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REVIEW

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How to enhance MSCs therapeutic properties? An insight on potentiation methods

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Abstract

Mesenchymal stem/stromal cells (MSCs) have emerged as a promising tool in the field of regenerative medicine due to their unique therapeutic properties as they can differentiate into multiple cell types and exert paracrine effects. However, despite encouraging results obtained in preclinical studies, clinical trials have not achieved the same levels of efficacy. To improve the therapeutic properties of MSCs, several strategies have been explored. Therefore, in this review, the therapeutic properties of MSCs will be analyzed, and an update and overview of the most prominent approaches used to enhance their therapeutic capabilities will be provided. These approaches include using drugs, molecules, strategies based on biomaterials, and modification parameters in culture. The strategy described shows several common factors among those affected by these strategies that lead to an enhancement of the MSCs therapeutic properties such as the activation of the PI3K/AKT pathway and the increased expression of Heat Shock Proteins and Hypoxia-Inducible Factor. The combined effect of these elements shift MSCs towards a glycolytic state, suggesting this shift is essential for their enhancement.

Keywords Mesenchymal stem cells, Therapeutic properties, Potentiation, Regenerative medicine, Immunomodulatory, Anti-inflammatory

†Patricia Luz-Crawford, Ana María Vega-Letter and Claudia Altamirano these have equals contribution to this work.

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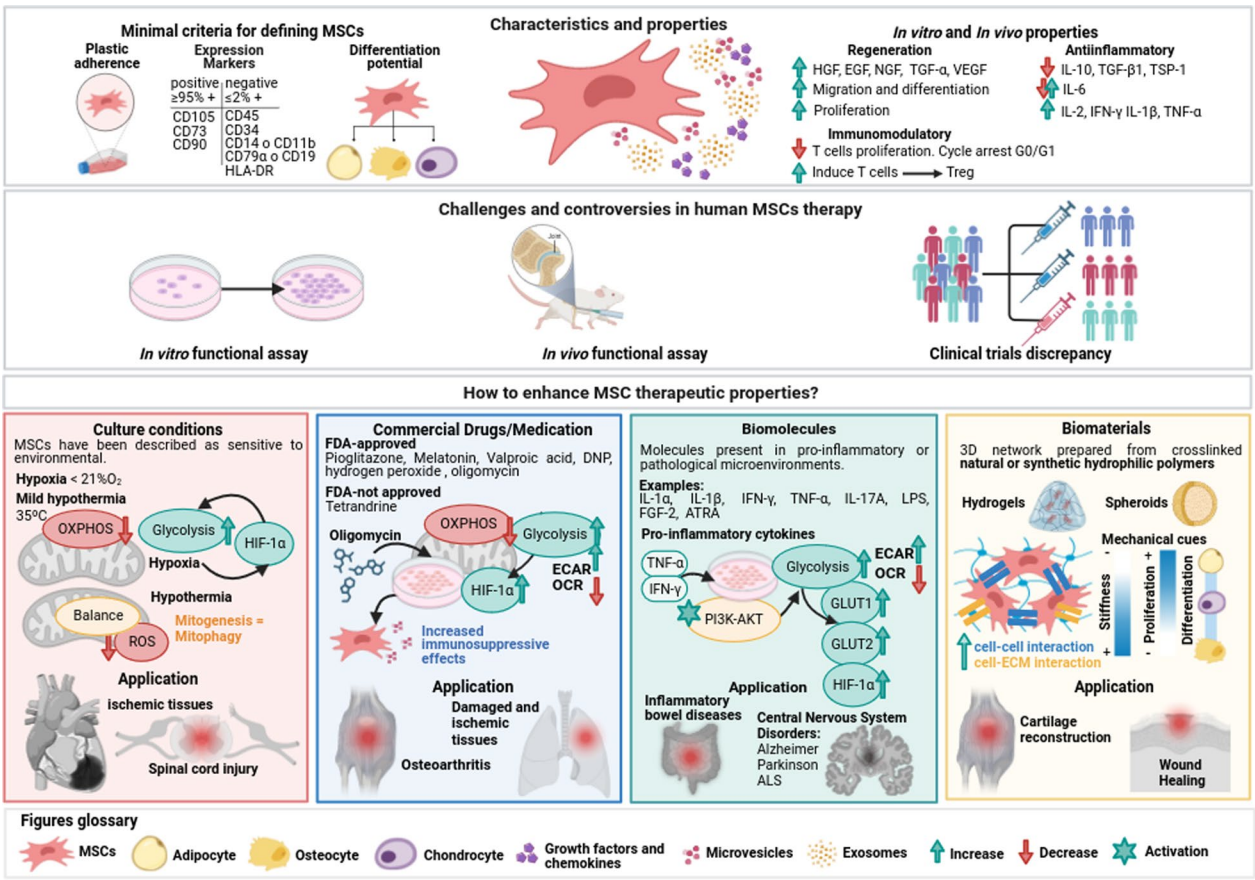
Graphical abstract



Background

Different therapies have long been at the forefront of medical research, primarily acellular therapies such as the use of medications, physical therapies or even surgeries [1–3]. Despite them being extensively researched in medicine, they often provide only temporary relief and can cause severe side effects [1]. As a result, scientists are increasingly turning to cellular therapies, particularly mesenchymal stem/stromal cells (MSCs), as promising alternatives for treating various diseases.

MSCs possess unique properties: they can differentiate into multiple cell types and modulate the immune response, making them an attractive candidate for therapeutic applications. Numerous studies in vitro demonstrate this potential, however, when it comes to translating these results into in vivo models or clinical studies, the outcome has often shown inconsistency [4], possibly due to less controlled environments impacting MSC effectiveness.

Researchers are exploring different approaches to enhance the therapeutic properties of MSCs in vitro in the hope of increasing the effect when administered in patients. Some strategies involve mimicking the natural niche of these cells or adding drugs and molecules [5–7]. These novelty approaches aim to program or priming MSCs to modify their viability, metabolism, and even the composition of the soluble factors secretion, thereby influencing their regenerative or immunomodulatory capacities [5, 7] to improve performance in clinical trials.

This review explores strategies to enhance MSCs' therapeutic potential, focusing on their natural properties and how various approaches can boost their regenerative and immunosuppressive abilities, aiming to optimize MSC-based therapies.

Mesenchymal stem/stromal cells

Characteristics and properties

MSCs are defined as multipotent cells of mesodermal origin, found in various tissues like adipose tissue (ASC), dental pulp, endometrium, bone marrow (BM-MSC), and umbilical cord (UC-MSC), among others [8]. The International Society for Cellular Therapy established three criteria to define MSCs: (1) the ability to adhere to plastic under cell culture conditions, (2) the capacity to differentiate into three specific mesodermal lineages (chondrogenic, adipogenic, and osteogenic) and (3) must express specific surface markers (CD105, CD73, CD90), but should not present hematopoietic surface markers (CD45, CD34, CD14, CD11b, CD19, and HLA-DR) [8]. These cells exhibit a high capacity for self-renewal and immunosuppressive, anti-inflammatory and regenerative properties. Despite maintaining fundamental characteristics, MSCs derived from different tissues exhibit variations that influence their selection for research and therapy. Kern et al. found that UC-MSCs demonstrated greater expanding potential, reaching up to 10 passages in vitro, while BM-MSCs and ASCs reached a maximum of 7 or 8 passages [9].

In terms of differentiation capacity, these three sources of MSCs have been reported to possess the ability to differentiate into various mesodermal lineages to varying degrees. In the context of osteogenic and chondrogenic differentiation, UC-MSCs have demonstrated superior performance compared to others [10]. It is suggested that the regulation of differentiation into these lineages involves signaling pathways such as AKT, associated with cell growth and proliferation [11], and more specifically, Notch, which has been reported to play a crucial role in osteogenic regulation and osteoblast proliferation [12]. On the other hand, BM-MSCs also exhibit a significant capacity for differentiation into osteogenic lineages, although not to the same extent as UC-MSCs but greater than ASCs [10, 13]. The regulation of this differentiation and bone formation in BM-MSCs is primarily attributed to the TAK1-NF-κB pathway, along with the production of osteoprotegerin by these MSCs [14]. In the case of ASCs, it has been reported that these cells possess a greater capacity for differentiation into the adipogenic lineage compared to MSCs derived from other tissues, due to their tissue of origin, which was attributed to an increase in the expression of adipogenic genes such as PPARG and LPL [13].

Concerning therapeutic properties, UC-MSCs exhibit superior immunoregulatory potential, particularly in inhibiting the proliferation of T lymphocytes, in addition to their differentiation capacity crucial for regeneration and reprogramming [15]. Furthermore, UC-MSCs have been described to possess a greater anti-inflammatory potential due to their secretome, such as anti-inflammatory cytokines and Ang-1 [16].

MSC's Advantages against typical treatments or therapies

Current therapies for various diseases predominantly focus on symptom management through approaches like immunosuppressive drugs, physical therapy, or surgery. These methods aim to alleviate pain or control symptoms, but generally do not address the underlying causes or reverse the disease progression [1]. Among them, the most commonly used are medications, invasive surgeries, or physical therapy [1]. Over the past decades, MSCs have been a growing focus of study because it has consistently been shown that they can achieve what more basic or common therapies cannot.

MSCs as a cellular therapy have shown increasing advantages that set them apart not only from the aforementioned treatments but also from other cell-based therapies. These cells maintain a genetic profile similar to embryonic stem cells, thus avoiding the formation of teratomas [17]. Additionally, several isolation sources correspond to tissues considered surgical waste, such as those obtained from liposuction or the umbilical cord after childbirth [17]. It is worth noting that MSCs are immune-privileged cells, meaning that the chances of triggering an immune rejection response are low since they have a low expression of major histocompatibility complex class 1 (MHC-1) and lack HLA-DR [18]. It has also been described that MSCs are capable of migrating to the site of damage due to the presence of chemokines, making them an efficient mechanism for repair and modulation [19].

Therapeutic properties

MSCs exhibit a wide range of therapeutic effects, with their regenerative, anti-inflammatory, and immunomodulatory properties being of particular interest. This review explores the clinical applications of MSCs, focusing on emerging strategies in the field. We will emphasize regenerative medicine due to its growing importance and the current scarcity of effective regenerative therapies. The following section provides a summary of these therapeutic effects and includes a table (Table 1) detailing the sources, doses, and observed effects of MSCs in the studies reviewed.

Anti-inflammatory and immunomodulatory capacities

It has been described that MSCs are capable of reducing the inflammatory response through the secretion of soluble factors such as IL-10, TGF-B1, IL-6, and TSP-1 [20]. Oh et al. evaluated the anti-inflammatory effect of MSCs in a model of corneal loss with active inflammation caused by chemical burns. They observed a reduction in

Table 1 Evaluation of MSCs from different sources and their effect on in vitro, ex vivo or in vivo assays

MSCs	Culture condition	Passage number	Dose or seeding density	Disease type pathology	Therapeutic effect	Assay type	References
Rat primary MSCs	- Expansion media (no defined) - 37 °C - 5% CO_2	3	- 2×10^6 cells in 200 uL - Direct administration on cornea - 1 Dose	Chemical burned corneas (Wound healing)	- Improved corneal surface (reduced opacity and neovascularization - Reduced T CD4 infiltration & consequent inflammation - Reduced proinflammatory cytokines & increased antiinflammatory production	In vivo (murine)	[20]
Amniotic MSCs Bone marrow MSCs	- DMEM/F12 +FCS	5	- 1×10^6 - Intravenous tail - 1 Dose	Chronic obstructive pulmonary disease (Inflammatory disease)	- Reduced leukocyte infiltration & consequent inflammation - Reduced proinflammatory cytokines - Reduced collagen deposition & consequent fibrosis (only Amniotic MSCs)	In vivo (murine)	[21]
Bone marrow MSCs	- DMEM +FBS +Penicillin/Streptomycin - 37 °C - 5% CO_2	No described	- 1×10^6 - Intravenous - 1 Dose	Rheumatoid arthritis (Autoimmune/Inflammatory disease)	- Reduced paw swelling - Reduced proinflammatory cytokines & increased antiinflammatory production - Reduced pathology markers (TLR-2, MMP3, Comp-1)	In vivo (murine)	[22]
Adipose tissue MSCs	- DMEM +FBS +L-Glutamine +Penicillin/Streptomycin - 37 °C - 5% CO_2		1)In vivo: - $1\times10^{5-6}$ - Intraperitoneally - 1 Dose 2)Ex vivo: - 5×10^4 MSCs - 1×10^5 macrophage	Colitis (Inflammatory disease)	- Increased survival rate for animal model - Recovery from weight loss for animal model - Reduced proinflammatory cytokines and chemokines production - Inhibition of proinflammatory cytokines produced by macrophages (cell–cell or conditioned media) - Inhibition of differentiation of autoreactive / inflammatory Th1 cell - Increase of IL-10 producing T cells and Treg cells - Reduced macrophage, lymphocyte and neutrophils infiltration	In vivo (murine) Ex vivo (murine)	[23]

Table 1 (continued)

MSCs	Culture condition	Passage number	Dose or seeding density	Disease type pathology	Therapeutic effect	Assay type	References
Bone marrow MSCs	- α-MEM +L-Glutamin +2-mercaptophenol +inositol folic acid - 37 °C - 5% CO_2	No described	- Co-cultured with T CD3 cells at Ratio 1:5	Evaluation Treg recruitment/ function (Inflammatory disease)	- Recruit, regulation and maintenance of Treg function overtime	In vitro	[24]
Placental MSCs	- DMEM/F12 +FBS +L-Glutamat +Penicillin/Streptomycin - 37 °C	2	- MSCs co-cultured with macrophages in Ratio 1×10^5: 1×10^6	Evaluation Immunomodulatory effect over M1 macrophage (Inflammatory disease)	- Modulation of expression M2-like macrophages - Increased production of antiinflammatory cytokines by macrophages after co-culture - Increased phagocytic activity of macrophages	In vitro	[26]
Adipose tissue MSCs Bone marrow MSCs	- α-MEM +Penicillin/Streptomycin +HPL +Heparin	1 to 5	1)Differentiation: - 5×10^3 cell/cm^2 2)Bone formation & engraftment: - 2×10^6 cell/biomaterial implant	Bone repair (Degenerative disease)	- Adipose tissue MSCs perform better osteogenic differentiation - Bone marrow MSCs perform better ectopic bone formation - Adipose tissue MSCs increase neovascularization - Bone marrow passage 2 forms bone in vivo model but not at passage 5	In vitro In vivo (murine)	[29]
Amniotic MSCs Adipose tissue MSCs	1)Amniotic MSCs: - DMEM +FBS 1)Adipose MSCs: - MesenPro RS	2	Seeding density: 2.1×10^4 / cm^2	Evaluation differentiation capacity (Degenerative disease)	- Amniotic MSCs performed accelerated and enhanced chondrogenic and osteogenic differentiation - Adipose tissue MSCs turns into premature hypertrophy thus less amenable for regenerative therapy - Amniotic MSC performed greater ECM production	In vitro	[30]
Bone marrow MSCs	- α-MEM +FBS - 37 °C - 5% CO_2	3	- 2×10^6 - Intraarticular - 1 Dose	Meniscus injury Osteoarthritis (Degenerative disease)	- Promotes meniscus regeneration - Increase expression of genes associated to cartilage development - Inhibits osteoarthritis development	In vivo (murine)	[31]

Table 1 (continued)

MSCs	Culture condition	Passage number	Dose or seeding density	Disease type pathology	Therapeutic effect	Assay type	References
Adipose tissue MSCs Bone marrow MSCs	No described	No described	- 2×10^6 - Intravenous - 1 Dose	Ischemic heart disease (Degenerative disease)	- Cardiac dysfunction due to ischemic necrosis was improved (Restored RR & QT interval) - Reduced fibrotic area and cardiomyocyte disorganization - Induction of vessel formation - Reduced collagen deposition - No significative difference between both sources of MSCs	In vivo (murine)	[32]

the levels of IL-2 and IFN-γ derived from local inflammation when MSCs were applied in their pathological model [20]. Likewise, in a model of pulmonary disease with severe inflammation, intravenous administration of MSCs reduced the infiltration of other inflammatory cells such as leukocytes, and the concentration of pro-inflammatory cytokines, including IL-6 [21]. This effect of reducing pro-inflammatory cytokines (especially IL-6 or TGF-B) is consistently maintained when using MSCs in other types of pathologies, as described by Abdelmawgoud et al. in a model of rheumatoid arthritis with chronic inflammation [22]. This decrease in inflammation has also been described in other models as colitis, where MSCs inhibit the secretion of inflammatory cytokines by Th1 lymphocytes by inducing the secretion of IL-10 through their interaction with resident macrophages [23]. MSCs also help transform T cells into regulatory T cells (Tregs) by releasing PGE2 and TGF-B1. These specialized Tregs effectively reduce inflammation as part of their natural function [24]. Similarly, these cells are capable of modulating a phenotype switch in macrophages, which are significantly involved in inflammatory processes due to their cytokines secretion such as IL-6, shifting them from a pro-inflammatory (M1) to an anti-inflammatory (M2) type [25, 26]. This occurs, as described by Mittal et al., due to the stimulation that MSCs undergo when exposed to an environment rich in pro-inflammatory cytokines and chemokines [25]. This stimulation leads to the secretion of TSG-6 and PGE2 by MSCs, promoting the transition from M1 to M2 macrophages [25].

Regeneration properties

MSCs show great promise in regenerative medicine due to their ability to differentiate into various cell types, making them ideal for regenerating damaged tissues. However, their primary mechanism for tissue recovery involves not only direct differentiation but also the secretion of paracrine factors that enhance the function of damaged cells [27]. Some of these factors include growth factors such as hepatocyte growth factor (HGF), epidermal growth factor (EGF), nerve growth factor (NGF), transforming growth factor-alpha (TGF-α), and vascular endothelial growth factor (VEGF), which promote cell proliferation, while others are associated with increased cell survival, such as HGF [27]. MSCs also release cytokines like IL-6, activating the JAK/STAT pathway for self-potentiation into chondrocytes [27]. This is crucial in degenerative cartilage conditions like osteoarthritis, where resident chondrocytes experience significant loss or apoptosis [28]. However, the regenerative properties can vary depending on the tissue from which these MSCs are isolated. In the case of ASCs, they have higher potential for differentiation into adipogenic lineages but lower potential for osteogenic lineages compared to BM-MSCs [29]. In contrast, other MSCs have demonstrated a high capacity for differentiation into osteogenic and chondrogenic lineages [30], which makes them an ideal approach for the development of targeted therapies for pathologies such as osteoarthritis. Regardless, when BM-MSCs were evaluated in a model of meniscus degeneration, it was observed an increased collagen II expression which indicated an inhibition of the progression of degeneration [31]. Similar regenerative effects were observed in models of tissue damage, such as a murine cardiac ischemia where ASCs and BM-MSCs demonstrated comparable tissue regeneration properties, highlighting their therapeutic potential [32].

Disadvantages and clinical trials discrepancy

A significant disadvantage is that when MSCs are expanded in vitro at higher passages, they may undergo cell senescence. This process can cause changes in their qualities, diminishing their effectiveness as a treatment and even undergoing malignant transformation [33]. Although all those in vitro and in vivo results appear promising, clinical trials have shown inconsistent outcomes compared to them, which is suggested is caused by the low cell survival when faced with a complex living system without controlled conditions [4]. This has led to a constant search for methods that can enhance their therapeutic capabilities, whether they are through direct cell contact or paracrine effects.

Potentiation in vitro

MSCs have been described as sensitive to environmental and cellular signals. Therefore, different approaches have been used to assess if biomolecules or change in conditions increase the potential concerning basal cultured MSCs. To underscore the novelty of this review in relation to previously published summaries, here we discuss the use of biomolecules or drugs [7, 34], biomaterials [35] or mimicking the MSCs's natural niche by altering culture conditions [36] on the therapeutic capacities of these cells and how are these improvements triggered by signals, pathways or metabolism changes (Table 2).

Culture conditions

Hypoxia is frequently employed to enhance MSCs therapeutic properties, mimicking their native low-oxygen niche (5–10%), since culturing at higher oxygen levels (20%) alters their properties [37]. Zhu et al. found that MSCs cultured in hypoxia showed increased survival post-transplantation and higher ATP production linked to elevated glycogen levels and enhanced activity of glycolytic enzymes like hexokinase (HKII) and glucose transporter 1, induced by HIF-1α [5]. These effects are

Table 2 Methods of potentiation for improving MSCs therapeutic properties

Group of enhancement	Potentiation method	MSCs	Results	References
Culture conditions	Hypoxic culture	BM-MSC	Enhanced limb regeneration in ischemic limb model Promotes ATP production	[5]
			Improved organ function Reduced inflammatory mediators Increased growth factor expression Reduced fibrotic tissue	[40]
		UC-MSC	Improved migration Enhanced survival Increased tissue preservation Enhanced axonal regeneration	[36]
	Reduced culture temperature (35 °C)	ASC	Prevents apoptosis Allows long-term proliferation Reduced inflammatory phenotype	[45]
Commercial drugs/medication	Tetrandrine	BM-MSC	Higher production of PEG2 Higher Immunomodulatory effects against natural killer cells Induction of Treg differentiation	[56]
	Melatonin		Improved self-renewal Improved differentiation capacity Reduced senescence Improved regeneration capacity through passaged cells	[6]
	2,4-dinitrophenol		Increased viability Enhanced homing Enhanced secretion of growth factors Increased angiogenesis Improved regeneration capacity	[58]
	Hydrogen peroxide		Increased survival Enhanced migration	[60]
	Oligomycin	BM-MSC UC-MSC	Enhanced immunosuppressive capacity	[48]
	Valproic acid	UC-MSC	Reduction of disease progression Enhanced engraftment Increased regenerative effect	[52]
	Pioglitazone	ASC	Improved differentiation of MSC Improved proliferation of damage tissue cells Repair of damaged tissue, Improved regeneration	[34]
Biomolecules	IL-1α IL-1β	BM-MSC	Promote survival Promoted migration Promotes secretion of neuroprotective factors Promotes functional recovery of stroke model Reduced brain damage of stroke model	[62]
	IFN-γ		Improved survival Inhibition of proliferation of T cells Increased immunomodulatory activity overall Enhanced migration Diminished damage	[7]
	All-trans retinoic acid		Increase PEG2 Enhanced angiogenesis Enhanced regeneration of wound Higher epithelialization	[73]
	IL-17A		Inhibition of proliferation of T cells Induction of Tregs	[68]
	IFN-γ TNF- α	BM-MSC	Inhibition of proliferation of T cells Increased secretion of antiinflammatory cytokines	[63]
		UC-MSC BM-MSC	Improved immunosuppressive properties	[48]
	Lipopolysaccharides	ASC	Increased secretion of growth factors Enhanced liver regeneration	[70]
	FGF-2	SHED	Increased vascularization Increased secretion of growth factors	[71]

Table 2 (continued)

Group of enhancement	Potentiation method	MSCs	Results	References
Biomaterials	Hydrogel	UC-MSC	Enhanced viability Increased chondrogenesis Increased collagen II expression	[75]
		BM-MSC	Enhanced viability Increased ECM production Enhanced differentiation	[35]
			Enhanced neovascularization Reduced inflammation Increased ECM production Enhanced wound regeneration	[76]
	Spheroid	BM-MSC	Enhanced angiogenesis Increased anti-inflammatory mediators	[78]
		ASC	Enhanced expansion in vitro Enhanced migration Reduced senescence Enhanced wound regeneration	[77]
		UC-MSC	Increased anti-inflammatory mediators Increased pro-regenerative factors	[79]

attributed to the stabilization of HIF-1α in hypoxia, that leads to the inactivation of prolyl hydroxylases, and its translocation to the nucleus [38]. There, HIF-1α forms the HIF-1α/HIF-1β complex that interacts with transcription factor binding sites within the promoter regions of target genes, referred to as hypoxia response elements (HRE) [39]. This activation leads to the transcription of genes associated with glycolytic activity, as mentioned before. Similarly, Zhilai et al. and Lan et al. observed positive effects when applying hypoxia on MSCs [36, 40]. Zhilai demonstrated that hypoxia enhanced cell survival and migration to the injury site in a spinal cord injury model. This preserved tissue integrity, promoted axonal regeneration, and involved HIF-1α stabilizing to regulate CXCR4 expression, aiding in cell migration [41]. This leads to an increase in the presence of the receptor on the membrane of MSCs, which can recognize Stromal cell-derived factor-1 (SDF1), a chemokine present in areas of damage and inflammation, and migrate rapidly [41]. Additionally, the ability of HIF-1α to enhance the survival of MSCs is attributed to its interaction with Bcl-2, a protein capable of inhibiting apoptosis and regulating cell proliferation, by binding with a hypoxia-response element in the Bcl-xL promoter [42]. On the other hand, Lan et al. observed improvements in the immunomodulatory properties of MSCs cultured under hypoxia, as well as indications of regenerative effects, such as a decrease in fibrotic tissue in their pulmonary fibrosis model [40]. This occurs through transcriptional regulation by HIF-1α, causing an increase in the expression of cytoprotective genes such as the previously described Bcl-2, as well as VEGF, EPOR and HO-1 [40]. It has also been shown that under hypoxic culture conditions, MSCs increase their proliferation rate through the upregulation of S-phase cells and maintain their stemness as under low oxygen culture their expression of HIF increases which upregulates the expression of transcription factors essential for maintaining their stemness such as Sox2, Nanog and C-Myc [37]. Considering the differentiation properties, Kwon et al. evaluated if MSCs cultured in hypoxia conditions could maintain their osteogenic potential. It was shown that under hypoxia not only did they maintain their differentiation potential but also increased their differentiation into osteoblasts [37]. This improvement in differentiation is attributed to transcriptional regulation by HIF-1α on CCN1, which interacts with the c-Jun/AP-1 pathway, a pathway that has been mentioned to promote osteogenic differentiation in MSCs [43]. Research has shown that the secretory capacity of MSCs remains comparable under both normal and hypoxic conditions. It has been described that under hypoxic conditions, there is an increased secretion of proteins related to outgrowth, inhibition of apoptosis, angiogenic effect as well as an enhanced anti-inflammatory effect attributed to IL-1Ra and HGF through miRNAs [44].

Tirza et al. investigated the impact of short-term hypothermia at 35 °C on ASCs in cell culture, which was shown to enhance ASCs viability by reducing apoptosis and oxidative stress, promoting long-term proliferation without premature senescence [45]. One of the reasons for these improvements is described as a lower temperature allowing mitochondria to maintain a better state

by balancing between mitogenesis and mitophagy, thus avoiding a cycle of oxidative stress [46].

Commercial drugs/medication

Pioglitazone, a thiazolidinedione drug used to treat type 2 diabetes increases glucose uptake [34]. It has been shown by Hong et al. that pioglitazone can improve the differentiation and regenerative capacity of ASCs by enhancing the proliferative capacity of resident cells in the damaged tissue [34]. This drug is known to be an agonist of PPAR-γ, a nuclear receptor whose activation has been reported to be important for MSCs to exert their therapeutic potential by activation of gene transcription related to lipid and glucose metabolism (LPL, GLUT4, and adipocyte fatty acid binding protein) [34, 47].

Our team has also shown the enhancement effect using Oligomycin, a drug able to induce glycolytic metabolism [48]. It increases glucose transporters (GLUT1, GLUT2) and MCT4 (monocarboxylate Transporter 4) expression, which translates into an increased glucose uptake and lactate export, thus promoting glycolysis and increasing MSCs immunosuppressive effect on proinflammatory T lymphocyte populations [48].

Another drug for enhancement is Melatonin, a hormone often prescribed for regulating sleep. Shuai et al. demonstrated that its use on MSCs increased self-renewal capacity, enhanced differentiation and regenerative potential, and reduced senescence [6]. This can be attributed to antioxidant properties of Melatonin, which protects against oxidative stress by preserving mitochondrial integrity [49]. The melatonin effect described herein is initiated by upregulation of heat shock 70 kDa protein 1L (HSPA1L), a protein governing cell growth and signal transduction, and forming complexes with cellular prion protein (PrPc), a crucial element in the self-renewal and proliferation of MSCs [50]. This novel complex translocates to mitochondria, binding to COX4IA, enhancing the proton electrochemical gradient and mitochondrial membrane potential. Lower membrane potential has been correlated with oxidative phosphorylation (OXPHOS) deficiencies, causing increased reactive oxygen species (ROS) production which translates to triggering senescence [50, 51]. Therefore, achieving an augmentation in membrane potential turns into antioxidant protection and an overall enhancement in cellular functionality.

Lastly, Valproic acid (VPA) used in UC-MSCs culture not only halted disease progression but also promoted regeneration as well as it promoted engraftment [37, 52]. VPA acts as a histone deacetylase inhibitor, enhancing the priming potential of sphingosine-1-phosphate (S1P), resulting in a heightened expression of the CXCR4 signaling pathway, increased proliferation and elevated expression of pluripotency markers, like SOX2 previously here described [52]. The activation of CXCR4 causes the activation of G protein subunits and pathways such as ERK1/2 and PI3K/Akt, leading to cell migration and proliferation in non-hematopoietic cells [53]. Furthermore, the regenerative capacity attributed to VPA is linked to the histone deacetylase inhibitor's ability to upregulate the osteogenic potential of MSCs by stabilizing CCN1 protein, thus modulating differentiation potential through the αvβ3/ILK signal pathway [54, 55].

Tetrandrine is a bisbenzylisoquinoline alkaloid drug derived from plants and commonly used in Chinese medicine [56]. It has shown to down-regulate protein kinase C (PKC) signaling, reducing IL-2 secretion, and inhibiting IL-2-dependent T lymphocyte proliferation when used on MSCs culture. Furthermore, these MSCs increase prostaglandin E2 (PGE2) secretion via the NF-κB/COX-2 signaling pathway [56] which inhibits the secretion of TNF-α by activated macrophages and induces a shift in macrophage phenotype from M1 to M2 [56, 57]. Yang et al. observed that using Tetrandrine induced a higher secretion of PGE2, a prostaglandin that regulates multiple immune cells, affecting their migration, maturation, and activation [56]. Consequently, this pretreatment resulted in enhanced in vitro immunomodulatory capacity of MSCs on natural killer cells and T lymphocyte populations, inducing a Treg phenotype [56].

Khan et al. studied 2,4-dinitrophenol (DNP) as a hypoxia mimetic agent for BM-MSCs. They found that DNP increased cell viability, boosted growth factor secretion, and enhanced angiogenic and regenerative capacities [58]. Since DNP acts as a hypoxia mimetic agent, it has been shown to increase expression of HIF-1α and HIF-2α [59]. Similarly, the use of hydrogen peroxide as an inducer of oxidative stress, increased the survival and migration of BM-MSCs described by Bianchi and Mezzpelle [53]. This effect occurs similarly to what is observed with the use of VPA, as it has been demonstrated that hydrogen peroxide increases the expression of CXCR4 and potentially activates the ERK1/2 pathway, which as mentioned earlier, regulates migration and proliferation in non-hematopoietic cells, including MSCs [60].

Biomolecules

This review highlights that MSCs require a proinflammatory stimulus to exhibit their therapeutic effects [61]. Based on this, the potential of pre-treating MSCs with proinflammatory molecules has been evaluated to enhance their therapeutic properties.

Redondo-Castro et al. investigated IL-1α and IL-1β as pre-conditioning agents for BM-MSCs. These cytokines interact with MSC membrane receptors IL-1R and

IL-1RAcP, activating NF-κB pathways to express proinflammatory or anti-inflammatory genes [62]. Redondo-Castro showed this pre-conditioning not only increased the survival and migration capacity of the MSCs to the damaged area but also enhanced the secretion of protective factors to the injured tissue and promoted its recovery [62].

Duijvestein et al. and Francois et al. investigated pretreating MSCs with IFN-γ. Duijvestein focused on the cytokine alone, while Francois combined it with TNF-α. Both studies found enhanced immunomodulatory capabilities, specifically in inhibiting T cell proliferation by degrading tryptophan, essential for these cells [7, 63]. This effect triggered by IFN-γ is attributed to the activation of the JAK/STAT signaling pathway upon its recognition by a membrane receptor [64]. This involves the dimerization of STAT1 and its recruitment to the nucleus, where it binds to elements in the promoter region of the gene encoding for IDO, causing an increase in its production [65]. Using this same combination of cytokines, Contreras-Lopez et al. demonstrated an increase in glycolytic metabolism, as evidenced by an increase in the extracellular acidification rate (ECAR) and decrease in the oxygen consumption rate (OCR) of MSCs [48]. This was attributed to an increase in the expression of glucose transporters GLUT1/GLUT2 and the induction of HIF-1α [66]. Additionally, the activation of the PI3K-AKT pathway was shown to promote the activation of the enzyme HKII, an important indicator of a shift to a glycolytic metabolism [67].

Another interleukin used to enhance the immunomodulatory effects of MSCs is IL-17A. This interleukin, employed by Sivanathan et al., directly improved the immunoregulatory capacity of BM-MSCs with an increased inhibition of T cells and inducing a Treg population [68]. To initiate this effect mediated by IL-17, the cytokine binds to an IL-17RA/IL-17RC receptor located on the cell membrane [69]. These triggers ACT1, an ubiquitin ligase that has been shown to act as an activator of the NF-κB, AKT, and ERK pathways, which were described earlier as pathways that culminate in the increased expression of anti-inflammatory genes or reduce those proinflammatory [68, 69].

Continuing with molecules displaying proinflammatory activity, Lee et al. used Lipopolysaccharide (LPS) as a molecule for cell preconditioning of ASCs. This leads to an increase in growth factor secretion by the MSCs and an improvement in their regenerative capacity [70]. For this, LPS interacts with a membrane receptor, TLR4, and activates NF-κB in a MyD88-dependent manner, triggering a change in the expression of growth factors or genes associated with the therapeutic potential of MSCs [70]. Gorin et al. studied the impact of preconditioning dental pulp mesenchymal stem cells (SHED) with FGF-2, a growth factor known for its roles in cell proliferation, migration, and differentiation across tissues. FGF-2 increased SHED's revascularization ability and boosted secretion of growth factors like VEGF and HGF, highlighting their pivotal role in enhancing MSC therapeutic functions [71]. The regulatory effect of FGF-2 on the secretion of other growth factors is suggested to occur due to the activation of the MAPK pathway from the interaction between FGF-2 and a dual tyrosine kinase receptor on the membrane, leading to a change in the mRNA expression of VEGF and HGF in endothelial or stromal cells such as MSCs [72]. All-trans retinoic acid (ATRA) is a retinoic acid that has been described to play crucial roles in cellular processes such as apoptosis, differentiation, and immune function. Additionally, it has been reported to induce the expression of HIF-1α, a transcription factor associated with hypoxic conditions that, as mentioned earlier in this article, enhances the therapeutic properties of MSCs [73]. Considering this characteristic, Pourjafar et al. proposed that ATRA could enhance MSCs' therapeutic properties. In vitro, pretreating BM-MSCs with ATRA increased PGE2 production, enhancing angiogenic and regenerative capabilities in an in vivo model of superficial epithelial damage [73].

Biomaterials

Biomaterials have been a novel approach to enhance the therapeutic properties of MSCs, as it embed cells in a 3D matrix capable of holding large quantities of fluids constituted by crosslinked natural or synthetic hydrophilic polymers [74]. This provides the MSC with a controlled microenvironment with desired characteristics.

Meng et al. developed a 3D culture with a hydrogel that presented an affinity peptide sequence. Using this for growth and administration of BM-MSCs, it improved their viability, differentiation capacity, and production, specifically the production of extracellular matrix (ECM) [35]. Similarly, Chen et al. used UC-MSCs in a hydrogel matrix and observed improved viability, increased differentiation capacity and higher expression of type 2 collagen, the main component of the ECM [75]. Although not evaluated in these studies, another study focused on determining the effect on the regenerative capacity of BM-MSCs demonstrated that these cells had a higher anti-inflammatory and regenerative potential [76]. These findings suggest that embedding MSCs in hydrogels shields them from harsh conditions in damaged areas, enabling them to exert therapeutic effects through paracrine mechanisms. Hydrogels mimic natural environments, providing essential elements like biomolecules and antioxidants that support MSCs proliferation, differentiation, and overall therapeutic effectiveness.

Finally, another method in terms of biomaterials is the use of 3D culture in the form of spheroids. A study conducted by Cheng et al. showed that using this type of culture improved the self-renewal of ASCs, their differentiation capacity, and enhanced regenerative capacity even at high cell passages [77]. These findings align with the concept that enhancing genes linked to pluripotency can boost MSCs' therapeutic efficacy. Costa et al. demonstrated that spheroid culture induced a hypoxic environment, enhancing BM-MSCs' angiogenic potential by upregulating crucial genes like VEGF and HGF [78]. Moreover, a concomitant augmentation in anti-inflammatory mediators, exemplified by TSG-6 and PTGS2, was discerned, thus indicating that this culturing methodology concurrently potentiated MSCs anti-inflammatory properties [78, 79]. This effect has been attributed to the ability of this type of culture to increase the expression of glycolytic enzymes such as PDK1 and SLC2A1, leading to an increase in the secretion of the mentioned immunomodulatory factors, as well as the secretion of pro-regenerative factors such as VEGF, FGF2, and HGF [79].

Perspective

Comparing methods to enhance MSCs, our novelty review approach, reveals commonalities in boosting cellular pathways and molecule expression, which collectively enhance their therapeutic properties (Fig. 1). For instance, the PI3K/AKT pathway is enhanced both by the use of biomolecules like TNF-α and IFN-γ and by factors in *vitro* culture such as hypoxia and hypothermia, which lead to an increased expression of Heat Shock Proteins (HSP) and HIF, respectively [67, 80–82]. Similarly, drugs can trigger PI3K/AKT pathway activation through HSP and HIF, thereby increasing cell viability [81]. This pathway activation, in turn, increases the expression of VEGF which is a crucial factor for the high angiogenic and regenerative properties of MSC [80], and HKII [67], an enzyme critical for glycolytic pathway activation, both directly associated with improved immunomodulatory capacities of these cells [67, 83]. However, both effects can also be attributed to the increased expression of HIF mentioned earlier and the expression of multiple glucose transporters [80, 84]. Additionally, both PI3K/AKT activation and increased HSP expression trigger the

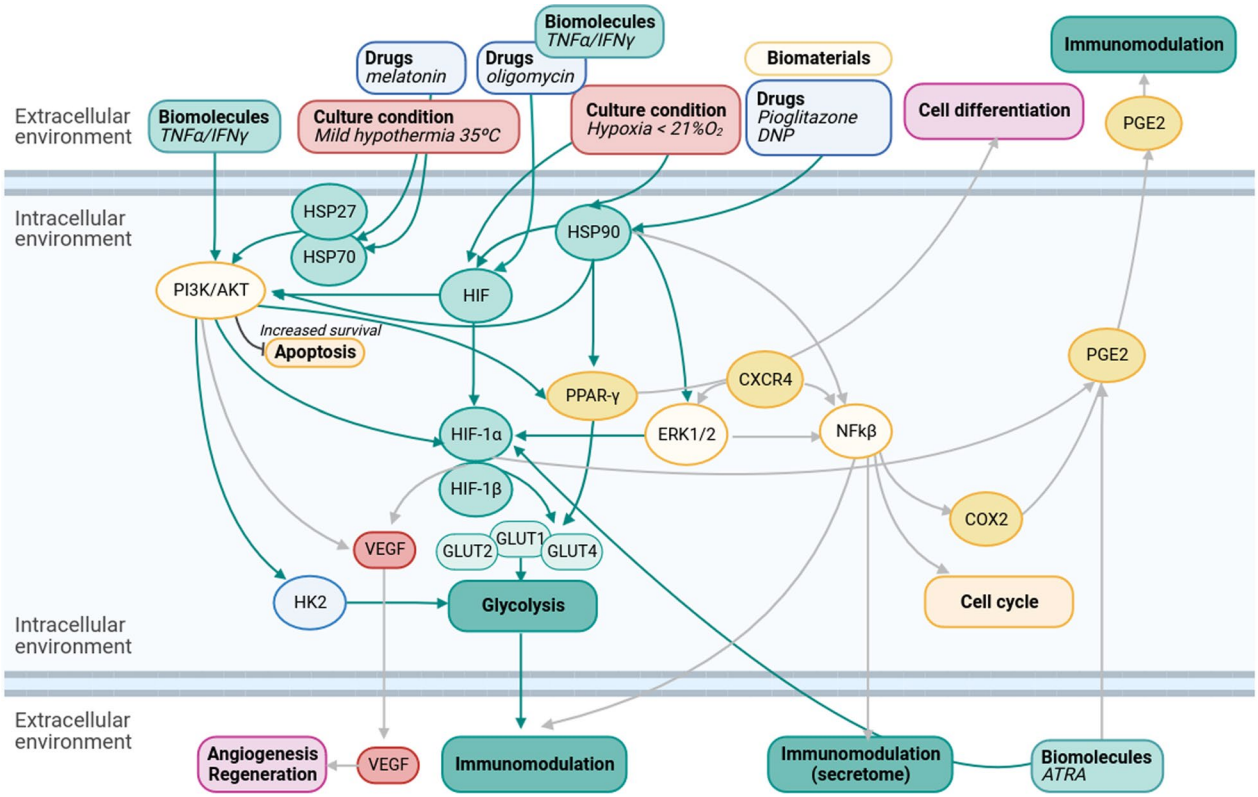


Fig. 1 Effects of different methods of MSCs enhancement. This schematic outlines how different potentiation methods discussed in the review enhance MSCs' immunomodulation, regeneration, angiogenesis, and differentiation. Colors match those in the graphical abstract, with green arrows highlighting pathways converging on glycolysis as a key metabolic pathway for enhancing MSC therapeutic properties. Created using Biorender.com

expression of PPAR-γ and the activation of the ERK1/2 pathway [83, 85]. The latter not only increases HIF-1 expression but also, together with CXCR4, activates NF-κB [86, 87]. NF-κB is an important factor in enhancing the immunomodulatory properties of MSCs through COX2 and PGE2 expression and cell cycle modulation [84, 88]. Furthermore, PPAR-γ is crucial for MSC differentiation and capable of shifting metabolism towards glycolysis by increasing the expression of certain glucose transporters [89]. It is then evident that the common factor among all potentiation methods appears to be a metabolic shift in MSCs towards a more glycolytic state, driven by PI3K/AKT pathway activation, HSP or HIF expression. This metabolic shift into a glycolytic metabolism is suggested to be the primary mediator for the potentiation of MSCs.

Conclusion

The strategies discussed in this review offer insights into optimizing the therapeutic potential of MSCs for tissue regeneration and disease treatment. By focusing on cell culture conditions, commercial drugs, molecule preconditioning, and biomaterials, researchers can enhance the therapeutic properties of MSCs and maximize their efficacy. Optimizing cell culture conditions have shown to preserve MSCs pluripotency and improve their therapeutic properties while commercial drugs enhance their self-renewal, differentiation and regenerative capacities. Similarly, the use of biomolecules has improved MSCs immunomodulatory or regenerative potential and biomaterials increase their viability, differentiation capacity and production of ECM. Not only understanding the most commonly employed enhancement methods but also elucidating the pathways and mechanisms that underpin these positive outcomes in the therapeutic properties of MSCs and exploring how these mechanisms may be interrelated supports the understanding of critical alterations o changes required to advance MSC-centered cell therapy development. Here, we highlight how diverse methodologies trigger metabolic shifts in MSCs, which are then linked to generating improved proliferative, differentiation, immunomodulatory, anti-inflammatory, and regenerative properties.

Overall, the strategies discussed in this review highlight the importance of optimizing various factors to enhance the therapeutic properties of MSCs as a way to improve treatments in regenerative medicine and disease management. Further exploration and refinement of these strategies are needed and will undoubtedly contribute to the advancement of MSC-based therapies in the future.

List of Abbreviations

3D	Three-dimensional
AKT	Protein kinase B
AP-1	Activator protein 1
ASCs	Adipose-derived stem cells
ATRA	All-trans retinoic acid
Bcl-2	B-cell lymphoma 2
BM-MSCs	Bone marrow-derived MSCs
CCN1	Cellular communication network factor 1
CD105	Cluster of differentiation 105
CD11b	Cluster of differentiation 11b
CD14	Cluster of differentiation 14
CD19	Cluster of differentiation 19
CD34	Cluster of differentiation 34
CD45	Cluster of differentiation 45
CD73	Cluster of differentiation 73
CD90	Cluster of differentiation 90
C-Myc	Cellular Myelocytomatosis
COX	Cytochrome c oxidase
COX4IA	Cytochrome c oxidase subunit 4 isoform 1
CXCR4	C-X-C chemokine receptor type 4
DNP	Dinitrophenol
ECAR	Extracellular acidification rate
ECM	Extracellular matrix
EGF	Epidermal growth factor
EPOR	Erythropoietin receptor
ERK	Extracellular signal-regulated kinase
FDA	Food and Drug Administration
FGF-2	Fibroblast growth factor 2
GLUT4	Glucose transporter type 4
HGF	Hepatocyte growth factor
HIF-1α	Hypoxia-inducible factor 1-alpha
HIF-1β	Hypoxia-inducible factor 1-beta
HIF-2α	Hypoxia-inducible factor 2-alpha
HKII	Hexokinase Type 2
HLA-DR	Human leukocyte antigen—antigen D related
HO-1	Heme oxygenase 1
HSPA1L	Heat shock 70 kDa protein 1L
IDO	Indoleamine 2,3-dioxygenase
IFN-γ	Interferon-gamma
IL-1	Interleukin-1
IL-10	Interleukin-10
IL-17	Interleukin-17
IL-17A	Interleukin-17A
IL-17RA	Interleukin-17 receptor A
IL-17RC	Interleukin-17 receptor C
IL-1R	Interleukin-1 receptor
IL-1ra	Interleukin-1 receptor antagonist
IL-1RAcP	Interleukin-1 receptor accessory protein
IL-1α	Interleukin-1 alpha
IL-1β	Interleukin-1 beta
IL-2	Interleukin-2
IL-6	Interleukin-6
ILK	Integrin-linked kinase
JAK	Janus kinase
kDa	Kilodalton
LPL	Lipoprotein Lipase
LPS	Lipopolysaccharide
M1 macrophage	Classically activated macrophage
M2 macrophage	Alternatively activated macrophage
MAPK	Mitogen-activated protein kinase
MCT4	Monocarboxylate Transporter 4
MHC-1	Major histocompatibility complex class I
miRNAs	MicroRNAs
mRNA	Messenger RNA
MSCs	Mesenchymal stem/stromal cells
MyD88	Myeloid differentiation primary response 88
NF-κB	Nuclear factor kappa B
NGF	Nerve growth factor
OCR	Oxygen consumption rate
Oct-4	Octamer-binding transcription factor 4
OXPHOS	Oxidative phosphorylation
PDK1	Phosphoinositide dependent protein kinase 1
PGE2	Prostaglandin E2

PI3K	Phosphatidylinositol-3 kinase
PKC	Protein kinase C
PPAR-γ	Peroxisome proliferator-activated receptor gamma
PrPc	Cellular prion protein
PTGS2	Prostaglandin endoperoxide synthase 2
ROS	Reactive oxygen species
SDF1	Stromal cell-derived factor 1
SHED	Stem cells from human exfoliated deciduous teeth
SLC2A1	Solute carrier family 2 member 1
SOX2	Sex-determining region Y-box 2
STAT	Signal transducer and activator of transcription
STAT1	Signal transducer and activator of transcription 1
TAK1-NF-κB	Transforming growth factor beta-activated kinase 1-nuclear factor kappa B
TGF-B	Transforming growth factor beta
TGF-B1	Transforming growth factor beta 1
TGF-α	Transforming growth factor alpha
Th1	T helper type 1
TLR4	Toll-like receptor 4
TNF-α	Tumor necrosis factor alpha
Tregs	Regulatory T cells
TSG-6	Tumor necrosis factor-inducible gene 6 protein
TSP-1	Thrombospondin-1
UC-MSCs	Umbilical cord-derived mesenchymal stem cells
VEGF	Vascular endothelial growth factor
VPA	Valproic acid
αvβ3	Alpha v beta 3 integrin

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Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publications
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