population. They are primarily characterized by their fibroblastic morphology, preferential plastic adherence, trilineage differentiation potential, and surface marker expression of CD44, CD73, CD90, CD105, *etc.*, albeit without hematopoietic stem cell-specific markers, *i.e.*, CD45, CD34, CD14, CD11b, *etc.* according to the minimal criteria defined by the International Society for Cellular Therapy[1]. They can be isolated and purified from several organs and tissues, *i.e.*, adipose, muscle, placenta, *etc.*, and hence, diverge in their characteristics, such as proliferation rate, self-renewal, paracrine activity, and differentiation potential[2]. For instance, umbilical cord-derived MSCs (UC-MSCs) have a higher propensity of primitive cell population than their counterparts derived from the bone marrow (BM-MSCs). Similarly, adipose tissue-derived MSCs (Ad-MSCs) differ in their immunoregulatory characteristics from their counterparts in BM-MSCs. However, based on their cell biology, superior paracrine behavior, and anti-inflammatory and immunomodulatory characteristics, MSCs isolated from the bone marrow and umbilical cord remain the most well-studied cells in clinical studies[3].

Despite encouraging data from experimental and clinical studies[3,4], massive donor cell death primarily influences the efficacy of cell-based treatment, poor engraftment rate, and low differentiation rate. Some studies have reported that as high as 99% of donor cells undergo apoptosis and necrosis during the acute post-transplantation phase[5,6]. The drastic diminution in the number of surviving donor cells has a three-pronged effect on the intervention outcome; firstly, dead cell debris accentuates the acute phase inflammatory response at the injury site. Secondly, reducing the number of surviving donor cells lowers the reparability and paracrine activity of the transplanted cells. Lastly, extensive cell death necessitates a larger cell dose for optimal therapeutic outcomes, thus raising logistic implications. Hence, donor cell survival post-transplantation remains a significant determinant of the efficacy of the cell-based intervention. The issue may be addressed by augmenting the tissue microenvironment by manipulating it to favor the donor cells or priming them to become more resistant to apoptosis and ferroptosis[7,8]. The editorial by Wan *et al*[9] elegantly delves into strategies to pre-treat MSCs to enhance their stemness, biology, and reparability characteristics. We have previously reported different strategies encompassing physical, chemical, pharmacological, or genetic manipulation of MSCS to develop 'super stem cells" that are primed to endure the harsh microenvironment in the injured tissue, resist apoptosis, and undergo differentiation besides expressing a plethora of bioactive molecules as part of their paracrine activities[10]. Our editorial delves into the pretreatment approaches of MSCs to enhance their survival, stemness, and functional characteristics, as summarized in Figure 1.

PRETREATMENT APPROACHES OF MSCS

Pretreatment approach using physical methods

Physical manipulation protocols constitute the most prevalent methods to pre-treat stem cells for their improved survival and functionality post-transplantation in the harsh microenvironment of injured tissues. One of these approaches is to expose the MSCs to hypoxic culture conditions as part of hypoxic preconditioning that successfully initiates pro-survival signaling downstream of hypoxia-inducible factor (HIF)-1α activation[11]. The level of hypoxia used ranges from 1% to 5% oxygen in various studies, simulating the condition of their natural habitat and that of the ischemic injured tissue microenvironment[12]. These hypoxic priming protocols, either continuous exposure to hypoxia from a few hours to 1-3

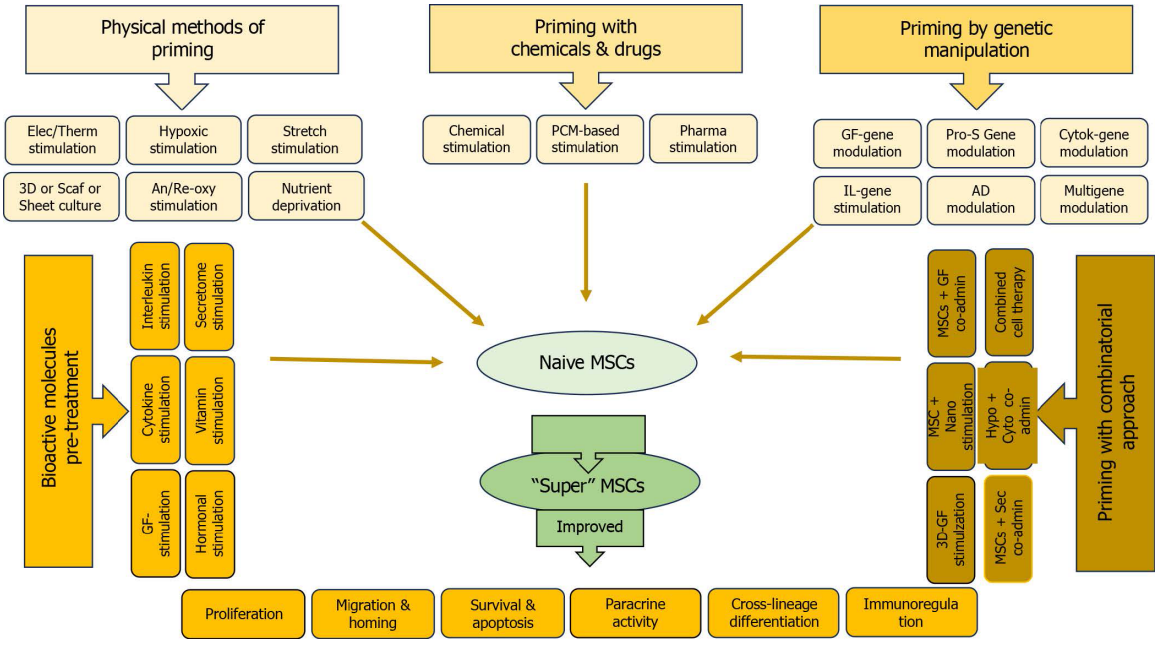

Figure 1 Summary of mesenchymal stem cell priming strategies to develop "super mesenchymal stem cells" with enhanced stemness, biology, and functionality. AD: Adrenomedullin; An: Anoxia; Cytok: Cytokine; GF: Growth factor; Hypo: Hypoxia; IL: Interleukin; MSC: Mesenchymal stem cell; PCM: Preconditioning mimetics; Pharma: Pharmacological; Pro-S: Pro survival; Scaf: Scaffold; Sec: Secretome; Therm: Thermal.

d[13-15] or intermittent hypoxia and reoxygenation cycles[16], have been shown to enhance their *in vitro* proliferation, paracrine activity, differentiation potential, and alleviation of senescence. There is no consensus regarding the level of hypoxia or the duration of exposure. Elucidating the molecular mechanisms, these studies have revealed activation and involvement of multiple signaling pathways and induction of pro-survival of molecules, including Akt, B-cell lymphoma-2 (Bcl-2), Bcl-Xl, BAG-1, Pim-1, *etc.*, and inhibition of pro-apoptotic molecules Bad, Bax, *etc.* Besides, hypoxia-induced priming also increases the higher expression of chemokine receptors, *i.e.*, C-X-C chemokine receptor type 4 (CXCR4), CXCR7, *etc.*, contributing to their emigrational and homing activity.

In one of the elegant studies from Haider's group, repeated short intermittent cycles of the anoxia-reoxygenation-based preconditioning protocol were optimized to address the issue of poor cell survival[17]. Mechanistic studies revealed the activation of Akt(ser473) and Erk1/2(Thr202/Tyr204), which also involved HIF-1α nuclear translocation with the concomitant activity of hypoxemic, *i.e.*, miRNA-210[17]. Furthermore, real-time polymerase chain reaction array for rat apoptotic genes, computational target gene analyses, and luciferase reporter assay identified FLICE-associated huge protein/caspase-8-associated protein-2 suppression in the preconditioned cells as the downstream target gene of miRNA-210 that was responsible for cytoprotective effects of preconditioning. The same research group further analyzed the significance of hypoxamir-107 in cytoprotection and its simultaneous induction with miRNA-210 in MSCs to mimic cytoprotection by ischemic preconditioning[18]. In one of the well-designed studies, Kim *et al*[19] genetically modified BM-MSCs for miRNA-210 expression to mimic the ischemic preconditioning effects for their improved survival post-engraftment in the experimentally infarcted myocardium. Interestingly, MSCs delivered miRNA-210 to the host cardiomyocytes through gap junctions to contribute to the functional recovery of the ischemic myocardium. To validate this observation *in vitro*, the transfer of miRNA-210 from MSCs to the co-cultured cardiomyocytes was abrogated by heptanol pre-treatment. A recent study has reported that hypoxic pre-treatment of MSCs significantly alters their derivative exosomal payload and accentuates their efficacy[11]. Molecularly, these exosomal payload changes have been attributed to HIF-1α induction, which modulates the expression of multiple genes regulating cell biology and functions. Put together, hypoxic/anoxic preconditioning is considered the most favored and extensively studied preconditioning. It is safe and has the potential to be safely advanced to clinical applications.

Other physical methods used for priming include electrical stimulation, stretch pre-treatment, thermal and laser pre-treatment, and, more recently, 3D culture, which incidentally simulates the natural niche condition of MSCs. Electrical stimulation protocol conditions MSCs to enhance their proliferation, migration, differentiation, and scaffold adherence [20]. The effect of physiological level electrical stimulation has been shown to impact slowly; three days of culture has little effect compared to seven days and fourteen days to stimulate osteogenic, chondrogenic, and neural differentiation of the MSCs[21,22]. Similarly, the impact of electric stimulation is also determined by whether it is continuous or pulsed. One of the exciting applications of electrical stimulation has been to acclimatize MSCs to the cardiac environment for myocardial repair and regeneration[23]. Molecular studies showed that electrical stimulation enhanced phosphorylation of Akt, focal adhesion kinase, glycogen synthase kinase, and abrogated caspase-3 cleavage. Interestingly, inhibition of AKT or focal adhesion kinase abolished the pro-survival effects of electrical stimulation. The connective tissue growth factor was cytoprotective, promoting cell adhesion to support cell survival.

Akin to electrical stimulation, mechanical stretch, and other mechanics, including compressive and hydrostatic pressure, cell biology and characteristics, such as cell survival, migration, proliferation, differentiation, *etc.*, are influenced. The underlying principle is that mechanical forces experienced by the cells are converted into signals leading to specific biological responses. For example, the effects of mechanical stretch differ in 2D and 3D culture conditions[24]. One study showed cyclical mechanical stretch-induced HIF-1α-mediated osteogenic differentiation of BM-MSCs in 2D culture conditions[25]. On the other hand, human MSCs cultured in 3D extracellular matrix-like fibrous structures significantly enhanced collagen and mineral deposition, thus increasing the rate of osteogenesis[26]. It has been reported that 5%-10% mechanical strain increased collagen synthesis without changing the surface marker changes. On the contrary, 5%-15% strain for three days significantly enhanced their proliferation[27]. Microscopically, cyclical mechanical stretch aligns the cells perpendicular to the stretch axis and remains aligned, modifying their cytoskeletal orientation. Some more relevant studies have been included in Table 1, along with their summary of results.

These data from the physical pre-treatment of MSCs show that the potential of optimizing these strategies is undoubtedly there. Still, a standard protocol for each one of the strategies needs to be optimized before any of these approaches can be used in clinical settings. However, the pre-clinical studies have proven their therapeutic efficacy and safety, with hypoxia pretreatment leading the race and set for use alone or in combination with other strategies. Some typical studies using physical manipulation of MSCs with respective salient findings have been summarized in Table 1.

Pretreatment approach using chemicals and drugs

Since the publication of early reports about organ preconditioning with cyclical intermittent ischemia-reperfusion protocol[54] and the subsequent use of preconditioning mimetics[55,56], the preconditioning approach has been successfully extrapolated to cellular and sub-cellular priming of stem cells[7]. An ever-expanding list of chemicals, preconditioning mimetics, and pharmacological agents have been reported to prime MSCs successfully for *in vitro* mechanistic studies and *in vivo* studies in different experimental animal models to elucidate their safety and efficacy. Some typical examples of these chemical and pharmacological priming agents for MSCs have been listed in Table 2.

Although using chemicals is considered a simple and preferred approach for MSC priming, approved drugs are advantageous because they are safe for human use and can be used for stem cell priming in clinics. Amongst the list of priming agents mentioned above, statins are gaining popularity[57]. Statins are a group of 3-hydroxy-3-methylglutaryl-CoA reductase inhibitors approved for cholesterol lowering. The commonly reported statins used as pre-conditioning mimetics, including lovastatin, simvastatin, atorvastatin, rosuvastatin, *etc.*, have been successfully used for MSCs priming to accentuate their stemness and functionality. For example, atorvastatin-primed MSCs survived better in the infarcted porcine heart and improved myocardial function and morphology of the infarcted heart *via* activation of endothelial nitric oxide synthase[58]. The primed MSCs also reduced cardiomyocyte apoptosis and suppressed the acute-phase inflammatory response in the ischemic myocardium[59]. Similar data have also been reported with the use of other members of the statin group, such as simvastatin[60].

Niagara *et al*[61] effectively primed skeletal myoblasts with diazoxide, a known mitochondrial ATP-sensitive potassium channel opener, to enhance their resistance to apoptosis under oxidative stress by stabilizing mitochondrial and cellular functions and improving their viability post-engraftment in the infarcted myocardium. Molecular studies showed the activation of Akt and interleukin (IL)-11/Stat3 signaling pathways with a critical role for miRNA-21 as the underlying mechanism of diazoxide-based cell priming[61-63]. The primed cells also secreted copious paracrine secretions, thus contributing to angiomyogenic repair of the infarcted myocardium. These data were consistent with the group's published data on diazoxide priming of other cell types, wherein the cell survival was attributed to the activation of PI3K/Akt/nuclear factor kappa B signaling[41,64]. After the publication of these initial studies, pharmacological agents belonging to diverse drug groups have been successfully used for MSC priming, especially for experimental animal studies. Tadalafil is an essential preconditioning mimetic for priming stem cells[65], One-time treatment of MSCs with tadalafil has been shown to protect the cells for 36 h due to enhanced 3',5'-cyclic guanosine monophosphate activity and activation of protein kinase G1. The primed cells showed a significant increase in cell proliferation and post-engraftment survival in experimentally infarcted rat hearts. The tadalafil-primed cells have also been shown to home into the ischemic myocardium and neurological recovery in ischemic heart and embolic stroke animal models, respectively[66, 67]. A recently published review comprehensively describes the therapeutic potential of tadalafil-based priming of the cells and the underlying mechanism[68].

An exciting application of the MSCs priming strategy has been using trace elements with Zinc due to its significant role in cell growth and proliferation. Sahibdad *et al*[69] have reported that pre-treatment of MSCs at lower concentrations had pro-proliferative actions besides cell adhesion. Still, at higher concentrations, it showed concentration-dependent cytotoxicity[69]. Elucidating the molecular mechanism, they found upregulation of multiple cell cycle regulating genes, *i.e.*, CDC20, CDK1, CCNA2, CDCA2, besides the induction of HIF-1α and genes involved in self-renewal, *i.e.*, Oct4, Sox2, Nanog, *etc.* For further reading, please refer to the literature review published by Noronha *et al*[70].

Pre-treatment with growth factors, cytokines, and other bioactive molecules

Pre-treatment of MSCs with bioactive molecules encompassing growth factors, cytokines, interleukins, interferon (IFN), hormones, vitamins, *etc.*, is commonly used to prime the cells. A list of widely used bioactive molecules reported by different research groups, including, among many, as listed in Table 2. These bioactive molecules are used either alone or in combination, leading to the induction of diverse signaling molecules, *i.e.*, Akt/ERK1/2, MAPK, eIF4E, p44, p70S6K, eIF48, p44/42, mechanistic target of rapamycin, S6RP, *etc.* Induction of these signaling molecules contributes to functional changes ranging from altered metabolism to cell proliferation, survival, migration and homing, differentiation, paracrine activity changes, angiogenesis, *etc.*

Table 1 Typical pre-clinical and clinical studies involving mesenchymal stem cells primed using different strategies

Ref.	Model type	Cell source and pre-treatment	Main findings
Pre-treatment of MSCs by physical manipulation			
Kim *et al*[17], 2009	*In vitro* studies	BM-MSCs pre-treated with IP (with two cycles of 30 min anoxia/reoxygenation)	IP significantly reduced apoptosis in MSCs through activation of Akt [Ser(473)] and ERK1/2 [Thr(202)/Tyr(204)] and nuclear translocation of HIF-α. There was simultaneous induction of miR-210 in the IP MSCs. RT-PCR array for rat apoptotic genes, computational target gene analyses, and luciferase reporter assay identified FLICE-associated huge protein/caspase-8-associated protein-2 in (PC)MSCs as the target gene of miR-210, responsible for improved cell survival
Fang *et al*[24], 2019	*In vitro* study	Stretch pre-treatment of AD-MSCs	Human ADSCs were subjected to cyclic stretch stimulation, significantly promoting their proliferation, adhesion, and migration. It also reduced cellular apoptosis but inhibited adipogenesis and increased osteogenesis. Long-term stretch promotes cell aging but without any size or morphological changes. Stretching also caused the induction of PI3K/AKT and MAPK pathways
Bianconi *et al*[28], 2023	A femur defect rat model	ElecS in 2D and 3D cultures	A femur defect rat model was used to assess the healing effects of MSCs pre-treated by ElecS in 2D and 3D cultures for one hour/day for seven days. The bone healing effect was evaluated at one, four-, and eight weeks post-surgery. In all groups, the percentage of new bone increased, while fibrous tissue and CD68+ cell count were reduced. However, these and other healing features, like mineral density, bending stiffness, the amount of new bone and cartilage, and the gene expression of osteogenic markers, did not significantly differ between groups
Li *et al*[29], 2023	*In vitro* study	UC-MSCs pre-exposed to (2% O_2) hypoxia + treatment with IL-1β, TNF-α, INF-γ for 24 h	Combined pretreatment with hypoxia and inflammatory factors changed UC-MSC morphology without changing viability, proliferation, or size. Also, pretreatment did not alter surface marker expression or mitochondrial function and integrity. However, pretreatment promoted UC-MSC apoptosis and senescence. Interestingly, immune regulation-related genes and protein expression significantly increased. These molecular changes increased peripheral blood mononuclear cell and NK cell proliferation and reduced NK cell-induced toxicity
Liu *et al*[30], 2021	Mice model of ICH-induced brain injury using collagenase IV	Hypoxic OM-MSCs treated with hypoxia (3% O_2) for 48 h	This study investigated the neuroprotective effects of hypoxia-preconditioned OM-MSCs in treating ICH in mice. Hypoxia-pretreated OM-MSCs reduced microglial activation and IL-1β and TNF-α. Also, there was a significant reduction in pyroptosis and pyroptosis-associated proteins in peri-hematoma brain tissues. Molecular studies showed reduced microglial NLRP3 expression, caspase-1, and reduced membrane pores on microglia after ICH
Dong *et al*[31], 2021	*In vitro* study	Macrophages co-cultured with BM-derived MSCs with mechanical stretch	Macrophages co-cultured with MSCs with mechanical stretch efficiently induced osteogenic differentiation of MSCs. Cyclical stretch caused macrophages to be polarized to the anti-inflammatory M2 phenotype, inducing IL-10 and TGF-β expression. YAP activation and nuclear translocation also caused BMP2 expression to facilitate MSC osteogenesis
Romanek *et al*[32], 2018	*In vitro* study	Porcine MSCs under HHP of 20, 30, 40, 50, or 60 MPa (1 h at 24 °C)	Porcine BM-MSCs were cultured *in vitro* and, before cryopreservation, subjected to HHP, *i.e.*, 20, 30, 40, 50, or 60 MPa for 1 h at 24 °C. Immediately after thawing and on day 8, the cells were assessed for survival and proliferation. MSCs subjected to 40, 50, and 60 MPa showed improved survival *vs* the control, while cells exposed to 40 MPa HHP had higher proliferation than the control group after eight days of culture
Hu *et al*[33], 2016	*In vitro* and *in vivo* study	Cynomolgus monkey MSCs were exposed to 0.5% oxygen for 24 h	MSCs were exposed to 0.5% oxygen (HP-MSCs) for 24 h and later used to treat MI in cynomolgus monkeys. Hypoxia pretreatment increased the expression of pro-survival/pro-angiogenic factors *in vitro*. Post-transplantation, they reduced the infarct size and LVEF on day 90 after treatment compared to the control group. This was also accompanied by higher cardiomyocyte proliferation, blood vessel density, myocardial glucose uptake, and engraftment of the transplanted cells. There were no arrhythmogenic changes
Sun *et al*[34], 2016	*In vitro* study	BM-derived MSCs and ligament tissue-derived fibroblast under the uniflex/bioflex culture system	The cells were uniaxially or radially stretched under 5%, 10%, and 15% strains at 0.1, 0.5, and 1.0 Hz. Exposure to uniaxial stretch (15% at 0.5 Hz; 10% at 1.0 Hz) increased proliferation and collagen production in fibroblast. On the other hand, the uniaxial strains (5%, 10%, and 15%) at 0.5 Hz and 10% strain at

			1.0 Hz were favorable for MSCs. Radial strain did not affect fibroblast, but radial strains 5%, 10%, and 15% at 0.1 Hz increase MSC proliferation. The data supported the differential response of the cells depending on cell type under different types of mechanical stress
Wang *et al*[35], 2013	*In vitro* study	Cyclical compressive stress on rat BM-derived MSCs	Dynamic cyclical compressive stress remarkably increased MSCs quantity and viability during early chondrogenesis, increasing cyclin D1, CDK4, and Col2α1. MEK/ERK and p38 MAPK were not activated, but BMP signaling was activated in mechanotransduction for chondrogenic proliferation
Pre-treatment with chemicals and pharmacological agents			
Qazi *et al*[36], 2022	Rat model of MI	3D-cultured rat BM-MSCs and treated with zebularine	MSCs were cultured on a collagen scaffold and, after treatment with zebularine for cardiac differentiation, were used to treat the rat MI model. Compared to the MI control, there was a significant improvement in cardiac function in the zebularine-treated scaffold-cultured group. A significant reduction in a fibrotic scar and an improvement in LV wall thickness preserved LV remodeling. Blood vessel density in and around the infarct area was also improved
Aslam *et al*[37], 2020	*In vitro* studies	UC-MSCs pre-treated with IH	The scratch assay showed a decreased scratch area in the case of IH-treated MSCs at 24 h, extending to complete closure of the scratch area at 48 h. Histological analysis showed reduced inflammation and completely remodeled epidermis and dermis without scar formation. There was a time-dependent reduction in IL-1β and IL-6. There was a simultaneous increase in Bcl-2 and TGF-β, VEGF, Bcl-2, and MMP-9, with increased angiogenesis and reduced inflammation and apoptosis
Li *et al*[38], 2015	Rat model of AMI	Atorvastatin-treated rat BM-MSCs	Atorvastatin pretreatment induced CXCR4 expression in MSCs and supported their emigrational potential. When delivered intravenously in a rat model of AMI, the cells homed into the infarcted myocardium, participated in myocardial repair, and preserved global cardiac function
Liu *et al*[39], 2014	Rat model of AMI	HIF-α prolyl hydroxylase inhibitor DMOG	DMOG pre-treatment of BM-MSCs significantly enhanced the expression of pro-survival and pro-angiogenic factors, including HIF-1α, VEGF, glucose transporter 1, and phospho-Akt. DMOG-treated MSCs also survived better than naïve MSCs post-engraftment in the rat model of AMI, in addition to increasing blood vessel density in and around the infarcted myocardium
Shinmura *et al*[40], 2011	Nude rat model of AMI	Pioglitazone pre-treated human MSCs	MSCs pretreated with pioglitazone were injected two weeks after MI. Pretreatment with pioglitazone significantly improved change in LVFS. Immunohistochemistry showed increased cardiomyogenic transdifferentiation of the transplanted cells and improved global cardiac function
Suzuki *et al*[41], 2010	*In vitro* studies	Diazoxide pre-treated BM-MSCs	Treatment of MSCs with DZ (200 μM) induced NF-κB-dependent miR-146a expression to support cell survival. Abrogation of miR-146a expression using an antisense miR-146a inhibitor abolished DZ-induced cytoprotective effects. The computational analysis demonstrated a consensus putative target site of miR-146a in the 3′ untranslated region of Fas mRNA regulating cell apoptosis. A Luciferase reporter assay revealed forced expression of miR-146a downregulated Fas expression
Pre-treatment of MSCs with cytokines and growth factors			
Chen *et al*[42], 2023	*In vitro* studies	hUC-MSCs pre-treated with IFN-γ & TNF-α alone or combined in a colitis mice model	Treatment with IFN-γ alone increased PD-L1 in hUC-MSCs, while TNF-α alone did not. Co-treatment with IFN-γ and TNF-α increased PD-L1 expression. IFN-γ also activated the JAK/STAT1 signaling pathway, increased the IRF1 transcription factor, promoted the binding of IRF1 and the PD-L1 gene promoter, and finally increased PD-L1 mRNA. TNF-α significantly enhanced IFN-γ-induced JAK/STAT1/IRF1 activation. TNF-α increased IFN-γ receptors *via* the NF-κB signaling pathway, significantly enhancing IFN-γ signaling. Finally, co-treatment inhibited lymphocyte proliferation, reduced mucosal damage, inflammatory cell infiltration, and up-regulation of inflammatory factors in colitis mice
Chen *et al*[43], 2023	*In vitro* studies	Rat BM-MSCs treated with SDF-1α	CXCR4 expression was observed on BM-MSCs by immunofluorescence staining. Treatment with SDF-1α increased collagen X and MMP13 expression during cartilage differentiation but with no change in collagen II or aggrecan. SDF-1α treated MSCs were validated in primary chondrocytes. SDF-1α increased p-GSK3β and β-catenin in MSCs. Abrogation of this pathway using ICG-001 (5 μmol/L) abrogated the SDF-1α-mediated up-regulation of

			collagen X and MMP13 expression in MSCs
Esmaeili *et al*[44], 2021	Rat model of AMI	Rat BM-MSCs overexpressing VEGF and pre-treated with SDF-1α	SDF-1α pre-treatment significantly reduced LDH release in MSCs, significantly thus increasing their survival more than the naïve control MSCs. The LVEF was improved considerably with a concomitant reduction in the infarct size in the animals in SDF-1α pretreated cells compared to the other treatment groups of animals
Lu *et al*[45], 2009	*In vitro* studies and Rat model of AMI	BM-derived Sca-1+ cells exposed to OGD and pre-treatment with IGF-1	Exposure to OGD for up to 12 h activated Erk1/2 in Sca-1(+) cells. Moreover, higher intracellular calcium with simultaneous PKC activation was also observed. Pretreatment with nifedipine or dantrolene reduced cellular calcium, abrogated PKC, and Erk1/2 activation. Pretreatment with 100 nM IGF-1 increased cell resistance to ischemia *via* Erk1/2 activation to improve their survival under OGD and post-engraftment of the infarcted heart
Hahn *et al*[46], 2008	Rat model of AMI	Rat BM-MSCs pre-treated with FGF-2 and IGF-1	Pre-treatment of MSCs with bFGF + IGF1 increased the expression of cardiac transcription factors and survival. Transplantation of the pre-treated cells in a rat myocardial infarction model reduced infarct size and improved global cardiac function compared to untreated MSCs. Pre-treatment with growth factors enhanced gap junction formation in the transplanted MSCs without any arrhythmias
Pre-treatment of MSCs by genetic manipulation			
Li *et al*[47], 2018	Isoproterenol-induced heart failure model in rats	MSCs overexpressing ADM	Transplantation of ADM-MSCs significantly improved heart function and reduced the size of the fibrotic area. Fluorescence microscopy revealed that ADM-MSCs survived considerably better in the heart. ADM-MSC treatment also improved heart function through enhanced antifibrotic activity
Gómez-Mauricio *et al*[48], 2016	Porcine heart model of MI	Porcine adipose tissue-derived MSCs genetically modified for HGF-1 and IGF-1	I/M delivery of MSCs with IGF-1 and HGF-1 was safe. Inflammation was significantly reduced in some myocardial sections analyzed. There was a significant increase in blood vessel density in ischemic tissue. Although cardiac function parameters were not significantly improved, cell retention and IGF-1 overexpression were confirmed within the myocardium. Concomitant IGF-1- and HGF overexpression promoted a synergistic effect
Gnecchi *et al*[49], 2009	Rat model of acute MI	MSCs overexpressing Akt1	Akt-MSCs, or PBS, were used to treat rats with experimental MI. High energy metabolism and basal 2-DG uptake were evaluated on isolated hearts using phosphorus-31 NMR 72 h and two weeks after MI. Treatment with Akt-MSCs increased 2-DG uptake in the residual intact myocardium *vs* PBS or the naïve MSC treatment. Also, Akt-MSC-treated hearts had normal pH and functional recovery after MI, thus showing that Akt-MSCs preserved normal metabolism and pH in the surviving myocardium
Haider *et al*[50], 2008	Rat model of acute MI	Rat BM-MSCs overexpressing IGF-1	Overexpression of IGF-1 led to enhanced phosphoinositide 3-kinase, Akt, and Bcl-xL and inhibition of glycogen synthase kinase 3beta besides the release of SDF-1α in BM-MSCs. Intramyocardially transplantation of IGF-expressing MSCs of the transplanted MSCs with massive mobilization and homing of ckit(+), MDR1(+), CD31(+), and CD34(+) cells into the infarcted heart. Infarction size was significantly reduced *vs* control. There was extensive angiomyogenesis in the infarcted heart and improved LVEF
Pre-treatment of stem cells in clinical trials			
Bartunek *et al*[51], 2013	C-CURE Clinicaltrial.gov ID: NCT00810238	hBM-MSCs pre-treated with a cocktail of bioactive molecules	In 100% of cases, treatment using MSCs pre-treated with a cardiopoietic cocktail was without complications. Cardiopoietic cell therapy did not induce systemic toxicity. LVEF was significantly improved compared to the standard therapy without cell treatment. Cell therapy also increased the 6-min walk distance, improving the New York Heart Association functional class, quality of life, and physical performance
Bartunek *et al*[52], 2016	CHART-1 Clinicaltrial.gov ID: NCT01768702	Cardiopoietic MSCs C3BS-CQR-1	Patients (n = 351) with symptomatic advanced HF with reduced LVEF (< 35%) were randomized to receive C3BS-CQR-1 or a sham procedure. Treatment with C3BS-CQR-1 resulted in a significant progressive reduction in LVEDV and LVESV during a 52-wk follow-up. Interestingly, the most considerable reverse remodeling was observed in the patients receiving moderate injections (< 20)
Qayyum *et al*[53], 2017	MyStromalCell trial. ClinicalTrials.gov ID: NCT01449032	VEGF-A165-stimulated adipose-derived stromal cells ASCs	The MyStromalCell trial is a randomized, double-blind, placebo-controlled study in sixty patients with refractory angina, CCS/NYHA class II-III, LVEF > 40%, and at least one significant

	coronary artery stenosis. ASCs were culture-expanded and primed with VEGF-A165. Six months of follow-up showed that the treatment was safe and improved exercise tolerance compared to placebo

HIF: Hypoxia-inducible factor; ADM: Adrenomedullin; ASCs: Adipose-derived stromal cells; DMOG: Dimethyloxalylglycine; ElecS: Electrical stimulation; IL-1β: Interleukin-1β; HGF-1: Hepatocyte growth factor-1; hUC-MSCs: Human umbilical-cord-derived mesenchymal stem cells; ICH: Intracerebral hemorrhage; IGF-1: Insulin-like growth factor-1; INF-γ: Interferon-γ; IP: Ischemic preconditioning; HHP: High hydrostatic pressure; LDH: Lactate dehydrogenase; LVEF: Left ventricular ejection fraction; NK cells: Natural killer cells; NLRP3: Nod-like receptor family protein 3; OM-MSCs: Olfactory mucosa mesenchymal stem cells; SDF-1α: Stromal cell-derived factor-1α; UC-MSCs: Umbilical cord mesenchymal stem cells; VEGF: Vascular endothelial growth factor; TGF-β: Transforming growth factor-β; TNF-α: Tumor necrosis factor-α; IH: Isorhamnetin; IRF1: Interferon regulatory factor 1; OGD: Oxygen and glucose deprivation; PBS: Phosphate-buffered saline; 2-DG: 2-deoxy-glucose; MSC: Mesenchymal stem cell; BM-MSC: Bone marrow-derived mesenchymal stem cell; RT-PCR: Real-time polymerase chain reaction; FLICE: FADD-like interleukin-1β-converting enzyme; AD-MSC: Adipose tissue-derived mesenchymal stem cell; MMP: Matrix metalloproteinase; Bcl-2: B-cell lymphoma-2; CXCR4: C-X-C chemokine receptor type 4; MI: Myocardial infarction; NF-κB: Nuclear factor kappa B; PD-L1: Program death ligand-1; GSK: Glycogen synthase kinase; FGF-2: Fibroblast growth factor-2; ADM: Adrenomedullin; HF: Heart failure; HIF: Hypoxia-inducible factor; LVEDV: Left ventricular enddiastolic volume; LVESV: Left ventricular endsystolic volume.

Table 2 Typical examples of growth factors, bioactive molecules, chemicals, and pharmacological agents used to pre-treat mesenchymal stem cells

GF, cytokines, & other bioactive molecules	Transgenes for genetic modulation of MSCs	Chemicals and pharmacological agents
Adrenomedullin	Adrenomedullin	Cobalt chloride
Angiopoietin-1	Akt	2,4-dinitrophenol
Angiotensin-II	Akt + angiopoietin	Aliskiren
Basic fibroblast GF	Bcl2	Atorvastatin
Epidermal GF	CXCR4, CXCR7	Diazoxide
GCSF	GATA4	Deferoxamine
Hepatocyte GF-1	Focal adhesion kinase	Dimethyloxalylglycine
Insulin-like GF-1	Hepatocyte GF-1	Glucagon-like protein-1
Interferon-γ	Hypoxia-inducible factor	Hydrogen peroxide
Interleukin1-β	Insulin-like GF-1	Lipopolysaccharide
Interleukin 6	Integrin subunit-alpha4	Nicorandil
Melatonin	Kallikerin-1	Pioglitazone
Oxytocin	cMyc	Salvianolic acid
Platelet-derived GF	Oct4	Simvastatin
Stromal cell-derived factor 1α	Protein kinase C	Sevoflurane
Thymosin β4	Sox2	Sodium butyrate
Transforming GF-β	Stromal cell-derived factor 1α	Tadalafil
Tumor necrosis factor-α	Tumor necrosis factor-α receptor	Trimetazidine
Vascular endothelial GF	Vascular endothelial GF	Trace elements like Zn
Vitamin E	Various microRNAs	Valproic acid

Bcl2: B-cell lymphoma-2; CXCR4: C-X-C chemokine receptor type 4; GCSF: Granulocyte colony-stimulating factor; GF: Growth factor; GATA4: GATA-binding protein 4; MSC: Mesenchymal stem cell.

For example, with specific receptors on the MSCs' surface membrane, oxytocin hormone has been used to pre-treat them to improve their functionality[71]. Functional and molecular studies revealed phosphorylation of Akt/ERK1/2 proteins for their role in migration, proliferation, and cytoprotection in pre-treated cells subjected to hypoxia and serum deprivation insults. There was also a significant increase in the proangiogenic and anti-apoptotic proteins. Similarly, Zhang *et al*[72] used IFN-γ for priming MSCs to attenuate experimental liver injury *via* induction of indoleamine 2,3-dioxygenase, which enhanced the protective autophagy mechanistic target of rapamycin pathway inhibition and activation of the AMPK pathway. On the same note, adipocytokines, including the proinflammatory interleukins, have been shown to contribute significantly to bone homeostasis[73]. These examples illustrate the role of a diverse array of

bioactive molecules studied in experimental settings.

Encouraged by the cellular preconditioning data, Suzuki *et al*[41] observed a dual role for connexin-43 during insulin-like growth factor (IGF-1)-based priming of BM-derived Sca-1+ cells. Treatment of Sca-1+ cells not only improved their rate of cardiogenic differentiation, but it was also cytoprotective due to the induction and translocation of connexin-43 onto the mitochondrial inner membrane in the preconditioned Sca-1+ cells[41]. Based on these novel findings, they designed the protocol for subcellular preconditioning *via* mitochondria-specific targeting of transgenic connexin-43 in Sca-1+ cells[74,75]. It was observed that Sca-1+ cells with mitochondria-specific connexin-43 overexpression were resistant to glucose-oxygen deprivation due to reduced accumulation of cytosolic cytochrome-c in the cytoplasm and lower caspase-3 activity, thus leading to their improved survival. Computational analysis revealed a Bcl-2 homology domain-3 motif in Cx-43 with a conserved pattern of amino acids akin to the Bcl-2 family, the inhibitors of cytochrome-c release from mitochondria into the cytoplasm. These data were later supported with *in vivo* assessment of subcellular preconditioning for successfully protecting the transplanted cells post-engraftment in a rodent model of acute myocardial infarction[76]. It was observed that cell-based therapy using sub-cellularly preconditioned cells successfully reduced infarct size and preserved global cardiac function.

Priming of MSCs by genetic modification

Due to the robust nature of MSCs, they can withstand the rigors of genetic modulation protocols without altering their stemness and differentiation potential. Hence, they are considered one of the best candidate cell types for combining cell therapy and gene therapy to exploit the best of the two therapeutic strategies. This combinatorial approach also allows stem cell priming *via* transferring a transgene or a set of genes of interest into the MSCs for transient or long-term expression[77]. The genetically modified cells then serve as "tiny little factories" that transiently or persistently release the gene expression product as a part of the releasate or the secretome. The releasate is rich in bioactive molecules, dominated by the transgene expression product, and may act in a paracrine or autocrine manner to initiate cell signaling, impacting the cell's stemness and functionality. One of the limiting factors in this approach of MSC priming is the availability and propensity of the specific receptors on the effector cells. The use of receptor-engineered cells can overcome this limiting factor. Also, diverse protocols based on viral and non-viral vectors have been optimized to transfer the transgene with their respective advantages and limitations[78], with significant advancements for clinical applications[79].

Genetic modulation of MSCs induces and amplifies the temporospatial expression of different proteins and soluble factors with diverse uses, such as growth factors, cytokines, chemokines, transcription factors, enzymes, and microRNAs, which enhance the regenerative potential of MSCs[80]. A long list of transgenes has been reported for genetic modulation of MSCs, encompassing pro-survival molecules to the ones supporting vasculogenic and myogenic differentiation of the cells (Table 2). Besides broadening their therapeutic capabilities, it enhances their survival post-engraftment, a problem that significantly impacts the therapeutic outcome[81].

The protocol of genetic modulation generally involves *ex-vivo* manipulation of *in vitro* expanded cells. The transgenes/s selection for the genetic modulation is made based on the desired outcome. Some typical examples of MSCs' genetic modulation for experimental and clinical studies have been given in Table 1 with a summary of the results. Meanwhile, non-viral delivery vector-based genes will remain episomal, yielding transient and low-level expression. On the contrary, viral delivery vectors, *i.e.,* lentivirus and retrovirus, are considered more efficient and yield long-term expression due to integrating the delivered genes with the host genome[82].

One of the most significant applications of genetic modulation has been reprogramming somatic cells to pluripotency, which has revolutionized cell-based therapy[83]. The reprogramming protocol has also successfully reprogrammed MSCs to develop induced pluripotent stem cells to treat infarcted myocardium[84]. The reprogrammed cells are considered surrogate embryonic stem cells and are fast-emerging for theragnostic applications and disease modeling. Another important application of genetic modulation of MSCs is loading specific cargo of the transgene expression product in their derivate exosomes[85]. It has been observed that the exosomes released from the genetically modulated cells are rich in the gene expression product. Hence, genetic priming of MSCs is becoming an excellent tool to engineer the derived exosomes the cells release as the insoluble fraction of their paracrine activity.

Combinatorial approaches in pre-treatment of MSCs

Unlike the strategies mentioned above, Li *et al*[29] in this issue report a preconditioning protocol that employs a combinatorial approach of culturing UC-MSCs in a pro-inflammatory milieu including a cocktail of IL-1β, tumor necrosis factor-α (TNF-α), and INF-γ, under hypoxia (2% O_2) for 24 h. The authors report improving the preconditioned cells' immunoregulation-related gene and protein profiles, thus enhancing their immunoregulatory properties compared to the naïve (non-preconditioned) UC-MSCs. More importantly, the preconditioned UC-MSCs' immunoregulatory properties were accentuated without conceding their biological characteristics, *i.e.,* surface marker expression, proliferation, mitochondrial integrity, *etc.* The conditioned medium from the preconditioned cells is rich in soluble immunomodulatory factors, *i.e.,* indoleamine 2,3-dioxygenase, prostaglandin E2, transforming growth factor (TGF)-β1, TNF-stimulated gene-6, and IL-10. The data from co-culture experiments involving natural killer cells or peripheral blood mononuclear cells with preconditioned UC-MSCs is intriguing and depicts significantly reduced activity of pro-inflammatory cells. These data are consistent with the prior studies, which used a similar approach to precondition BM-MSCs and Ad-MSCs. For example, using a proinflammatory milieu akin to the one used by Li *et al*[29], Rodriguez *et al*[86], they reported upregulation of anti-inflammatory genes in the preconditioned cells cultured under normoxia in a pro-inflammatory milieu for 48 h showed robust suppression of T-cell activity. On the same note, Gorgun *et al*[87] supplemented a mix of TNF-α and IL-1β (without IFN-γ) to the medium under 2% O_2 culture conditions to study the proangiogenic activity of extracellular vehicles (EVs) derived from preconditioned Ad-MSCs[87]. Analyzing the payload of EVs, the authors have proposed their use for anti-inflamaging. Interestingly, Ragni *et al*[88] used only IFN-γ to create an inflammatory milieu under

normoxia to precondition Ad-MSCs; payload profiled their derived EVs and reported their anti-inflammatory effects on the synovial fluid macrophages[88]. In summary, the common feature of these studies is the inflammatory milieu-based preconditioning protocol, either in the presence or absence of hypoxia, to accentuate their immunosuppressive potential.

Despite the combinatorial preconditioning approach reported by Li *et al*[29], which has provided exciting and clinically relevant data, the study has limitations. For example, no rationale is offered to use a cocktail of three pro-inflammatory molecules under hypoxia, unlike the prior reports. It would have been prudent to compare the effect of each pro-inflammatory molecule under normoxia and hypoxia for preconditioning to appreciate how adding each molecule to the cocktail adds to the priming efficiency of the inflammatory milieu with and without hypoxia. Secondly, an attempt must be made to elucidate the molecular mechanism and recognize the signaling pathways underlying the combinatorial preconditioning strategy. Understanding the molecular mechanism will go a long way in refining the preconditioning protocol besides discriminating the individual contribution of each pro-inflammatory cytokine in the cocktail in the presence and absence of hypoxia. It will also be interesting to compare the combinatorial preconditioning protocol with either of the non-combinative protocols, especially sub-cellular preconditioning[74,76], in a parallel set of experiments. Intriguingly, authors have reported that preconditioning increased apoptosis and senescence in the preconditioned cells; however, their anti-apoptotic capacity was increased. The authors did not attempt to elucidate whether the preconditioned cells could resist apoptosis upon subsequent exposure to oxidative stress.

There have been other similar combinatorial priming approaches using growth factors reported using growth factors and hypoxia. For example, Caroti *et al*[89] pre-treated MSCs with 10 ng/mL basic fibroblast growth factor (bFGF) followed by hypoxic culturing. The combined treatment with bFGF and hypoxic culture led to a 2.8 times higher proliferation rate and reduced senescence while maintaining their multipotentiality for up to 11th passage. In another study adopting a combinatorial strategy, Tu *et al*[90] treated MSCs with IFN-γ and TNF-α, which increased factor H, an inhibitor of complement activation, secretion in the pre-treated cells. Furthermore, the primed MSCs4 caused an anti-inflammatory response by promoting M1 to M2 macrophage transition in 2D culture. The same priming strategy in 3D culture for four days suppressed the release of indoleamine 2,3-dioxygenase, TGF-β, and IL-6 with a concomitant increase in their anti-inflammatory and immunomodulatory activity[91].

Priming MSCs from the clinical perspective

Another significant area for improvement in MSCs' pre-treatment strategies to develop super stem cells is the paucity of *in vivo* data on whether the priming of stem cells enhances their efficiency after delivery to patients in clinical settings [92]. The extrapolation of translational data is a prerequisite to observing the *in vivo* behavior of the cells that may or may not withstand the "*in vivo* hazards" of the human biological system, an aspect of cell-based therapy that is challenging to approximate during *in vitro* experimentation and experimental animal modeling. Hence, many position papers are being published to stress the need to move pre-treatment strategies to clinical settings for enhanced stemness features and reparability of MSCs[93,94]. On the practical side, some clinical trials have reported using pre-treated "primed" cells for based therapy. Bartunek *et al*[51] were the first to implement the paradigm of lineage guidance in clinical trials. They reported a multicenter, randomized trial C-CURE (Cardiopoietic stem Cell therapy in heart failure; ClinicalTrials.gov Identifier: NCT00810238) in 240 heart failure patients[95]. Extrapolating their translational study data to support cardiomyogenic lineage commitment using a cocktail of cardiopoietic factors, including TGF-β1, bone morphogenetic protein-2/4, FGF-2/4, IL-6, IGF-1/2, vascular endothelial growth factor A (VEGF-A), EGF and activin-A (which promoted nuclear translocation of cardiac transcription factors, *i.e.*, Nkx2.5, Gata4, *etc.*), the clinical trial used the cardiomyogenic lineage committed MSCs. More importantly, the pre-treatment strategy with bioactive molecules showed no evidence of increased cardiac or systemic toxicity, while their reparability was significantly improved.

A subsequent CHART-1 study (Clinicaltrials.gov ID: NCT01768702 and EudraCT ID: 2011-001117-13) was conducted by the same research group on a larger cohort of patients with symptomatic advanced heart failure with reduced left ventricular ejection fraction (LVEF) (< 35%)[52]. Follow-up results at 39 wk from 271 patients (n = 120 C3BS-CQR-1, n = 151 sham) revealed that the primary outcome was neutral. At the same time, exploratory analyses showed the benefit of cell treatment on the primary composite in patients with baseline LV end-diastolic volume in 60% of patients[96]. There was no difference in serious adverse events. A 52-wk follow-up after treatment with C3BS-CQR-1 resulted in a significant progressive reduction in left ventricular end-diastolic and left ventricular end-systolic volumes[97]. Interestingly, the most considerable reverse remodeling was observed in the patients receiving moderate injections (< 20).

Sponsored by Inbo Han, CHA University, Republic of Koreas, another phase I/II study was registered (ClinicalTrials.gov ID: NCT05011474) entitled "Safety and Efficacy Study of Matrilin-3 Pretreated Autologous Adipose-Derived Mesenchymal Stem Cells Implantation in Chronic Low Back Pain Patients with Lumbar Intervertebral Disc Degeneration (MANT3_ASC)". The Matrilin-3 treatment was expected to enhance spheroid formation, and treatment was expected to reduce low back pain and improve disability in the patients. The expected completion was in 2022, but no results have been posted as yet.

Qayyum *et al*[53] used pro-angiogenic VEGF-165 to prime adipose tissue-derived stromal cells (ASCs) for use during the MyStromalCell trial (ClinicalTrials.gov ID: NCT01449032), a randomized double-masked placebo-controlled study. The trials involved sixty patients with unstable angina, CCS/NYHA class II-III, LVEF > 40%, and at least one significant coronary artery stenosis. A six-month follow-up showed that VEGF165 primed ASC-based treatment was safe with enhanced exercise capacity *vs* placebo. A three-year follow-up revealed that the exercise performance remained unchanged in VEGF165-primed ASCs-treated patients, while it declined in the placebo-treated patients[98].

Another study was sponsored by the Federal Research Clinical Center of the Federal Medical & Biological Agency, Russia, entitled "Clinical Study of the Efficacy and Safety of the Application of Allogeneic Mesenchymal (Stromal) Cells of Bone Marrow, Cultured Under the Hypoxia in the Treatment of Patients With Severe Pulmonary Emphysema" was designed and registered (Clinicaltrial.gov ID: NCT01849159). The treatment group received an intravenous infusion of

MSCs pre-conditioned under 1% oxygen. Infusions were performed every two months for one year. However, the study was later withdrawn. On the same note, a clinical study is anticipated to involve 200 patients with ischemic heart disease (ClinicalTrials.gov Identifier: NCT02504437). The study entitled "Therapy of Preconditioned Autologous BMMSCs for Patients With Ischemic Heart Disease (TPAABPIHD)" was designed to use hypoxia pre-treated and endothelial-induced BM-MSCs and was expected to be completed in 2017. However, no results are available as no data from the study were published.

CONCLUSION

After more than two decades of extensive cell-based therapy efforts, searching for an ideal stem cell type is still in process. Embryonic, extraembryonic, and adult tissue-derived stem cells have been used for cell-based therapy, but each has some limitations. These limitations have been further exposed when used to treat pathological conditions with complex underlying mechanisms. Induced pluripotent stem cells have given new impetus to cell-based therapy but suffer from safety issues, which hamper their progress in the clinic[99]. Therefore, despite progressing to advanced phases of clinical trials, the reported safety and efficacy parameters have been encouraging but modest at best[4]. This may be attributed to many challenges and unanswered questions, mainly centering on stem cells lacking the desired features. Living bio-drugs are different from ordinary pharmaceuticals and biopharmaceuticals and, hence, need to be optimized for their biology, stemness, and functionality. One of the strategies to overcome these limitations and to impart the desired characteristics, MSCs need to be modulated with one or more priming strategies to develop "super stem cells" with enhanced stemness, better survival, superior cell biology, accentuated paracrine activity with specific secretome composition and exosomal cargo, and higher differentiation potential. Huang *et al*[100] and Jauković *et al*[101] have provided a comprehensive account of the literature to show the significance of shear stress and treatment with soluble mediators in priming MSCs. These priming strategies, together with other physical methods, genetic manipulation, pharmacological pre-treatment, growth factor preconditioning, 3D-culturing, *etc.* (Figure 1 and Table 1), may lead to the development of "super MSCs" that will be the next-generation cells to advance the field of regenerative medicine[102]. For further reading on the physical and genetic methods, the readers can refer to the studies in Table 3. Additionally, the priming of MSCs may contribute to manipulating their derivative exosomes in terms of their payload profile and surface modification for targeted delivery of exosomes with well-defined payloads as part of the cell-free therapy approach. It is a fast-emerging field with potential for clinical usage, but it is out of the scope of this editorial. For further reading on this topic, it has been reviewed in-depth by Choi *et al*[117].

More interesting is the emerging strategy of gene manipulation of exosomes and loading them with pharmacological agents for targeted delivery to the recipient tissues and organs in the body for cell-free therapy[118-120]. All these strategies have already been used and assessed in the preclinical and translational experimental models[121]. The time is now ripe to take a leap and advance them to the next step in the clinics. These functionally revamped cells will hold more incredible promise than their naive cell counterparts.

Table 3 Studies for further reading on physical manipulation and genetic priming strategies

Ref.	Model type	Cell source and pre-treatment	Main findings
Pre-treatment of MSCs by physical manipulation			
Izadpanah *et al*[103], 2022	*In vitro*	5-Aza treatment + static and microfluidic cell culture systems	5-Aza induced cardiac-specific markers in MSCs, but this induction was significantly increased after exposure to both 5-Aza and shear stress, showing their synergistic effects *vs* 5-Aza or in shear stress-only groups. These results demonstrated that MSCs' exposure to 5-Aza and shear stress is required for high-level cardiac gene expression
Manjua *et al*[104], 2021	*In vitro*/*in vivo* models for angiogenesis	MSCs exposed to magnetic pre-treatment	MSCs cultured on polyvinylalcohol and gelatin-based scaffolds containing iron oxide nanoparticles were exposed to a magnetic field. The cells showed significantly increased VEGF-A production and altered their morphology and alignment. MSCs' angiogenic potential was observed by the increase in angiogenic response using conditioned media *in vitro* and *in vivo*
Helms *et al*[105], 2020	*In vitro*	AD-SCs pre-treated with TSB or mechanical stimulation or their combined action	The study was intended to show if mechanical stimulation can support or replace TSB-induced differentiation of Ad-SCs. ASC or pre-differentiated SMC exposed to pulsatile perfusion for ten days with or without TSB resulted in collagen-I expression and circumferential orientation of the cells around the lumen. Molecular studies showed upregulation of αSMA and calponin expression. On the other hand, contractility and smoothelin expression required both mechanical and TSB stimulation
Vaez *et al*[106], 2018		BM-MSCs in static 2D and microfluidic cell culture	There was a clear but insignificant difference between the beating rate of APCs and CNCs in both 2D and the microfluidic

		systems	system during 30 d. Data from RT-PCR showed GATA4, Nkx2.5, CX43, cTnI, cTnT, and β-MHC induction during four weeks more in microfluidic chips than those co-cultured in 24-well plates. Combined shear stress and co-culture with cardiomyocytes significantly enhanced the differentiation rate *vs* co-culture alone
Popa *et al*[107], 2016	*In vitro*	hAD-SCs pre-treated by MNPs integrated in κC hydrogels	The MNP concentration in the κC hydrogels significantly influenced the cell viability, cell content, and metabolic activity. The optimal MNP concentration was 5% in κC. Exposure to magnetic actuation further altered their gene expression profile, favoring chondrogenic phenotype induction
Shi *et al*[108], 2011	*In vitro*	MSCs' exposure to CCMT	RhoA activity after CCMT stimulation was reduced. Pre-treatment of CCMT-stimulated MSCs with LPA, a RhoA activator, recovered ALP activity and Runx2 expression. In contrast, pre-treatment with C3 toxin, a RhoA inhibitor, reduced ALP activity with a concomitant reduction in Runx2. These results showed inhibition of Runx2 expression after the RhoA-ERK1/2 pathway mediates CCMT stimulation
Liu *et al*[109], 2011		hMSCs under perfusion culture system to produce FSS	hMSCs subjected to a perfusion culture system to produce FSS, which activated ERK1/2. The pre-treatment enhanced the pro-osteogenic gene expression profile in the cells *via* activating NF-κB and BMP. FSS inducing the osteogenic differentiation of hMSCs will provide new targets for osteoporosis and related bone-wasting diseases
Kasten *et al*[110], 2010	*In vitro*	BM-MSCs subjected integrin integrin-induced and inhomogeneous magnetic force exposure	Exposure to inhomogeneous magnetic forces increased Sox 9 (a marker of chondrogenesis) and decreased ALP expression. Molecular studies showed that VEGF induction induced by physical forces involved Akt activation. The results showed that the biological functions of MSCs can be stimulated by pretreatment with integrin-mediated mechanical forces and inhomogeneous magnetic field exposure
Pre-treatment of MSCs by genetic manipulation			
Li *et al*[111], 2023	*In vitro* and mice model of PD	hMSCs overexpressing VEGF189	hMSC overexpressing VEGF189-GFP significantly increased VEGF expression and slightly increased viability of the cells *vs* naïve cells. Transplantation of VEGF expressing MSCs significantly improved mechanical allodynia and inhibited the site's TRPV1 expression. TRPV1 agonists could partially block such pain relief effects. There was no tumorigenicity or neuron degeneration in hMSCs expressing VEGF189-GFP
Yu *et al*[112], 2023	*In vitro* and *in vivo* mice model of alkali-burned cornea	AD-MSCs overexpressing IGF-1	Treatment with MSCs overexpressing IGF-1 significantly recovered corneal morphology and function *vs* control and IGF-1 protein eyedrops. The healing of corneal epithelium and limbus, the inhibition of corneal stromal fibrosis, angiogenesis, and lymphangiogenesis, and the repair of corneal nerves were observed. *In vitro* experiments showed that MSCs with IGF-1 promoted trigeminal ganglion cell activity and maintained limbal stem cells' stemness
Singh *et al*[113], 2018	*In vitro*	Pharmacological and genetic manipulation of MSCs to enhance survivin	Induction of survivin is essential for MSC survival, expansion, lineage commitment, and migrational potential. On the other hand, pharmacological or genetic blockade of survivin expression in mouse and human BM-MSC increased caspase 3 and 7 expression and reduced proliferation, resulting in fewer MSC and clonogenic colony-forming unit-fibroblasts, growth factor (*i.e.*, b-FGF or PDGF)-mediated survivin modulation represents a novel therapeutic strategy
Konoplyannikov *et al*[114], 2013	*In vitro* and *in vivo* in rat model of MI	Simultaneous overexpression of IGF-1, VEGF, sSDF-1a, HGF-1 in SKM	Overexpression of four growth factors led to the induction of multiple angiogenic and pro-survival factors, including secreted frizzled-related protein-1,2,4,5, matrix metalloproteinases-3 and 9, connexin-43, netrin-1, Nos-2, Wnt-3, Akt, MAPK42/44, Stat3, NFκB, HIF-1α, and protein kinase C. Transplantation of the genetically modified cells causes extensive neomyogenesis and angiogenesis in the infarcted heart, attenuating infarct size and improving global heart function at eight weeks *vs* control animals. There was also massive mobilization and homing of stem/progenitor cells from the peripheral circulation, the bone marrow, and the heart for participation in infarcted myocardium repair
Jiang *et al*[115], 2006	*In vitro* and *in vivo* study in rat model of MI	Rat BM-MSCs are co-overexpressing Ang-1 and Akt	MSCs co-overexpressing Ang-1 and Akt survived better under anoxia *vs* naïve MSCs. At two weeks after cell transplantation, MAAs survived significantly more than the naïve MSCs in the infarcted heart. The heart function indices were significantly improved LVEF and fractional shortening *vs* control

Ye *et al*[116], 2005	*In vitro* and *in vivo* using a rat model of acute MI	SKMs genetically modulated to overexpress VEGF	The genetically modified cells expressed copious amounts of VEGF. Transplantation of the cells into the infarcted heart significantly increased blood vessel density compared to control animals. LVEF and fractional shortening were improved considerably compared to control-treated animals, and regional flow improved

5-Aza: 5-azacytidine; AD-MSC: Adipose tissue-derived mesenchymal stem cell; AD-SC: Adipose tissue-derived stem cell; ALP: Alkaline phosphatase; Ang-1: Angiopoietin-1; APC: Almost pure cardiomyocytes; ASC: Adipose tissue-derived stromal cell; b-FGF: Basic fibroblast growth factor; BM-MSC: Bone marrow derived-stem cells; BMP: Bone morphogenetic protein; CCMT: Cyclic mechanical tension; CNC: Cardiac niche cell; FSS: Fluid shear stress; GATA4: GATA-binding protein 4; hAD-SC: Human adipose tissue-derived stem cells; HGF-1: Hepatocyte growth factor-1; HIF: Hypoxia-inducible factor; hMSC: Human mesenchymal stem cells; IGF-1: Insulin-like growth factor-1; LPA: Lysophosphatidic acid; LVEF: Left ventricular ejection fraction; MAA: Mesenchymal stem cells co-overexpressing angiopoietin-1 and Akt; MI: Myocardial infarction; MNP: Magnetic nanoparticles; MSC: Mesenchymal stem cell; NF-κB: Nuclear factor kappa B; PD: Parkinson's disease; PDGF: Platelet-derived growth factor; RT-PCR: Real-time polymerase chain reaction; Runx2: Runt-related transcription factor 2; SDF-1a: Stromal cell derived factor 1-alpha; SKM: Skeletal myoblasts; SMC: Smooth muscle cell; TRPV1: Transient receptor potential vanilloid 1; TSB: Transforming growth factor-β, sphingosylphosphorylcholine, and bone morphogenetic protein-4; VEGF-A: Vascular endothelial growth factor A; αSMA: Alpha smooth muscle actin; β-MHC: Beta myosin heavy chain; κC: κ-carrageenan.

FOOTNOTES

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