COMPREHENSIVE INVITED REVIEW

Methods and Limitations of Augmenting Mesenchymal Stem Cells for Therapeutic Applications

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Significance: Given their capacity for self-renewal, multilineage differentiation, and immunomodulatory potential, mesenchymal stem cells (MSCs) represent a promising modality of clinical therapy for both regenerative medicine and immune diseases. In this study, we review the key approaches and popular methods utilized to boost potency and modify functions of MSCs for clinical purposes as well as their associated limitations.

Recent Advances: Several major domains of cell modification strategies are currently employed by investigators to overcome these deficits and augment the therapeutic potential of MSCs. Priming MSCs with soluble factors or pharmacologic agents as well as manipulating oxygen availability in culture have been demonstrated to be effective biochemical methods to augment MSC potential. Distinct genetic and epigenetic methods have emerged in recent years to modify the genetic expression of target proteins and factors thereby modulating MSCs capacity for differentiation, migration, and proliferation. Physical methods utilizing three-dimensional culture methods and alternative cell delivery systems and scaffolds can be used to recapitulate the native MSC niche and augment their engraftment and viability for *in vivo* models.

Critical Issues: Unmodified MSCs have demonstrated only modest benefits in many preclinical and clinical studies due to issues with cell engraftment, viability, heterogeneity, and immunocompatibility between donor and recipient. Furthermore, unmodified MSCs can have low inherent therapeutic potential for which intensive research over the past few decades has been dedicated to improving cell functionality and potency.

Keywords: mesenchymal stem cell, stem cell therapy, cell-based therapy

SCOPE AND SIGNIFICANCE

Mesenchymal stem/stromal cells (MSCs) are pluripotent cells with a wide variety of independent functions and modulatory effects on other cells in their tissue microenvironment.[1] Thus, MSCs are a major source of preclinical and clinical study to improve treatment options for diseases ranging from ischemic pathologies such as myocardial infarction and critical limb ischemia, respiratory diseases such as pulmonary failure from COVID-19, and autoimmune diseases, including Crohn's disease and multiple sclerosis (Fig. 1).[1–7]

The aim of this review is to discuss popular strategies employed to augment MSC therapeutic actions for preclinical and clinical applications and their associated limitations.

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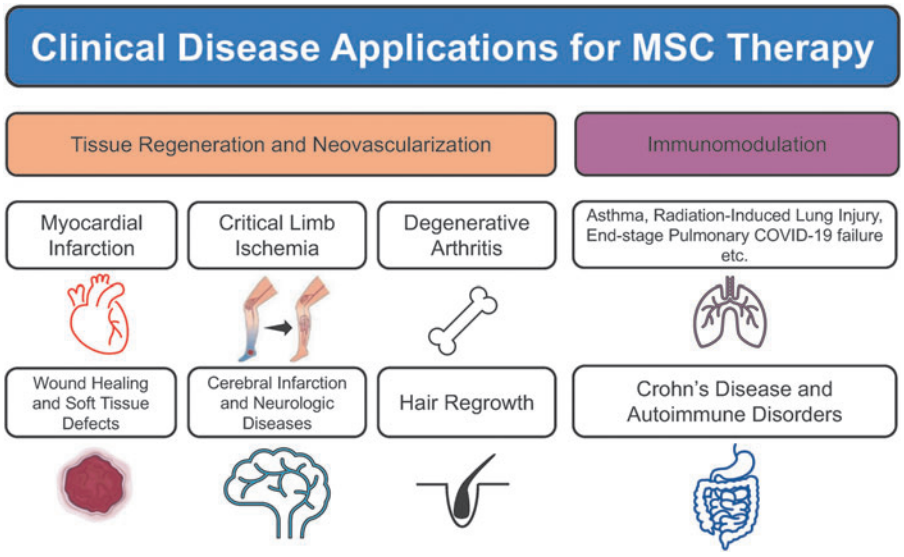

Figure 1. Examples of clinical disease applications for MSC therapy. MSC, mesenchymal stem cell.

TRANSLATIONAL RELEVANCE

MSC tissue regenerative functions, encompassing their ability for self-renewal and differentiation, have profound implications for future clinical applications. MSCs further enact a variety of paracrine effects in their local environment through the secretion of soluble factors and extracellular vesicles.[1] Moreover, their immunosuppressive properties and low immunogenicity contribute to a reduced or weakened immune response elicited by the implantation of allogeneic MSCs compared with other cell types.[8,9] Any method to improve stem cell potency and therapeutic efficacy as well as their survival and viability can have profound implications for improving stem cell-based therapy for a myriad of disease applications.

CLINICAL RELEVANCE

Despite their numerous benefits and acceptable safety profiles, unmodified MSCs have demonstrated only modest benefit in clinical trials compared to placebo treatments.[10–12] For example, MSC therapy for patients with critical limb ischemia has demonstrated little to no improvement in outcomes such as amputation-free survival rates, which many theorize may be due to low potency in the MSCs given.[10–12] Therefore, optimizing modifications of MSCs to overcome these challenges has become an intensified area of medical research in recent years.

BACKGROUND

Given their capacity for self-renewal, multilineage differentiation, and immunosuppressive properties, MSCs represent a promising modality of clinical therapy for both regenerative medicine and immune disease treatments (Fig. 2). These features of MSCs aid in the endogenous tissue repair machinery by replenishing resident cells in the wound bed, secreting a variety of soluble factors, and recruiting other cells involved in immunomodulation and tissue repair.[13] However, the effectiveness of stem cell therapy depends on efficient engraftment of cells to sites of disease to restore homeostasis and function, and thereby accomplish the desired therapeutic effect. Even when administered locally to diseased tissue sites, MSC therapy is significantly limited by the retention of administered cells, poor survival and viability, and indiscriminate or decreased migratory capacity.[13] Furthermore, significant heterogeneity in MSC donor cell populations can exist, which can result in issues with immunocompatibility between the donor and recipient.[1]

These challenges are further compounded by the local tissue microenvironment, which in diseased states can be inhospitable to MSCs due to reduced blood flow, tissue hypoxia, and widespread local inflammation in many disease states.[13,14] As a result, current methods have attempted to optimize both MSCs' nascent properties and their delivery conditions with consideration of the host environment to which they are transplanted to maximize their efficacy (Fig. 3).

BIOCHEMICAL APPROACHES

Priming with soluble factors

A well-studied strategy for improving MSCs potential is cell priming through exposure to

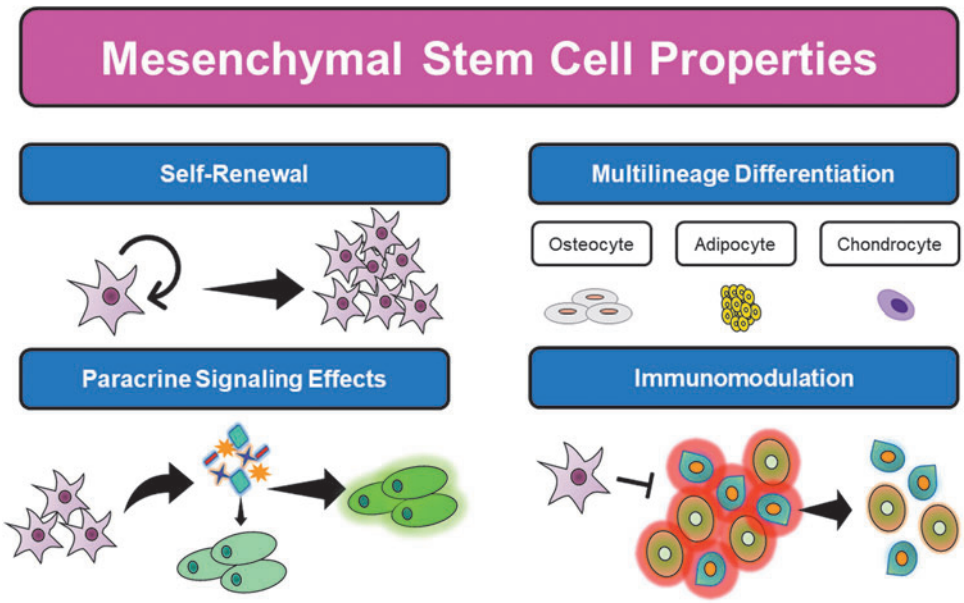

Figure 2. Overview of mesenchymal stem cell properties.

bioactive molecules before use (Fig. 4). For example, one limitation of adult stem cell treatment in the realm of cardiac disease has been due to these cells' limited plasticity and difficulty in isolating cardiac stem cells.[15] One study demonstrated that priming MSCs through culture in cardiogenic media enhanced MSC differentiation toward cardiomyogenic lineages thought to be related to the upregulation of molecular targets, including proteins Cx-43 and sarcomeric alpha-actinin.[2] Infusion of MSCs primed toward a cardiomyogenic program promoted myocardial protection compared to unprimed cells in a rodent model of acute myocardial infarction.[2] Priming MSCs can also be an effective method to improve MSC therapeutic potential in inhospitable or diseased tissue microenvironments.

Castilla et al[16] demonstrated that culturing bone marrow-derived MSCs (BM-MSCs) from diabetic mice in the presence of stromal cell-derived

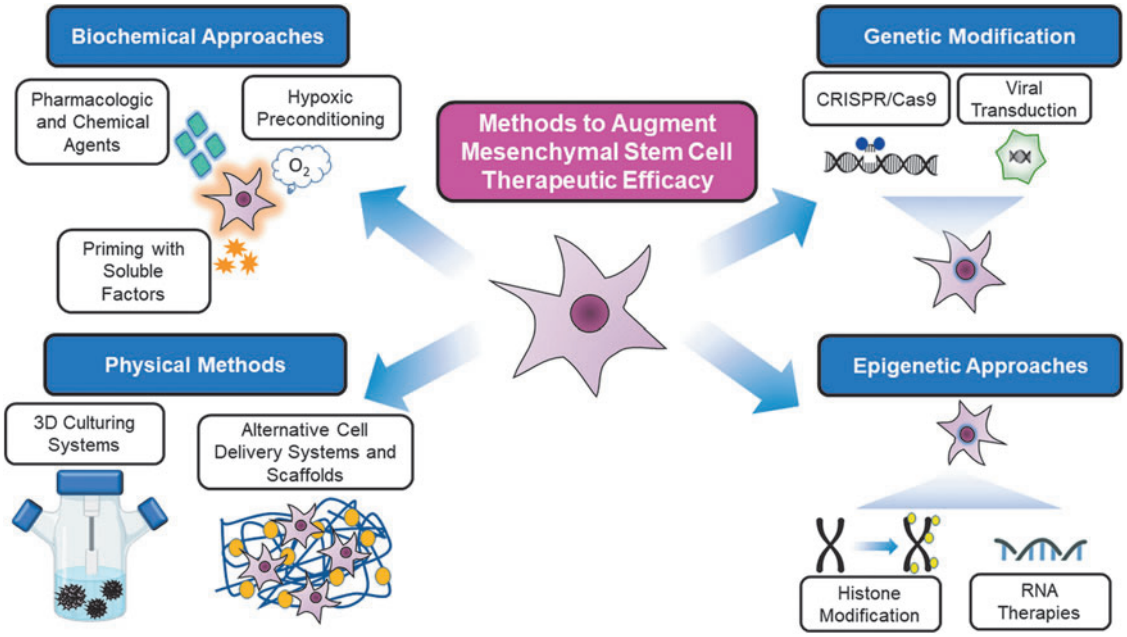

Figure 3. Schematic of common methods to augment mesenchymal stem cells for therapeutic applications.

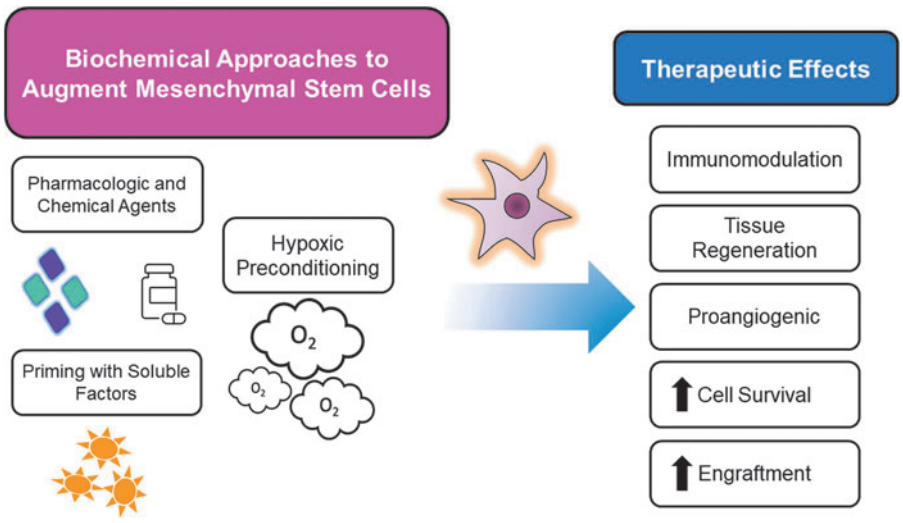

Figure 4. Effects of MSCs modified by differential priming methods.

factor 1 alpha (SDF1-α) improved neovascularization, endothelial progenitor cell recruitment, and the rate of wound healing compared to unprimed BM-MSCs in a murine model of diabetic wound healing. Similarly, others have demonstrated that adipose-derived MSCs (AD-MSCs) from diabetic mice exposed to platelet-derived growth factor (PDGF) displayed improvement in their trilineage differentiation potential and migratory capacity through PDGF-associated signaling receptor pathways.[17]

Recruitment of bone marrow-derived circulating stem/progenitor cells, including MSCs, to diseased tissue relies on critical interactions between circulating stem/progenitor cells and hyaluronan (HA) present in the extracellular matrix of wound tissues, which can also be improved through priming of molecular targets by exposure to soluble factors.[18] One previous preclinical study demonstrated that inducing overexpression of CD44 in rat-derived MSCs during exposure to PDGF enhanced *in vitro* migration through CD44-HA binding interactions and could be inhibited by anti-CD44 neutralizing antibodies.[19]

Modulation of the inflammatory milieu can be another method to prime MSCs toward their anti-inflammatory and immunomodulatory paracrine actions.[9] Previous studies have demonstrated that *in vitro* exposure of MSCs with interferon gamma can result in protrophic and anti-inflammatory changes to the MSC secretome.[9,20] Stimulation with similar cytokines can also improve MSC ability to empower resident cells through their paracrine effects.[21]

One study utilizing BM-MSCs demonstrated that preconditioning cells with interleukin 1 (IL-1) resulted in an upregulation of granulocyte colony-stimulating factor.[21] Exposure of microglial cells to the culture media of IL-1-preconditioned human MSCs resulted in an upregulation of the anti-inflammatory factor IL-10 compared to exposure with media from unprimed MSCs.[21] Thus, manipulating MSC exposure to differential inflammatory conditions and soluble factors can prove an effective strategy to augment their therapeutic potential.

Hypoxic preconditioning

An alternative method of MSC priming is through stimulation in hypoxic conditions. Unlike the normoxic conditions that MSCs are typically cultured in, the native MSC tissue microenvironment in adipose and bone marrow has an oxygen tension as low as 1–7%.[22] Furthermore, MSCs in clinical therapeutics are often conveyed to ischemic and hypoxic tissue sites that can be inhospitable to unmodified MSCs.[23] As a result, it is reasonable to expect that recapitulating the hypoxic MSC niche can optimize MSCs before cell-based therapy applications. One study found that incubation of MSCs under low oxygen tension stimulated the expression of glucose-regulated protein 78 through hypoxia-inducible factor-1 alpha (HIF-1α) molecular signaling, which resulted in improved proliferation and migratory potential.[24] Hypoxic preconditioning of MSCs further promoted their survival and angiogenic cytokine secretion when transplanted into ischemic tissues in a murine model of hindlimb ischemia.[24]

HIF-1α can also enhance MSCs' therapeutic effect by augmenting their resistance to hypoxic stress and decrease the generation of reactive oxygen species in hypoxic-ischemic tissue such as in applications for radiation-induced lung injury.[25]

Others have demonstrated that hypoxic stimulation of MSCs improve cell retention and viability by augmenting their metabolic potential, which can improve their functional capacity in tissue environments with limited nutrition and oxygen availability.[26,27] As a result, hypoxia serves as a simple method to increase the survival and retention of transplanted MSCs.

Chemical and pharmacological treatment

Another promising strategy of altering MSC function to improve their therapeutic actions and engraftment is through pretreatment of cells with pharmacologic and chemical agents before transplantation. An additional advantage of using such agents *in vitro* is achieving a modification of MSCs at doses that would be toxic or produce off-target side effects if administered systemically. For example, one significant obstacle to utilizing MSCs is the culture and expansion of sufficient quantities of cells to produce clinically relevant effects.

All-trans retinoic acid (ATRA) is a vitamin A metabolite and retinoic acid isomer and acts as a significant transcriptional regulator that has been used for a variety of diseases.[28] Pourjafar et al[28] demonstrated that pharmacologic treatment of MSCs with ATRA at varying concentrations improved MSC survival and proliferation *in vitro* through the upregulation of genes and trophic factors, including HIF-1α, C-X-C chemokine receptor type 4 (CXCR4), vascular endothelial growth factor (VEGF), and angiopoietin-2 and angiopoietin-4. Subsequent exposure of MSCs to ATRA stimulated their ability to induce angiogenesis and tube formation of endothelial cells derived from the modulation of these genes, as well as upregulation of prostaglandin E2 production and signaling pathways.[28] These proangiogenic and proliferative effects translated to increased collagenization and epithelialization, as well as increased vascular density of wounds treated with MSCs preconditioned with ATRA in a rat model of excisional wound healing.[28]

Other studies have demonstrated the benefit of pharmacologic agents to modulate the immunomodulatory potential of MSCs. Using a mouse model of asthma through constitutive overexpression of IL-13, one study demonstrated that human umbilical cord-derived MSCs pretreated with a ferroptosis inhibitor, Liproxstatin-1, were able to downregulate T helper Type 2 cells to reduce macrophage infiltration and chronic airway inflammation.[29] Although the exact mechanism was not described, Liproxstatin-1-primed MSCs were demonstrated to have multiple direct interactions with the inflammatory milieu, including suppression of M2 macrophage activation and reduced eosinophil infiltration.[29] As more pharmacologic and chemical agents continue to be discovered, future studies should characterize their effects on MSCs as potential targets for cell-based therapy applications.

Limitations

Unfortunately, priming with bioactive soluble factors, hypoxic culture conditions, and chemical agents can produce unintended, off-target downstream effects in cell populations and can be associated with only transient expression of the therapeutic targets (Fig. 5).[30] One such off-target effect can be the induction of differential major histocompatibility complex II expression, which

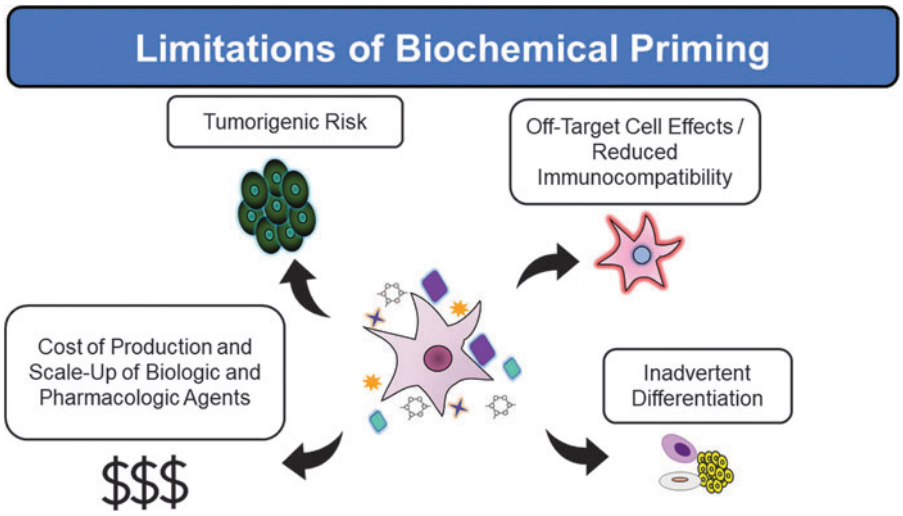

Figure 5. Drawbacks of utilizing biochemical methods to prime MSCs.

can result in reduced immunocompatibility and rejection of transplanted MSCs.[8,31] Furthermore, priming strategies can often be costly due to the price of producing soluble factors and pharmacologic agents and can be difficult to scale-up to manufacture sufficient quantities of cells for clinical applications.[32]

GENETIC MODIFICATION

Supercharging MSCs with therapeutic molecules by viral transduction

One of the most common methods of genetic engineering of MSCs is through the use of viral transduction. Adeno-associated viruses (AAV) have a favorable therapeutic profile and are one such safe FDA-approved vector currently utilized for gene therapy in humans, which have been successfully applied for MSCs.[33] Quiroz et al[14] demonstrated that modifying MSCs to overexpress an adhesion molecule, E-selectin, through an AAV vector improved their ability to induce neovascularization and functional recovery, and reduce muscle atrophy in a murine model of hindlimb ischemia. E-selectin overexpression as a target not only improved MSC engraftment but may also play roles in augmenting MSC ability to induce angiogenesis and beneficial immunomodulation in ischemic tissue.[14]

Other targets to augment MSC proliferative capacity have included viral transduction to overexpress growth regulators such as thioredoxin-1.[34] Suresh et al[34] demonstrated that MSCs transduced with AAV containing thioredoxin-1 displayed augmented cardiac function and angiogenesis for the treatment of myocardial infarction. Numerous studies have demonstrated promise of AAV for modification of MSCs to develop therapeutic properties for clinical applications.

Boosting MSCs with therapeutic factors by CRISPR/Cas9 genome editing

Another strategy becoming increasingly popular for genetic modification is the use of CRISPR/Cas9 technology. For stem cell therapy applications, CRISPR/Cas9 allows for highly specific and efficient genetic manipulation of MSCs for both gene silencing and activation.[35] Previous investigators have performed CRISPR/Cas9 gene editing to induce upregulation of HIF-1α, to improve the viability and potency of MSCs before transplantation into recipient sites with variable or low oxygen tension.[36] This improved the therapeutic efficacy of HIF-1α-modified MSCs and decreased disease burden in a murine model of Alzheimer's disease without affecting these cells' trilineage differentiation potential or viability thought to be derived from the improved retention and survival of these MSCs.[36] Others have demonstrated that targeted gene knock-in of factors such as PDGF of MSCs can improve their differentiation and regenerative capacity.[37]

Limitations

Several significant drawbacks exist with the use of genetic modification (Fig. 6). Both viral vectors

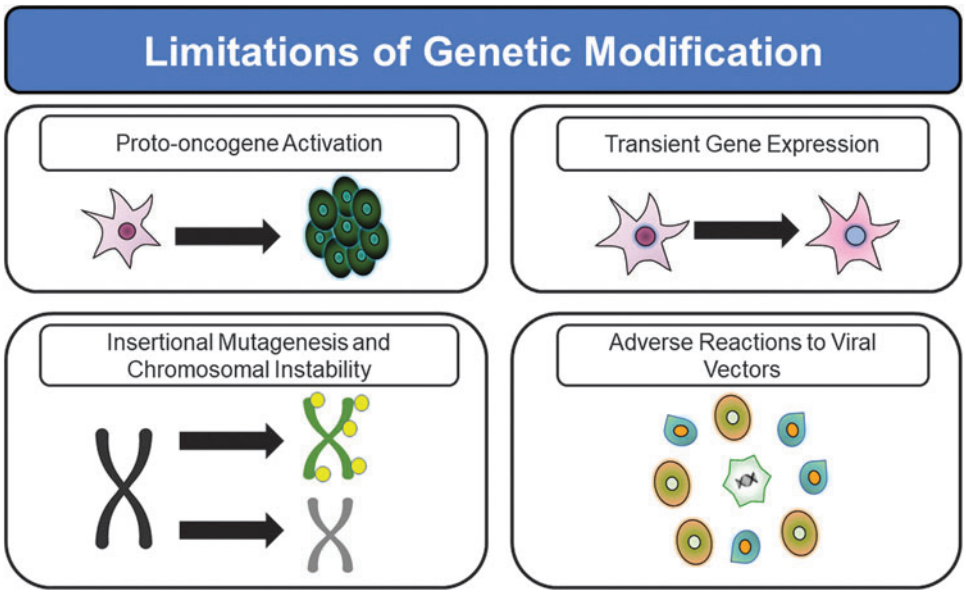

Figure 6. Common limitations of genetically modifying MSCs for clinical therapy using viral vectors and genome editing.

and transfection/transduction methods to deliver CRISPR/Cas9 constructs can result in low or transient gene expression, as well as risks associated with introducing genetic packages, including insertional mutagenesis and chromosomal instability.[38] However, for some clinical applications such as the induction of wound healing and augmentation of tissue vascularity, a transient genetic effect such as AAV-induced overexpression of MSC surface membrane-bound E-selectin can be a clinically desirable therapeutic design.[14,39] This is due to the fact that transient E-selectin overexpression avoids long-term chronic activation of healing and angiogenesis pathways.[14,39]

Such persistent activation beyond the point of complete healing and/or normal tissue perfusion would be counterproductive to the desired regenerative medicine benefit in MSC therapies and could lead to therapy with unwanted side effects. Genetic insertion can further result in epigenetic changes and inadvertent proto-oncogene activation.[38] Viral vectors can also propagate adverse immune reactions, which can limit the stability of transgene expression.[40,41]

EPIGENETIC APPROACHES

Histone modification

As more advanced technologies and understanding of genomic alterations emerge, epigenetic targets are becoming another area that holds major potential to augment MSCs (Fig. 7). For example, the role of Wnt signaling in regulating MSC differentiation in osteoblastic cell lineages has been previously characterized and implicated as a possible therapeutic target for orthopedic diseases such as osteoporosis where Wnt signaling in MSCs is suppressed.[42,43] To exploit this signaling pathway, Jing et al[42] demonstrated that augmenting GCN5 expression, a histone acetyltransferase, improved Wnt gene expression. In turn, this resulted in restored BM-MSC osteogenic differentiation and attenuated bone loss in mice with osteoporosis.[42]

Other strategies such as chemical inhibitors of histone demethylases have been utilized to rescue the osteogenic differentiation ability of MSCs and selectively induce their differentiation toward osteoblast instead of osteoclast populations in murine models of osteoporosis to prevent disease progression.[44] Influencing epigenetic regulation has the additional advantages of being reversible and stably passed down to subsequent cell lineages.[43,45]

RNA therapies

Another mechanism to potentiate MSCs' actions and therapeutic efficacy is through modulation of inhibitory RNAs such as small interfering, long noncoding, and microRNAs (miRNAs). This has been investigated in the arena of cardiac disease to examine miRNA targets related to VEGF signaling, to promote postmyocardial infarction angiogenesis.[46] Wen et al[46] demonstrated knockdown of miRNA-377 in MSCs can promote VEGF upregulation and postnatal vascularization in diseased myocardial infarction tissue in rats. Others have applied this methodology in experimental models of vascular insult, including intracerebral hemorrhage. Engineering MSCs to overexpress miRNA-21 resulted in augmented MSC survival and recovery of neurological function

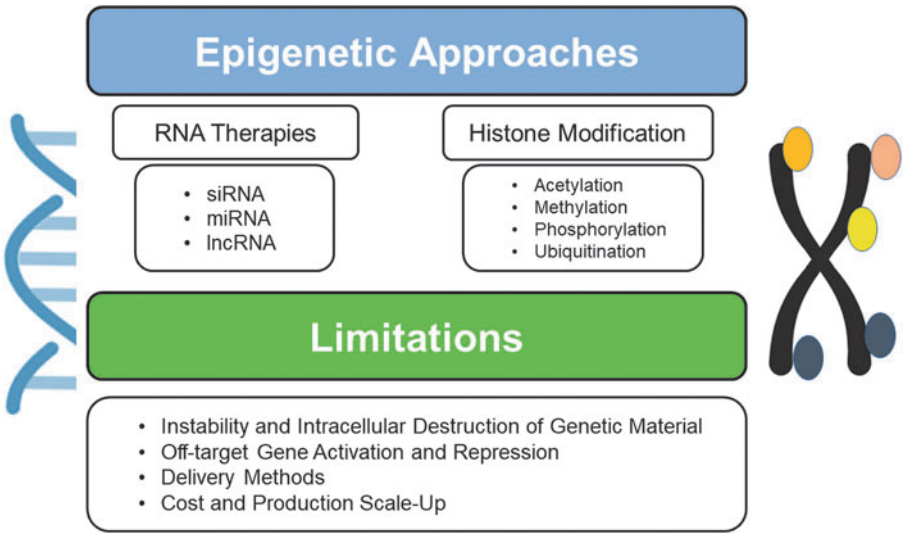

Figure 7. Epigenetic approaches to modify MSCs with their associated limitations.

in rats after intracerebral hemorrhage by activation of the nuclear factor-kappa B signaling pathway.[47]

Limitations

Shortcomings associated with epigenetic methods to modify MSCs can include their instability and destruction by *in vivo* nucleases (Fig. 7).[43] This can yield reduced treatment efficiency of carrier-free gene therapies due to difficulty with intracellular trafficking and degradation within lysosomal compartments.[43] As a result, modulation of epigenetic targets can often still require delivery by viral vectors or exosomes to achieve their therapeutic effects, and significant scale-up and manufacturing costs can still be incurred with these approaches.[48] Furthermore, epigenetic strategies must be carefully selected and constructed as they can bind multiple effector domains and promoter regions, which can result in off-target genetic activation and repression.[43]

PHYSICAL METHODS

Three-dimensional culturing systems

One emerging alternative to improve expansion and functionality of MSCs is 3D *in vitro* cell culture methods.[49] One drawback of traditional MSC-based therapy is the extensive 2D *in vitro* culture methods and resources needed to cultivate a sufficient quantity of cells required to induce a therapeutic effect. Furthermore, the time and typical protocols utilized to expand MSCs can result in cell populations with decreased stem cell potency, which may have reduced clinical efficacy. To more closely mimic the native MSC tissue microenvironment, newer 3D culturing methods have been introduced in the past decade. MSCs possess the ability to organize into 3D spheroid aggregates *in vivo*, which may recapitulate their function as limb precursors during mesenchymal condensation for early skeletal development.[49]

Cell aggregation in this manner enhances cell-cell interactions, which can trigger differential adhesion molecule expression, altered cell morphology and polarization among cells.[50] For example, Lee et al[51] demonstrated E-cadherin as a major calcium-dependent adhesion molecule playing a pivotal function in the formation of MSC spheroids. Subsequent E-cadherin activation was demonstrated to regulate both paracrine functions and self-renewal of MSCs in spheroids through ERK/AKT signaling.[51] Cadherin expression has been shown to mediate the immunomodulatory effects of MSCs upon fibroblasts during inflammatory reactions.[52,53] Furthermore, modified cadherin surfaces in 2D culture have been demonstrated to regulate MSC differentiation and cell-cell adhesion *in vitro*.[54]

Three-dimensional culture methods include both static and dynamic culture, and typically rely more closely on manipulation of external gravitational forces than manipulation of cell contact with specific substrates (Fig. 8). Static culture environments utilize low-attachment surfaces, hanging-drop, forced aggregation, or magnetic levitation methods to promote MSCs to coalesce into

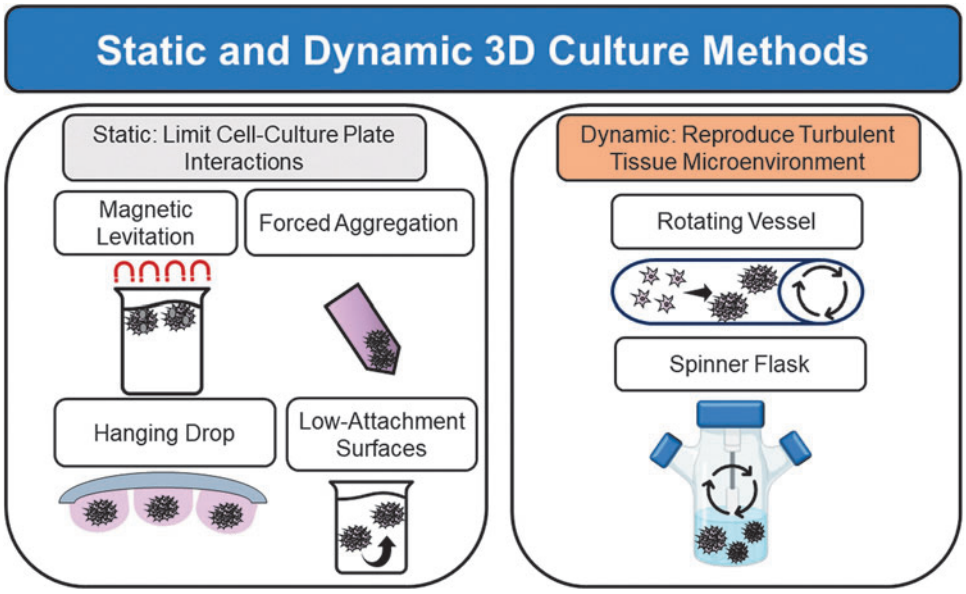

Figure 8. Three-dimensional culture methods to produce MSC spheroids *in vitro*.

spheroids by decreasing their interaction with tissue culture plate surfaces.[21,55,56] Dynamic culture methods attempt to recapitulate the natural turbulent tissue microenvironment through continuous mixing using spinner flasks or rotating wall vessels.

One study comparing 2D culture versus dynamic culture systems demonstrated improved adipogenic and osteogenic differentiation capacity of MSC spheroids cultured in dynamic 3D environments.[57] However, osteogenic differentiation potential was reduced in these cells due to decreased type I collagen expression and reduced integrin/type I collagen signaling interactions between cell and the extracellular matrix.[57,58] Future advances and therapies utilizing MSCs should carefully consider the advantages associated with 3D culture systems to augment their intended clinical effects.

Alternative cell delivery systems and scaffolds

Another method of augmenting MSC potential by physical means is through cell delivery systems to improve their engraftment and viability. Scaffold-based platforms utilizing both natural and synthetic biomaterials can be seeded with MSCs to improve their integration in tissue sites where they are applied, particularly if the recipient tissue microenvironment is diseased or inhospitable to donor MSCs. MSCs cultured on chitosan-based scaffolds have been demonstrated to have increased chondrogenic differentiation potential, while still preserving markers of MSC stemness, including Oct4, Sox2, and Nanog.[59] However, these substrates should be carefully selected as they can also decrease the stemness of MSCs.

Wang et al[60] demonstrated that MSCs exposed to micropatterned polyethylene glycol demonstrated upregulated genetic expression of markers related to osteogenesis and adipogenesis, but downregulated expression of genes related to stemness such as THY1 and MEST. Chitosan and other molecular substrates can also be utilized to create hydrogels to enhance MSC delivery and survival. A protein-based hydrogel (SHIELD-2.5) was created by one group with a heightened degree of mechanical stiffness to improve protection of induced pluripotent stem cells from shear forces experienced during injection.[61] Utilizing this hydrogel delivery system increased stem cell engraftment and arteriogenesis.[61]

More recently, small-molecule hydrogels have begun to show early promise with the advantages of being more readily biodegradable and compatible compared to standard hydrogels.[62,63] In a murine model of hindlimb ischemia, Huang et al demonstrated that the use of a small-molecule hydrogel using disulfide bond links improved engraftment of human placenta-derived MSCs as well as their paracrine and proangiogenic functions, which resulted in the regeneration of muscle cells and restored perfusion.[59]

Utilizing other autologous substrates such as platelet-rich plasma (PRP) and fat grafting has shown significant promise to improve the tissue regenerative functions of MSCs. PRP holds potential as a substrate, given the regenerative properties of hundreds of signaling factors and bioactive proteins contained in platelet alpha granules, including PDGF and VEGF, which can augment the environment and tissue repair function of MSCs.[64–67]

Moreover, tissue scaffolds containing HA and/or collagen to approximate the cutaneous dermal bed have long been utilized for surgical reconstruction of soft tissue defects and wound repair and represent another strategy to augment substrates such as PRP.[68–71] For example, PRP has been combined with both HA and fat grafting for clinical applications ranging from wound repair to inflammatory diseases such as Hidradenitis suppurativa.[72–77] PRP can be further prepared as leukocyte poor and with low or high levels of fibrin, which can be optimized for the survival of hair follicle stem cells to treat conditions such as hair loss and androgenetic alopecia.[78–85]

To explore potential solutions for the treatment of osteochondral lesions related to trauma and joint degeneration, Scioli et al found that administration of PRP and recombinant insulin could enhance the osteogenic and chondrogenic differentiation capacity of AD-MSCs independent of insulin-like growth factor-1 receptor and mammalian target of rapamycin signaling.[86] Fat grafting has long been recognized for its clinical applications in tissue regeneration and reconstruction, in part, due to its significance as a source of and delivery method for AD-MSCs.[87–95] AD-MSC phenotype and viability can be influenced by the method of fat graft preparation (enzymatic versus mechanical), as well as delivery with the stromal-vascular fraction (SVF) from fat containing a heterogeneous cell population of pericytes, mast cells, endothelial cells, smooth muscle cells, preadipocytes, and AD-MSCs.[95–98]

In particular, isolation and delivery of the SVF offer the concerted benefits of these heterogeneous cell types in maintaining an environment capable of sustaining AD-MSCs, which may contribute to their clinical uses for tissue reconstruction and rejuvenation.[99–103] Utilizing a similar concept to deliver MSCs with their native tissue envi-

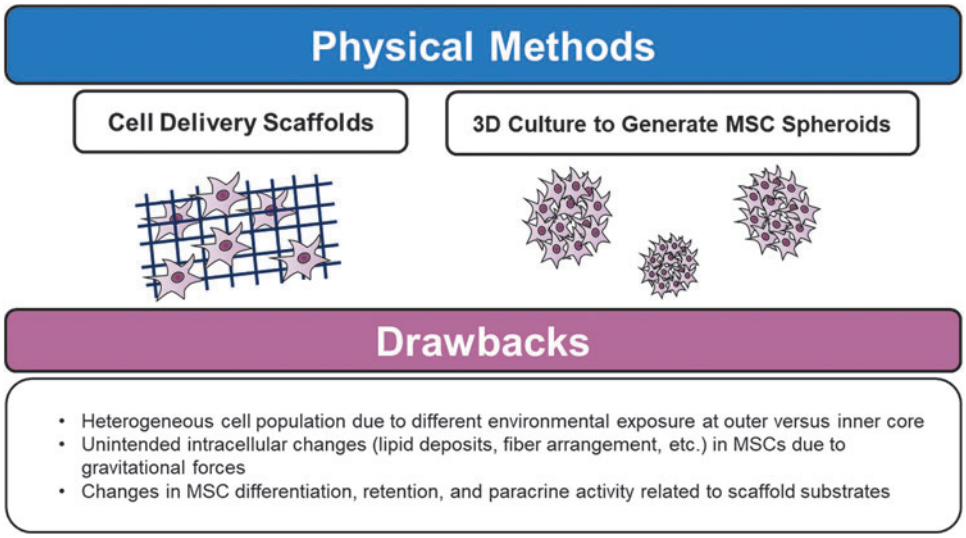

Figure 9. Recognized limitations of MSCs exposed to physical methods of modification.

ronment, others have demonstrated the potential of skin micrografts containing HF-MSCs as a targeted delivery method to regenerate hair and follicle growth in patients with hair loss and androgenetic alopecia.[104–106] Overall, research dedicated to optimizing scaffolds and delivery methods for cell-based therapy hold significant potential as another promising strategy to utilize MSCs for a wide variety of clinical applications.

Limitations

Despite their novelty and functional resemblance to native MSCs, MSC spheroids generated by 3D culture methods have several drawbacks (Fig. 9). Given their spherical arrangement, the environmental exposure to both oxygen and nutrient supply is unevenly distributed between cells at the outer versus inner layers, which can result in necrosis of spheroid cores.[107–109] This can result in heterogenous morphology, cellular adhesion molecule expression, paracrine activity, and decreased cellular metabolism among coalesced cells.[107,108,110,111]

Culturing with the effect of microgravitational forces as in these static and dynamic culture systems can also cause unintended changes in MSCs such as increased intracellular lipid deposition, decrease in RhoA activity, and interrupted F-actin fibers.[112] Similar challenges are faced by cell delivery scaffolds. The substrates utilized for cell delivery systems must be carefully selected with regard to their ability to stimulate cell differentiation, proliferation, retention, polarization, and cell-cell communication.

CONCLUSIONS & PERSPECTIVES

Although many MSC therapies have not yielded definitive, pronounced benefits in many clinical trials, intensive clinical and preclinical investigations are underway to optimize aspects of stem cell regenerative and immunomodulatory potential. As more functional targets and pathways continue to be discovered and refined to augment cell therapy potency, strategies to modify MSCs through biochemical, physical, genetic, and epigenetic methods will need to be continually optimized to achieve these intended clinical applications.

Modification of molecular targets to optimize the functional tissue repair and immunomodulatory machinery of MSCs may uncover the optimal method of utilizing MSCs to be a combination of these aforementioned strategies. One study demonstrated that the combination of a viral vector to induce overexpression of CXCR4 on umbilical cord-derived MSCs improved these cells' ability to bind a chitosan scaffold crosslinked to a bioactive factor (brain-derived neurotrophic factor) (114).

The overexpression of CXCR4 substantially augmented the ability of these MSCs to be seeded onto this scaffold, and the transplantation of this cell-scaffold implant into the brain cavity of rats with traumatic brain injury resulted in improved migratory and neuronal differentiation capacity of MSCs at the site of injury.[113] Similar strategies as this one combining multiple approaches may serve as an elegant method to harness the maximum therapeutic potential of MSCs and provide future hope for innumerable clinical therapeutics. However, future studies are still needed to address

limitations associated with cell-based therapy, despite these modifications (Fig. 10). For all *in vitro* manipulation and attempts at expansion of stem cells, an important concern is the lifespan and viability of MSCs before senescence.

The accumulation of age-related genomic changes can result in incremental loss of cellular functions and homeostasis with increasing passage numbers that future approaches could attempt to limit through specific genetic targets.[43] An equally important concern of cell-based therapy is the associated tumorigenic risk associated with implantation of modified, ectopic cells. Previous work has acknowledged the ability of MSCs to form teratomas at injection sites, and future work should consider the risks of insertional mutagenesis and alteration associated with priming of native cells to limit the generation of tumorigenesis.[114]

The tissue source from which MSCs are derived for therapy is another important technical consideration for any future experimental investigations into MSC modification. Stem cells derived from common sources such as umbilical, adipose, and bone marrow tissue contain heterogeneous genetic landscapes and functional characteristics that may not respond equivalently to the same therapeutic alterations.[49] As a result, the optimal conditions and stimuli must be tailored to each distinct origin of stem cells to establish cell-specific protocols and ensure the consistency of delivered stem cells.

As important as the origin of the stem cells utilized, clinical applications must also consider the tissue microenvironment to which cells are delivered. For example, application to ischemic tissue sites in extremities with critical limb ischemia should consider the relative hypothermia and lack of nutrient and blood supply in these areas.[60] Cell-specific manipulations to improve MSC proangiogenic effects and cell engraftment as well as the scaffolds with which they are delivered in can be optimized to overcome these factors in diseased host tissue.[14,60,115]

These alterations should also take into account the resultant immunogenic profile of MSCs to produce the most immunocompatible profile of donor cells to limit a robust recipient immune response.[31] One such emerging arena of medical research, given their favorable immunologic phenotype and lack of genomic materials, is the use of MSC-derived exosomes from modified MSCs.[47] As future investigations continue to delineate the advantages and disadvantages of both MSC and MSC-derived exosome therapy for both regenerative medicine and immunomodulatory applications, standard application-based assays will need to be developed to measure their desired clinical responses. Despite the significant limitations that still need to be overcome in scaling up and applying these methods to augment MSCs, MSC-based therapy holds tremendous promise in improving patient outcomes for a wide variety of regenerative purposes and disease treatments.

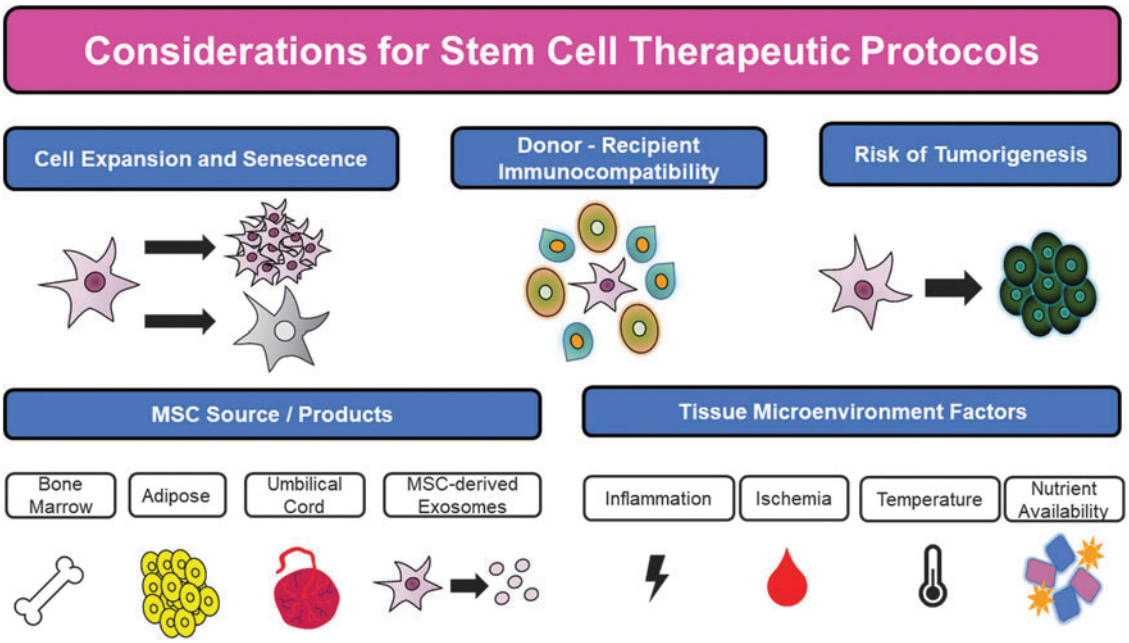

Figure 10. Factors for consideration in designing future MSC modification protocols for clinical applications.

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TAKE-HOME MESSAGES

- MSCs represent a promising modality of clinical therapy for both regenerative medicine and immune diseases.
- Unmodified MSCs have demonstrated only modest benefits in many preclinical and clinical studies due to issues with cell engraftment, viability, heterogeneity, and immunocompatibility between donor and recipient.
- Priming cells with soluble factors or pharmacologic agents as well as changing oxygen availability are effective biochemical methods to augment MSC potential.
- Both genetic and epigenetic methods have emerged in recent years to modify the genetic expression of target proteins and factors to modify or "supercharge" MSC capacity for differentiation, migration, proliferation, angiogenesis, and tissue repair.
- 3D culture methods and alternative cell delivery scaffolds can be utilized to recapitulate the native MSC environment and augment their retention and viability for clinical therapy.
- Future studies are still needed to address limitations associated with cell-based therapy, including cell expansion, immunocompatibility, tumorigenic risks, tissue sources of MSCs, and the host microenvironment to which MSCs are delivered.

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Abbreviations and Acronyms

3D = three dimensional
AAV = adeno-associated virus
AD-MSC = adipose-derived mesenchymal stem cell
ATRA = all-trans retinoic acid
BM-MSC = bone marrow-derived MSC
CXCR4 = C-X-C chemokine receptor type 4
HA = hyaluronan
HIF-1α = hypoxia-inducible factor-1 alpha
IL = interleukin
miRNA = microRNAs
MSC = mesenchymal stem cell
PDGF = platelet-derived growth factor
PRP = platelet-rich plasma
SDF1-α = stromal cell-derived factor 1 alpha
VEGF = vascular endothelial growth factor