ISSN 1948-0210 (online)

World Journal of *Stem Cells*

World J Stem Cells 2024 August 26; 16(8): 773-826

Published by Baishideng Publishing Group Inc

World Journal of Stem Cells

Contents

Monthly Volume 16 Number 8 August 26, 2024

World Journal of Stem Cells

Monthly Volume 16 Number 8 August 26, 2024

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RESPONSIBLE EDITORS FOR THIS ISSUE

NAME OF JOURNAL
World Journal of Stem Cells

ISSN
ISSN 1948-0210 (online)

LAUNCH DATE
December 31, 2009

FREQUENCY
Monthly

EDITORS-IN-CHIEF
Carlo Ventura, Shengwen Calvin Li

EDITORIAL BOARD MEMBERS
https://www.wjgnet.com/1948-0210/editorialboard.htm

PUBLICATION DATE
August 26, 2024

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World J Stem Cells 2024 August 26; 16(8): 773-779

DOI: 10.4252/wjsc.v16.i8.773

ISSN 1948-0210 (online)

EDITORIAL

Pretreatment can alleviate programmed cell death in mesenchymal stem cells

Xin-Xing Wan, Xi-Min Hu, Qi Zhang, Kun Xiong

Specialty type: Cell and tissue engineering

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Eid N

Received: May 13, 2024
Revised: July 4, 2024
Accepted: July 26, 2024
Published online: August 26, 2024
Processing time: 104 Days and 14.5 Hours

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Abstract

In this editorial, we delved into the article titled "Cellular preconditioning and mesenchymal stem cell ferroptosis." This groundbreaking study underscores a pivotal discovery: Ferroptosis, a type of programmed cell death, drastically reduces the viability of donor mesenchymal stem cells (MSCs) after engraftment, thereby undermining the therapeutic value of cell-based therapies. Furthermore, the article proposes that by manipulating ferroptosis mechanisms through preconditioning, we can potentially enhance the survival rate and functionality of MSCs, ultimately amplifying their therapeutic potential. Given the crucial role ferroptosis plays in shaping the therapeutic outcomes of MSCs, we deem it imperative to further investigate the intricate interplay between programmed cell death and the therapeutic effectiveness of MSCs.

Key Words: Mesenchymal stem cells; Programmed cell death; Apoptosis; Autophagy; Ferroptosis

Core Tip: As ferroptosis, a distinct form of programmed cell death, significantly impairs the post-engraftment viability of donor mesenchymal stem cells (MSCs), it assumes paramount importance in dictating the therapeutic efficacy of these cells. Recognizing the pivotal role ferroptosis exerts in shaping the outcome of MSC-based therapies, we underscore the urgency to delve deeper into the intricate relationship between this mode of cell death and the therapeutic potency of MSCs. In this context, we presented our viewpoint on the recently published editorial entitled "Cellular preconditioning and mesenchymal stem cell ferroptosis," offering insights into this intricate interplay.

Citation: Wan XX, Hu XM, Zhang Q, Xiong K. Pretreatment can alleviate programmed cell death in mesenchymal stem cells. *World J Stem Cells* 2024; 16(8): 773-779
URL: https://www.wjgnet.com/1948-0210/full/v16/i8/773.htm
DOI: https://dx.doi.org/10.4252/wjsc.v16.i8.773

INTRODUCTION

Mesenchymal stem cells (MSCs), derived from a diverse array of sources, exhibit remarkable ease in *in vitro* culturing, along with pluripotent differentiation capabilities and robust self-renewal potential[1]. Their therapeutic applications have been demonstrated to effectively alleviate various conditions, including diabetes and recalcitrant wounds, while offering promising immunosuppressive treatment strategies for a spectrum of inflammatory diseases[2,3]. Despite the ability of MSCs to modulate the organ microenvironment and enhance cellular differentiation and regeneration, their clinical success is still constrained by limited understanding of the underlying mechanisms *in vivo* and the absence of standardized criteria for patient selection[4,5]. Further research is urgently needed to address these challenges.

One of the significant challenges in MSC therapy is the decreased survival and increased cell death of MSCs following transplantation into recipients. To enhance the survival rate of grafted MSCs, it is crucial to minimize MSC death. As a potential solution, preconditioning strategies, such as cytokine preconditioning, can be employed to bolster the vitality of MSCs[6]. For instance, our laboratory has explored the transfection of c-Jun plasmid into human umbilical cord MSCs (HUCMSCs) to boost their wound healing capabilities by enhancing their survival rates[7]. Furthermore, we have utilized hydrocolloid dressings to cover MSCs, which facilitates vascular reconstruction by increasing the vitality of these cells[8]. These studies underscore the critical role of reducing MSC death in clinical MSC treatments.

DIFFERENT TYPES OF CELL DEATH

In general, MSC death occurs in two distinct manners. Firstly, there is a passive and unregulated process triggered by tissue damage, known as accidental cell death or necrosis. This occurs when MSCs are exposed to severe stress conditions, such as exposure to highly toxic compounds, starvation, DNA damage, and other factors. Under such conditions, MSCs are unable to maintain intracellular homeostasis, leading to rapid cellular swelling and rupture of the plasma membrane. This, in turn, results in the passive release of intracellular material into the microenvironment[9,10].

The second manner of MSC death involves highly regulated mechanisms that involve various signaling cascades to elicit different effector functions. This type of death is referred to as programmed cell death (PCD)[11,12]. PCD is a genetically determined and actively orderly process and is ubiquitous in the development of living organisms. It represents the suicide protection measures triggered by gene regulation in response to stimuli from both internal and external environmental factors[13]. This process involves the activation of specific molecular mechanisms and genetic programming, leading to the elimination of non-essential cells or cells destined for specialization from the body.

The various pathways of PCD, including apoptosis, necroptosis, autophagy, ferroptosis, pyroptosis, PANoptosis, *etc.* exhibit distinct morphological and biochemical characteristics[14-18]. By understanding these mechanisms, we can gain insights into the complex cellular processes that underlie organismal development and homeostasis. Unlike accidental cell death, PCD is a controlled process that is essential for maintaining tissue homeostasis and regulating cellular responses to various stimuli.

Both forms of MSC death have important implications for MSC therapy. Understanding the mechanisms underlying these processes is crucial for developing effective strategies to enhance the survival and functionality of MSCs and improve the outcomes of cell-based therapies.

Apoptosis, a fundamental biological process of the cell, serves as a crucial mechanism for eliminating unwanted or abnormal cells in multicellular organisms. It plays a pivotal role in the evolution of organisms, the maintenance of internal environmental stability, and the development of various systems. Beyond being a specific form of cell death, apoptosis holds significant biological importance and is governed by intricate molecular biological mechanisms. This process is tightly regulated by multiple genes that exhibit remarkable conservation across species, including the Bcl-2 family, the caspase family, oncogenes like *c-myc*, and the tumor suppressor gene *P53*[19,20]. These genes work in concert to orchestrate the orderly and controlled dismantling of cells, ensuring the precise removal of nonfunctional or potentially harmful cells from the body[21].

Necroptosis, also known as necrotic apoptosis, is a cellular self-destruction process triggered by either extracellular signals or intracellular signals[22,23]. During necroptosis, distinct morphological changes occur, including organelle swelling, cell membrane rupture, and the breakdown of the cytoplasm and nucleus[24,25]. Notably, necroptosis differs from apoptosis and other forms of PCD in its independence from caspase activity. Instead, it relies on receptor-interacting protein kinase 3-regulated phosphorylation of mixed lineage kinase domain-like protein. This phosphorylation triggers mixed lineage kinase domain-like protein to form a pore complex at the plasma membrane, resulting in the secretion of damage-associated molecular patterns, cell swelling, and ultimately, membrane rupture[26].

Whether autophagy is a PCD type is currently controversial. Although autophagy is sometimes called autophagic cell death, there are also many researchers who report autophagy as a type of PCD[27,28]. Cell death mediated by autophagy is characterized by cytoplasmic vacuolization, the formation of autophagic vesicles, and the lysosomal degradation of cellular material[29]. Autophagy can be classified into three types: Macroautophagy; microautophagy; and selective autophagy[30]. In macroautophagy, autophagosomes, which are double-membraned vesicles, envelope a portion of the cell. These autophagosomes then fuse with lysosomes to create autolysosome, where the contents are degraded by proteases. Microautophagy involves the direct interaction and fusion of vesicles containing organelles or inclusions with lysosomes. This process is more specific than macroautophagy and can be triggered by signaling molecules on the surface of damaged organelles. Selective autophagy, also known as chaperone-mediated autophagy, involves the fusion of cytoplasmic proteins with lysosomes *via* cytoplasmic chaperones[31]. These chaperones interact with pentapeptides within the amino acid sequence of the substrate proteins.

Pyroptosis is triggered by intracellular infections of bacteria, viruses, fungi, and protozoa in the presence of either pathogen-associated molecular patterns or cell-derived damage-associated molecular patterns[32]. It typically serves as the primary mode of cell death in response to pathogen infection and is commonly observed in cells of the innate immune system, such as monocytes, macrophages, and dendritic cells[33]. The classical pathway of pyroptosis unfolds through a two-step process. Initially, nuclear factor- kappaB is activated, initiating the expression of multiple proteins. Then, caspase-1 is activated to cleave gasdermin D and cytokines, pro-interleukin (IL)-1β and pro-IL-18. These cytokines are then proteolytically hydrolyzed into their proinflammatory forms, IL-1β and IL-18[34,35]. Additionally, caspase-1 cleaves the amino-terminal fragment of the key protein, gasdermin D, leading to its oligomerization and the formation of a pore in the cell membrane[36]. This pore formation results in cytokine secretion, water influx, and ultimately, cell rupture.

Ferroptosis exhibits no chromatin condensation or loss of plasma membrane integrity, yet it is characterized by mitochondrial condensation, reduction of mitochondrial cristae, and an increase in membrane density[37-39]. The process of iron death is primarily governed by iron homeostasis and oxidative stress pathways. Iron homeostasis is partially controlled by ferritin, and the levels of ferritin are modulated through "ferritin autophagy," a process facilitated by nuclear receptor coactivator 4 (NCOA4), which serves as a selective cargo receptor for ferritin[40]. Ferritin autophagy is a meticulously orchestrated process, which is distinctly marked by the liberation of iron. In this intricate pathway, the pivotal NCOA4 plays a crucial role by directly recognizing and engaging with ferritin heavy chain 1, the ferritin subtype harboring iron. Subsequently, NCOA4 facilitates the targeted delivery of iron-laden ferritin to autophagosomes, which then undergo lysosomal degradation, ultimately releasing the sequestered iron. Experimental findings indicate that iron death can be suppressed through the use of common iron chelators, such as desferrioxamine.

Multiple forms of PCD such as pyroptosis, apoptosis, and necroptosis occur extensively in complex crosstalk and coordination, which cause a newly emerging concept known as PANoptosis[41,42]. PANoptosis is usually triggered by infectious and inflammatory factors[43,44]. Like other PCD types, PANoptosis has a multimeric protein complex (*e.g.*, ZBP1-NLRP3), which was named the PANoptosome. However, the exact role of PANoptosis remains unclear.

PRETREATMENT CAN REDUCE PCD OF MSCS

The programmed death of MSCs results in a significant reduction in their transplantation efficiency, and a variety of factors can lead to PCD of MSCs. Most reported cell death in PCD is apoptosis, while necroptosis, PANoptosis and pyroptosis are rarely reported. To address this problem, various pretreatment has been used to enhance the survival of MSCs.

APOPTOSIS

Cytotoxicity, stimuli, virus infection, and radiation can all trigger apoptosis in MSCs. It has been demonstrated that pretreatment with drugs reduces the apoptosis of MSCs and improves the efficacy of MSCs. Recently, preconditioning with prostaglandin E1 (PGE1) has been shown to enhance the protein expression of hypoxia-inducible factor-1 alpha (HIF-1α) in MSCs[45]. This upregulation of HIF-1α not only mitigates MSCs apoptosis but also boosts the protein levels of C-X-C chemokine receptor type 4, thus enhancing MSCs migration and promoting the secretion of vascular endothelial growth factor. These findings suggest that PGE1 modulates the properties of MSCs through the regulation of the HIF pathway. This understanding provides valuable insights into how PGE1 preconditioning can potentially enhance the therapeutic benefits of MSCs in the treatment of pulmonary arterial hypertension.

Myricetin could alleviate hydrogen peroxide-induced senescence and apoptosis in rat nucleus pulposus-derived MSCs through the silent information regulator 1/proliferator-activated receptor gamma coactivator-1 alpha pathway[46]. Preconditioning MSCs with 1.5% oxygen significantly enhances their activities, effectively boosting proliferation, migration, angiogenesis, antioxidant, antiapoptotic, and antifibrotic properties[47]. Preconditioning MSCs with 2%

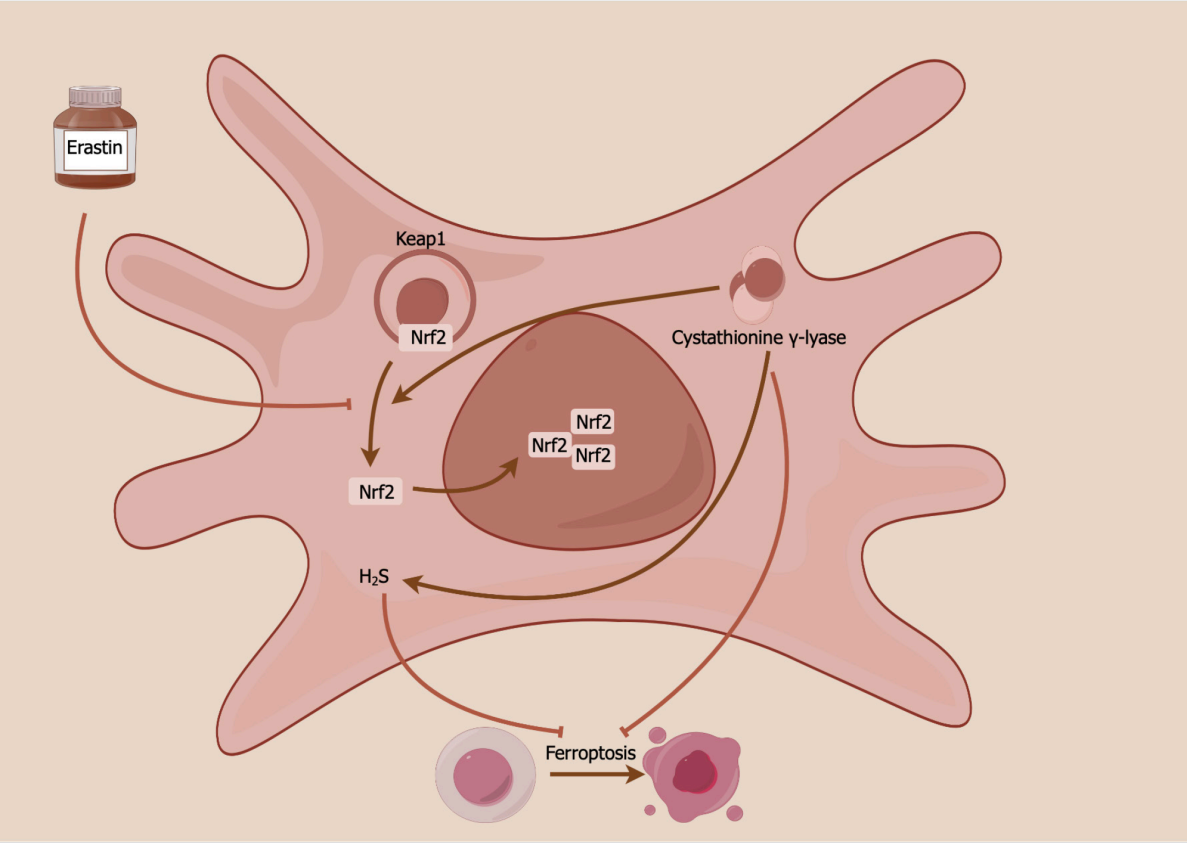

Figure 1 A novel ferroptosis mechanism of human umbilical cord mesenchymal stem cells through the cystathionine γ-lyase/hydrogen sulfide pathway. Cystathionine γ-lyase facilitates the liberation of nuclear factor erythroid 2 from the Keap1 complex within the cytoplasm, whereupon liberated nuclear factor erythroid 2 (Nrf2) is shuttled to the nucleus for subsequent activation. This process plays a pivotal role in inhibiting iron death in mesenchymal stem cells. Additionally, cystathionine γ-lyase enhances the direct suppression of iron death through the production of hydrogen sulfide (H_2S).

oxygen optimizes the expression of prion protein, activating prion protein-dependent Janus kinase 2 and signal transducer and activator of transcription 3 signaling pathways. This, in turn, upregulates the activity of superoxide dismutase and catalase, effectively inhibiting oxidative stress-induced apoptosis of MSCs and promoting the recovery of ischemic tissue[48].

AUTOPHAGY

The coordination of autophagy and apoptosis is the key to maintaining homeostasis in bone marrow MSCs (BM-MSCs). Hypoxic stress increases autophagy and apoptosis in BM-MSCs time dependently, and the increased autophagy and apoptosis in BM-MSCs induced by hypoxia are abolished by AMP-activated protein kinase (AMPK) inhibitor compound C[49]. Hypoxia pretreatment could promote BM-MSC survival by inducing autophagy through HIF-1α[50]. Besides, iron overload (IO) could significantly induce cell apoptosis and reduce cell viability in MSCs. This process is accompanied by extensive mitochondrial fragmentation and an enhancement of autophagy, both of which were dependent on reactive oxygen species (ROS). Notably, MSCs exposed to IO exhibited a marked decrease in ATP concentration, attributed to elevated ROS levels and a reduction in electron respiratory chain complex II/III activity. This depletion of ATP led to the phosphorylation of AMPK, which in turn triggered mitochondrial fission. Furthermore, genetic ablation of *AMPK* using CRISPR/Cas9 technology effectively mitigated cell apoptosis, enhanced cell viability and attenuated the mitochondrial fragmentation and autophagy triggered by IO in MSCs. These findings provide a deeper understanding of the cellular mechanisms underlying the deleterious effects of IO on MSCs and suggest potential therapeutic targets for mitigating these effects[51].

FERROPTOSIS

There is little research focused on ferroptosis in pretreated MSCs. A recently published article in the *World Journal of Stem Cells* showed a novel ferroptosis mechanism of MSCs through the cystathionine γ-lyase (CSE)/hydrogen sulfide (H_2S) pathway (Figure 1)[52]. MSC overexpression of CSE has been shown to enhance H_2S production and various ferroptosis-related indices, encompassing cell viability, iron concentration, ROS generation, cystine absorption, lipid peroxidation,

mitochondrial membrane density, and ferroptosis-related protein expression, in erastin-treated HUCMSCs. By modulating the CSE/H_2S pathway, ferroptosis in HUCMSCs was mitigated, subsequently leading to improved vascular remodeling in mice with hypoxia-induced pulmonary arterial hypertension. This occurs by maintaining a delicate balance between stem cell maintenance and differentiation. The CSE/H_2S pathway exerts its protective effects against ferroptosis in HUCMSCs through the mediation of S-sulfhydrated Keap1/nuclear factor erythroid 2-related factor 2 signaling[52,53].

CONCLUSION

Enhancing the transplantation efficacy of MSCs by decreasing programmed death of MSCs can significantly increase their survival rate, boost their differentiation potential, and modulate the immune microenvironment of the body. Although research on PCD types such as necroptosis, PANoptosis, and pyroptosis is still limited, MSC transplants can prevent various kinds of cells from necroptosis, PANoptosis and pyroptosis. As investigations progress, more types of PCD will be reported in MSCs. To improve the efficacy of MSCs, it is very important to inhibit PCD of MSCs, which can increase the survival of MSCs after transplants. Currently, the majority of preconditioning drugs aim to modulate apoptosis. However, with an enhanced comprehension of PCD, we can now target the prevention of various forms of cell death in MSCs. For example, the recently reported ferroptosis of MSCs suggests that we can target CSE to enhance the survival of MSCs. This represents a pivotal guideline in our efforts to enhance the therapeutic effectiveness of MSCs.

FOOTNOTES

Author contributions: Wan XX, Hu XM, and Zhang Q wrote the manuscript; Xiong K revised and supervised the manuscript; All authors read and agreed to the published version of the manuscript.

Supported by National Natural Science Foundation of China, No. 82372507.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: China

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S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Zhang XD

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World Journal of Stem Cells

Submit a Manuscript: https://www.f6publishing.com

World J Stem Cells 2024 August 26; 16(8): 780-783

DOI: 10.4252/wjsc.v16.i8.780

ISSN 1948-0210 (online)

EDITORIAL

Mesenchymal stem cells: A promising therapeutic avenue for non-alcoholic fatty liver disease

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Specialty type: Cell and tissue engineering

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification
Scientific Quality: Grade C
Novelty: Grade B
Creativity or Innovation: Grade B
Scientific Significance: Grade B

P-Reviewer: Hu HS

Received: June 28, 2024
Revised: July 24, 2024
Accepted: August 9, 2024
Published online: August 26, 2024
Processing time: 59 Days and 2.4 Hours

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Abstract

Non-alcoholic fatty liver disease (NAFLD) is a pressing global health concern that is associated with metabolic syndrome and obesity. On the basis of the insights provided by Jiang *et al*, this editorial presents an exploration of the potential of mesenchymal stem cells (MSCs) for NAFLD treatment. MSCs have numerous desirable characteristics, including immunomodulation, anti-inflammatory properties, and tissue regeneration promotion, rendering them attractive candidates for NAFLD treatment. Recent preclinical and early clinical studies have highlighted the efficacy of MSCs in improving liver function and reducing disease severity in NAFLD models. However, MSC heterogeneity, long-term safety concerns, and unoptimized therapeutic protocols remain substantial challenges. Addressing these challenges through standardized protocols and rigorous clinical trials is essential to the safe and successful application of MSCs in NAFLD management. Continued research into MSC mechanisms and therapeutic optimization is required to improve treatments for NAFLD and related liver diseases.

Key Words: Mesenchymal stem cells; Non-alcoholic fatty liver disease; Therapeutic potential; Liver fibrosis; Regenerative medicine; Stem cell therapy; Inflammation; Clinical trials

Core Tip: Mesenchymal stem cells (MSCs) constitute a promising therapy for non-alcoholic fatty liver disease. Expanding upon insights from the forthcoming study by Jiang *et al*. MSCs demonstrate potent immunomodulatory and anti-inflammatory effects and can promote liver tissue regeneration. Addressing challenges such as MSC heterogeneity and ensuring long-term safety through standardized protocols are crucial to harnessing the full therapeutic potential of MSCs in clinical settings.

Citation: Cheng CH, Hao WR, Cheng TH. Mesenchymal stem cells: A promising therapeutic avenue for non-alcoholic fatty liver disease. *World J Stem Cells* 2024; 16(8): 780-783
URL: https://www.wjgnet.com/1948-0210/full/v16/i8/780.htm
DOI: https://dx.doi.org/10.4252/wjsc.v16.i8.780

INTRODUCTION

Non-alcoholic fatty liver disease (NAFLD) is a pressing global health concern that is closely associated with metabolic syndrome, obesity, and type 2 diabetes mellitus[1-3]. NAFLD involves a spectrum of liver conditions characterized by excessive hepatic fat accumulation without substantial alcohol intake; these conditions include nonalcoholic steatohepatitis (NASH), fibrosis, cirrhosis, and hepatocellular carcinoma[3-5]. Treatment approaches for NAFLD primarily focus on lifestyle modifications and managing metabolic conditions but often fail to halt disease progression[6]. Therefore, novel therapeutic strategies are urgently required; mesenchymal stem cells (MSCs) constitute a promising option for NAFLD treatment[7]. The pathogenesis of NAFLD involves insulin resistance, oxidative stress, chronic inflammation, and genetic predisposition[8]. On the basis of insights from research, this editorial presents an exploration of the potential of MSCs for NAFLD treatment. MSCs are multipotent stromal cells capable of differentiating into various cell types and exerting strong immunomodulatory effects[1]. Studies have demonstrated that MSCs can ameliorate liver fibrosis and inflammation in preclinical NAFLD models[9,10]. MSCs exert their therapeutic effects through the paracrine secretion of anti-inflammatory cytokines such as interleukin (IL)-10 and transforming growth factor (TGF)-β, which modulate hepatic lipid metabolism and promote hepatocyte mitophagy, thereby reducing liver steatosis and oxidative stress[9,11,12]. However, translating MSC-based therapies from experimental settings to clinical applications presents substantial challenges, including the lack of standardized isolation protocols, uncertainty regarding their long-term safety, and lack of optimized delivery methods[1]. Overcoming these challenges is essential to the clinical application of MSC-based therapies and leveraging the regenerative and immunomodulatory properties of MSCs to address multiple facets of NAFLD pathology [1]. Jiang *et al*[1] demonstrated that MSCs have potential for NAFLD treatment because of their immunomodulatory, anti-inflammatory, and regenerative properties. Continued research into the mechanisms of action of MSCs and the refinement of therapeutic protocols may transform the management of NAFLD and related liver conditions. Accordingly, this editorial emphasizes the requirement for further investigation to overcome current challenges and realize the potential of MSCs in treating NAFLD.

THERAPEUTIC EFFECTS OF MSCs ON NAFLD

MSCs are multipotent stromal cells that can differentiate into various cell types, such as osteoblasts, chondrocytes, and adipocytes[1]. They can modulate immune responses, reduce inflammation, and promote tissue regeneration; they thus constitute an attractive option for treating liver diseases[4]. MSCs can be isolated from several tissues, including bone marrow, adipose tissue, and umbilical cord blood, and are thus highly accessible for therapeutic use[7]. Recent studies have demonstrated the potential of MSCs in ameliorating NAFLD. For example, one study revealed that human umbilical cord MSCs could effectively reduce diet-induced obesity and NASH-related fibrosis in mice by promoting fatty acid oxidation and reducing fatty acid synthesis[7]. Other studies have also demonstrated that MSC-derived exosomes could ameliorate experimental NASH through the nuclear factor erythroid 2-related factor/NAD(P)H: Quinone oxidoreductase 1 pathway, mediating anti-inflammatory and antifibrotic effects without the risks associated with direct stem cell transplantation[10,13]. Therefore, MSCs constitute a promising strategy for treating NAFLD through the targeting of key pathways involved in NAFLD pathogenesis. The primary mechanism of action of MSCs involves their ability to modulate hepatic lipid metabolism. Specifically, MSC-derived exosomes enhance fatty acid oxidation and suppress *de novo* lipogenesis in liver cells, thus effectively reducing hepatic steatosis[11,12]. This finding is consistent with those of studies demonstrating that interventions targeting metabolic pathways can restore lipid homeostasis in NAFLD models[14]. Moreover, MSCs exert potent anti-inflammatory effects within the liver by secreting cytokines such as IL-10 and TGF-β, which suppress Kupffer cell activation and hepatic inflammation; this is crucial for mitigating NAFLD progression[9]. A study on immune checkpoint blockade also highlighted the role of inflammatory pathways in NAFLD pathogenesis and suggested potential therapeutic avenues[15]. Additionally, MSCs can modulate the immune environment within the liver, shifting macrophage polarization and ameliorating the proinflammatory environment that exacerbates NAFLD. For

example, exosomes derived from human umbilical cord MSCs polarize profibrotic M2 macrophages without exacerbating liver fibrosis[16]. MSCs also secrete bioactive molecules such as hepatocyte growth factor and various microRNAs, which are crucial to liver regeneration and repair[17]. Furthermore, MSC-secreted miR-24-3p and miR-627-5p ameliorate NAFLD by targeting key pathways involved in lipid metabolism and inflammation[17,18]. MSCs also mitigate oxidative stress and endoplasmic reticulum stress in NAFLD by scavenging reactive oxygen species and enhancing antioxidant defenses, thereby protecting hepatocytes from oxidative damage and apoptosis[7,19,20]. Finally, MSCs are crucial to inhibiting the hepatic fibrosis associated with NAFLD; they can inhibit hepatic stellate cell activation and promote the breakdown of extracellular matrix components, which is essential for fibrosis resolution[9,13].

CLINICAL EVIDENCE AND CHALLENGES

NAFLD is characterized by hepatic fat accumulation, inflammation, and varying degrees of fibrosis and poses a substantial global health burden[1]. MSC-derived exosomes ameliorate hepatic steatosis through enhancing fatty acid oxidation and reducing *de novo* lipogenesis in hepatocytes[11]. MSCs also exert anti-inflammatory effects by secreting cytokines such as IL-10 and TGF-β, which suppress Kupffer cell activation and hepatic inflammation[9,16]. Moreover, MSCs mitigate oxidative stress by scavenging reactive oxygen species and inhibiting hepatic stellate cell activation, which is crucial to fibrosis development in NAFLD[13,19]. Clinical evidence supports the potential of MSC therapy to improve liver function and mitigate disease severity through these mechanisms[7,11]. Nevertheless, long-term safety concerns, such as adverse immune responses and tumorigenic risks, require comprehensive evaluation in clinical settings[13]. Additionally, optimizing treatment delivery and dosing strategies tailored to NAFLD pathology is crucial for maximizing therapeutic efficacy and minimizing adverse effects[1]. Future research should focus on elucidating specific mechanisms of MSC-mediated effects, including the role of MSC-derived exosomes in modulating hepatic pathways[10,18]. Robust clinical trials are required to establish the safety and efficacy of MSCs across diverse populations of patients with NAFLD [4]. Preclinical studies and early-phase clinical trials have demonstrated the potential of MSC therapy to ameliorate liver function and mitigate disease progression in NAFLD. However, several challenges remain. For example, the inherent heterogeneity of MSCs causes the therapeutic efficacy of these cells to vary considerably based on their source - bone marrow, adipose tissue, or the umbilical cord - and the methodologies used for their isolation and preparation. To overcome this variability, establishing standardized MSC isolation, culture, and delivery protocols is essential, as Jiang *et al*[1] emphasized and Korkida *et al*[4] indicated. The long-term safety of MSC therapy is also a critical concern. Potential risks, such as differentiation into unintended cell types, tumorigenicity, and immune reactions, must be meticulously evaluated. Rigorous preclinical and clinical studies are required to develop a comprehensive safety profile for MSC therapy[7,9,10]. Furthermore, determining the optimal dose, administration route, and frequency of MSC therapy is crucial to maximizing therapeutic benefits and minimizing risks. Although studies have demonstrated the potential of MSCs to treat NAFLD, optimal treatment protocols have not been established. However, research suggests that the route of administration (*e.g.*, intravenous *vs* direct hepatic injection) and the frequency of dosing considerably influence the efficacy and safety of MSC therapy[3,12]. These variables must be optimized to improve patient outcomes. MSCs exert their effects by secreting anti-inflammatory cytokines and growth factors that help reduce hepatic inflammation and fibrosis[16,17]. Future studies aimed at elucidating these mechanisms are essential to optimize MSC therapy for NAFLD [6]. In summary, although MSC therapy holds promise for the treatment of NAFLD, numerous challenges remain. Specifically, standardizing MSC preparation and delivery methods, ensuring long-term safety, and conducting large-scale clinical trials are essential prerequisites to the clinical application of MSCs to treat NAFLD. These efforts can promote the development of effective MSC treatments for NAFLD, which can improve patient outcomes and therapeutic strategies.

CONCLUSION

On the basis of the comprehensive review of Jiang *et al*[1], this editorial emphasizes the promise of MSCs in addressing the multifaceted pathogenesis of NAFLD. MSCs target critical pathways such as lipid metabolism, inflammation, oxidative stress, endoplasmic reticulum stress, and fibrosis; therefore, they constitute a novel and potent therapeutic option. Despite their therapeutic potential, MSC-based therapies have several challenges, such as a lack of standardized MSC isolation protocols, uncertain long-term safety, and a lack of understanding of the precise mechanisms through which MSCs exert their effects[4]. Nevertheless, integrating MSC therapy into clinical practice can offer new hope for effective treatments and improved patient outcomes for those with NAFLD. This editorial highlights the insights provided by Jiang *et al*[1] and recommends ongoing investigations and clinical trials into the use of MSCs to treat NAFLD.

FOOTNOTES

Author contributions: Cheng CH and Hao WR contributed equally to this editorial; Cheng CH, Hao WR, and Cheng TH have contributed to this editorial; Cheng CH and Hao WR primarily responsible for writing; Cheng TH overseeing revisions. All authors have read and approved the final manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Country of origin: Taiwan

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S-Editor: Wang JJ
L-Editor: A
P-Editor: Zhang XD

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World J Stem Cells 2024 August 26; 16(8): 784-798

DOI: 10.4252/wjsc.v16.i8.784

ISSN 1948-0210 (online)

MINIREVIEWS

Understanding and controlling the variables for stromal vascular fraction therapy

Naveen Jeyaraman, Sandeep Shrivastava, VR Ravi, Arulkumar Nallakumarasamy, Aditya Pundkar, Madhan Jeyaraman

Specialty type: Cell and tissue engineering

Provenance and peer review: Invited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade B, Grade C, Grade C, Grade D

Novelty: Grade B, Grade B, Grade B, Grade C

Creativity or Innovation: Grade B, Grade C, Grade C, Grade C

Scientific Significance: Grade B, Grade B, Grade B, Grade C

P-Reviewer: Li SC; Liu Y; Lu H; Mubarak M; Shen Y

Received: January 21, 2024

Revised: June 13, 2024

Accepted: July 25, 2024

Published online: August 26, 2024

Processing time: 217 Days and 15.6 Hours

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Abstract

In regenerative medicine, the isolation of mesenchymal stromal cells (MSCs) from the adipose tissue's stromal vascular fraction (SVF) is a critical area of study. Our review meticulously examines the isolation process of MSCs, starting with the extraction of adipose tissue. The choice of liposuction technique, anatomical site, and immediate processing are essential to maintain cell functionality. We delve into the intricacies of enzymatic digestion, emphasizing the fine-tuning of enzyme concentrations to maximize cell yield while preventing harm. The review then outlines the filtration and centrifugation techniques necessary for isolating a purified SVF, alongside cell viability assessments like flow cytometry, which are vital for confirming the efficacy of the isolated MSCs. We discuss the advantages and drawbacks of using autologous *vs* allogeneic SVF sources, touching upon immunocompatibility and logistical considerations, as well as the variability inherent in donor-derived cells. Anesthesia choices, the selection between hypodermic needles *vs* liposuction cannulas, and the role of adipose tissue lysers in achieving cellular dissociation are evaluated for their impact on SVF isolation. Centrifugation protocols are also analyzed for their part in ensuring the integrity of the SVF. The necessity for standardized MSC isolation protocols is highlighted,

promoting reproducibility and successful clinical application. We encourage ongoing research to deepen the understanding of MSC biology and therapeutic action, aiming to further the field of regenerative medicine. The review concludes with a call for rigorous research, interdisciplinary collaboration, and strict adherence to ethical and regulatory standards to safeguard patient safety and optimize treatment outcomes with MSCs.

Key Words: Mesenchymal stromal cells; Stromal vascular fraction; Adipose tissue; Autologous stromal vascular fraction; Stromal vascular fraction isolation

Core Tip: Stromal vascular fraction isolation is essential for extracting mesenchymal stromal cells (MSCs) in regenerative medicine. Optimizing this process requires improved liposuction techniques, immediate processing, precise enzymatic digestion, and efficient filtration and centrifugation. Quality control is verified through flow cytometry to ensure cell viability and purity. The necessity for standardized MSC isolation protocols is emphasized to ensure reproducibility and clinical success.

Citation: Jeyaraman N, Shrivastava S, Ravi V, Nallakumarasamy A, Pundkar A, Jeyaraman M. Understanding and controlling the variables for stromal vascular fraction therapy. *World J Stem Cells* 2024; 16(8): 784-798
URL: https://www.wjgnet.com/1948-0210/full/v16/i8/784.htm
DOI: https://dx.doi.org/10.4252/wjsc.v16.i8.784

INTRODUCTION

The extraction of mesenchymal stromal cells (MSCs) from stromal vascular fraction (SVF) is a cornerstone in the burgeoning field of regenerative medicine. The therapeutic efficacy of interventions utilizing MSCs is critically dependent on the successful isolation of these cells in terms of both quality and quantity[1]. SVF, sourced from adipose tissue, is a heterogeneous cellular conglomerate that provides a repository for MSCs, celebrated for their intrinsic regenerative capabilities. The complex process of MSC isolation from SVF demands a comprehensive understanding of various influential factors to optimize cell yield and ensure the development of effective regenerative therapies.

The technique of adipose tissue extraction through liposuction is pivotal in preserving the integrity and viability of the SVF[2-5]. The selection of a liposuction method that reduces adipocyte trauma is essential for maintaining a cell composition conducive to a substantial yield of MSCs. The choice of anatomical site for tissue harvest is also significant, as different regions may vary in MSC concentrations, affecting their regenerative potential. The critical time interval between adipose tissue collection and the initiation of SVF isolation inversely affects cell viability, necessitating optimized processing times to maintain MSC functionality[6-8]. Control of temperature during processing and transport is equally crucial to avert cell damage and maximize MSC yield.

Enzymatic digestion is a pivotal step in SVF isolation, and the choice and balance of enzymes, such as collagenase and dispase, are vital[8]. This stage must be meticulously controlled to prevent cellular damage that would reduce MSC viability and yield. The subsequent separation of SVF from the digested tissue matrix, utilizing precise filtration and centrifugation parameters, is critical for MSC recovery[9]. The washing and resuspension of isolated cells are further essential steps, requiring the use of isotonic buffers and appropriate culture media to support cell survival and maximize MSC yield[9]. Assessing cell viability and purity is fundamental, with techniques like the trypan blue exclusion test and flow cytometry providing crucial metrics of MSC quality[10]. The culture conditions post-isolation, including growth media composition, supplemental growth factors, and incubator environmental conditions, significantly impact MSC proliferation and yield[11]. The inherent biological variability among donors adds another dimension of complexity to MSC yield and quality, indicating the potential need for personalized isolation protocols[12].

This narrative review aims to systematically dissect the multifaceted process of MSC isolation from SVF, exploring the impact of each stage on the yield and viability of MSCs. It will delve into current methodologies and technologies, assess the influence of procedural variables, and consider donor variability on the quality of MSCs isolated. Furthermore, this review seeks to underscore the necessity of standardizing protocols to harness the full therapeutic potential of MSCs. Through this lens, the objectives of this review are to consolidate existing knowledge, identify gaps in the current understanding, and suggest directions for future research to refine MSC isolation techniques, thus facilitating the transition of MSCs from laboratory research to clinical application.

ADIPOSE TISSUE BIOLOGY

Adipose tissue, an intricate and specialized form of connective tissue, plays a quintessential role in the physiological landscape of mammals, offering a multifaceted suite of functions that transcend mere fat storage. Within this tissue, a

complex network comprising adipocytes, immune cells, vasculature, and an extracellular matrix (ECM) orchestrates a series of critical bodily functions. These include the regulation of energy balance, the secretion of hormones, the provision of thermal insulation, and the mechanical protection of organs. The primary cellular constituents of adipose tissue are the adipocytes, which are specialized in the storage and liberation of energy as triglycerides. These cells are structurally defined by a singular lipid droplet that occupies the majority of the cytoplasm, dynamically expanding and contracting in response to the organism's metabolic demands[11]. This capability to modulate lipid storage and mobilization positions adipocytes as pivotal actors in the maintenance of energy homeostasis, particularly evident during fluctuations in caloric intake and expenditure.

The dichotomy within adipose tissue is characterized by the distinction between white adipose tissue (WAT) and brown adipose tissue (BAT), which are anatomically and functionally distinct entities. WAT, the predominant form, is primarily tasked with energy storage. Its distribution is extensive, encompassing subcutaneous layers that insulate the body and visceral depots that enshroud internal organs, thereby serving as an energy reservoir. Beyond its storage capacity, WAT exerts profound endocrine effects through the secretion of adipokines, hormones that actively participate in the regulation of metabolic pathways, immune responses, and appetite[12]. In contrast, BAT serves a specialized function in thermoregulation, primarily through the process of non-shivering thermogenesis. This heat generation is a critical physiological response, especially in neonates and hibernating species, where maintenance of body temperature is vital for survival. The brown coloration of this tissue type is attributed to its high mitochondrial content, with mitochondria being rich in iron-containing cytochromes[12].

The dynamic nature of adipose tissue is further exemplified by its ability to remodel and adapt. Factors such as diet, physical activity levels, and hormonal milieu can precipitate structural changes within the tissue, manifesting either as hypertrophy of existing adipocytes or as hyperplasia, the formation of new fat cells[13]. In certain instances, this remodeling process involves the "browning" of white adipocytes, where they begin to mimic the thermogenic properties of brown adipocytes, a phenomenon that has garnered interest for its potential implications in energy expenditure and obesity management.

Adipose tissue's endocrine function is underscored by its role in the secretion of various adipokines, which have systemic effects on multiple organ systems[14]. These hormones play an instrumental role in maintaining metabolic equilibrium, influencing processes such as glucose uptake, fat oxidation, and appetite control. Disruption in the homeostatic secretion of adipokines has been implicated in the pathogenesis of metabolic disorders, highlighting the critical role of adipose tissue in the broader context of metabolic health. Within this complex tissue resides a reservoir of adipose-derived stem cells (ADSCs), which exhibit the potential to differentiate into a variety of cell types, including but not limited to, adipocytes, chondrocytes, and osteoblasts[15]. This multipotent characteristic of ADSCs, combined with their relative abundance and accessibility, renders them an attractive candidate for therapeutic applications in the burgeoning field of regenerative medicine. The regulation of adipose tissue is pivotal for metabolic health, with imbalances in this system contributing to the pathophysiology of obesity. Excessive accumulation of adipose tissue, particularly WAT, is associated with a spectrum of adverse health outcomes, including cardiovascular diseases, type 2 diabetes, and certain cancers. Conversely, therapeutic strategies that promote the activation of BAT or the browning of WAT have shown promise in combatting metabolic syndrome by increasing basal energy expenditure.

The exploration of adipose tissue biology is thus a field of immense importance, bearing significant implications for public health. The intricate mechanisms governing adipose tissue function are a subject of intensive study, promising to unveil a new understanding of obesity and metabolic diseases. This, in turn, is anticipated to catalyze the development of novel therapeutic interventions, tailored to exploit the regenerative and metabolic potential of adipose tissue in disease amelioration and prevention. Having explored the fundamental biology of adipose tissue, we now turn our attention to the specific regenerative products that can be derived from this versatile tissue source. These products form the basis for various therapeutic applications in regenerative medicine.

REGENERATIVE PRODUCTS OF ADIPOSE TISSUE

Adipose tissue, traditionally viewed as a passive storage site for triglycerides, has been reconceptualized as a dynamic and versatile source of regenerative materials. Its diverse cellular composition and rich milieu of bioactive compounds have positioned it at the forefront of regenerative medicine, offering a spectrum of applications from tissue repair to modulating inflammatory responses[13]. The therapeutic regenerative products derived from adipose tissue are tabulated in Table 1[16-22]. The regenerative potential of adipose tissue is primarily determined by ADSCs, which possess multipotent characteristics and can differentiate into various cell types including adipocytes, chondrocytes, osteoblasts, and endothelial cells[23,24]. These cells are not only remarkable for their differentiation capacity but also for their ease of harvest and subsequent expansion, which has spurred extensive research into their application in tissue engineering and cell-based therapies. The versatility of ADSCs lends itself to a myriad of regenerative medicine applications, from reconstructive surgeries to the treatment of chronic wounds, underscoring their significance as a therapeutic tool.

SVF represents another regenerative product obtained from adipose tissue[25]. This heterogeneous cell population, isolated through enzymatic digestion and centrifugation, encompasses a variety of cell types including ADSCs, endothelial progenitor cells, pericytes, fibroblasts, and immune cells. The SVF is particularly valued for its high content of growth factors and its ability to promote repair and regeneration across various tissue types. SVF acts *via* paracrine signaling by secreting growth factors, cytokines, and extracellular vesicles (EVs)[26]. It plays a crucial role in immunomodulation by secreting regenerative factors, modulating inflammation, and promoting tissue repair, including cartilage regeneration[27,28]. The chondrogenic differentiation of SVF cells enhances cartilage regeneration and supports

Table 1 Therapeutic regenerative products derived from adipose tissue

Regenerative product from adipose tissue	Description	Potential applications
Adipose-derived stem cells[16,17]	Multipotent stem cells capable of differentiating into multiple cell types	Tissue engineering, cell-based therapies
Stromal vascular fraction[18]	A heterogeneous cell population, including adipose-derived stem cells and other cell types	Wound healing, tissue repair, regenerative treatments
Extracellular vesicles[19]	Vesicles contain bioactive molecules like proteins and nucleic acids	Angiogenesis, immunomodulation, tissue healing
Adipokines[20]	Hormones and signaling molecules secreted by adipose tissue	Regulation of appetite, insulin sensitivity, inflammation, and lipid metabolism
ECM scaffolds[21]	Decellularized scaffolds maintain adipose tissue's structural components	Tissue engineering, support for tissue regeneration
Microfat and nanofat[22]	Adipose tissue forms rich in regenerative cells and growth factors	Soft tissue augmentation, wound healing, tissue repair

ECM: Extracellular matrix.

subchondral bone regeneration through the production of collagen type I[29]. Additionally, SVF mechanisms involve angiogenesis, where ADSCs produce vascular endothelial growth factor for migration and platelet-derived growth factor for proliferation, which supports endothelial cells in forming vasculature-like structures[25]. Furthermore, SVF contains multiple cell populations, including pericytes and ADSCs, which release growth factors and ECM particles, contributing to its regenerative potential[30,31]. These multifaceted actions of SVF underline its capability to mediate tissue repair, regulate inflammatory and immune responses, and promote chondrocyte proliferation and chondrogenesis[28,32]. Its application in wound healing and tissue repair is particularly promising, offering a minimally invasive option to stimulate the body's intrinsic repair mechanisms.

EVs derived from adipose tissue are yet another avenue through which adipose tissue contributes to regenerative processes[33]. Comprising exosomes and microvesicles, these EVs are laden with proteins, lipids, and nucleic acids that facilitate intercellular communication. Their role in regeneration is attributed to their ability to carry and transfer these bioactive molecules to target cells, thereby initiating processes such as angiogenesis, immunomodulation, and tissue repair. The therapeutic potential of adipose-derived EVs is vast, with research delving into their ability to enhance the body's repair mechanisms in a targeted and controlled manner.

The role of adipose tissue as an endocrine organ is characterized by its secretion of adipokines[34]. These signaling molecules, including adiponectin, leptin, and resistin, have been implicated in a host of physiological processes that extend to the regulation of appetite, insulin sensitivity, and lipid metabolism. Their contribution to regenerative processes, particularly tissue repair and angiogenesis, opens new therapeutic avenues, particularly in conditions where these processes are compromised, such as in diabetic wound healing. The development of adipose tissue-derived ECM scaffolds has introduced a novel approach to tissue engineering[35]. These scaffolds, created through the decellularization of adipose tissue, maintain the complex composition of growth factors and structural proteins inherent to the native tissue. They provide a conducive environment for cell attachment, proliferation, and differentiation, essential for the regeneration of damaged tissues.

The refinement of adipose tissue into microfat and nanofat grafts has expanded the utility of adipose tissue in regenerative medicine[36]. These preparations are rich in regenerative cells and growth factors and have shown effectiveness in soft tissue augmentation, wound healing, and tissue repair. The process of creating microfat and nanofat involves emulsification and filtration, which not only concentrates the regenerative components but also renders the fat more malleable for precise applications. The regenerative products derived from adipose tissue encapsulate a promising and rapidly evolving area of research, offering therapeutic possibilities that were previously unattainable. These products, each with their unique properties and mechanisms, present a diverse toolkit for clinicians and researchers aimed at promoting tissue repair, regenerating damaged tissues, and ultimately improving patient outcomes. As the field advances, ongoing research will be paramount to fully elucidate the mechanisms of action of these regenerative products and to optimize their clinical applications, thereby harnessing the full therapeutic potential of adipose tissue in regenerative medicine. To better understand the complexities of SVF isolation. Figure 1 illustrates the key variables that influence this process. Various methods of lipolysis for SVF-MSC isolation are depicted in Figure 2.

AUTOLOGOUS SOURCE OF SVF

The sourcing of SVF from autologous or allogeneic donors is a critical consideration in the field of regenerative medicine, each with distinct advantages and challenges. Autologous SVF, harvested from the patient's adipose tissue, is a cornerstone in tissue engineering due to its inherent compatibility with the recipient's immune system. In the context of autologous SVF, the process begins with the extraction of adipose tissue from the patient, typically through liposuction

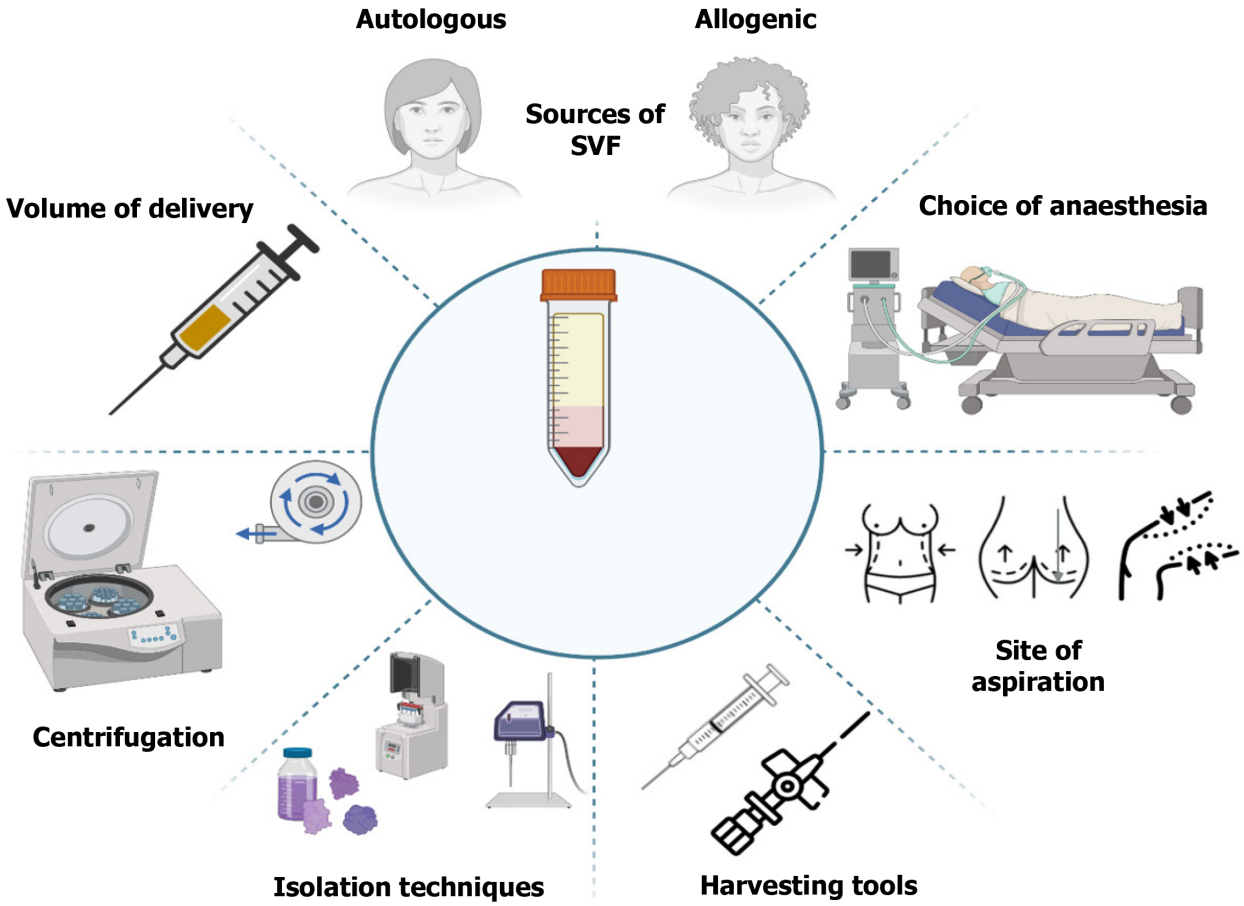

Figure 1 Variables of mesenchymal stem cell isolation in stromal vascular fraction derived from adipose tissue. SVF: Stromal vascular fraction.

[37]. This procedure, while minimally invasive, is a surgical intervention that requires careful consideration of the patient's overall health and comfort. Once the adipose tissue is collected, it undergoes processing to isolate the SVF. While the immunological advantages of autologous SVF are clear, there are practical considerations that must be weighed when choosing this approach.

The advantages of autologous SVF are rooted in its immunological profile, being derived from the patient's cells, thereby virtually eliminating the risk of immune rejection. This biocompatibility is of paramount importance in cell transplantation, as it ensures the integration of the transplanted cells without eliciting an adverse immune response. Furthermore, the autologous route circumvents the risks associated with disease transmission from donor to recipient, a concern particularly relevant in the context of infectious pathogens. Autologous SVF also allows for the possibility of personalized therapies. The cells can be manipulated or expanded *in vitro* to enhance their regenerative properties or to tailor them to address specific patient needs. This bespoke approach holds the potential for more effective and targeted regenerative outcomes.

However, the approach is not without its limitations. The requirement of a liposuction procedure for tissue harvesting introduces additional complexity to the treatment regimen. This step not only poses surgical risks but also may not be well-received by all patients, particularly those who are averse to invasive procedures or those with conditions that elevate surgical risk. Moreover, the variability in SVF yield and cellular potency is a significant factor; these attributes are influenced by the patient's age, comorbidities, lifestyle, and the intrinsic quality of the adipose tissue. Such variability necessitates a careful assessment of the harvested tissue's viability and may require subsequent interventions to secure a sufficient quantity of therapeutically effective cells.

In weighing the benefits and drawbacks of autologous SVF, it is essential to consider the individualized nature of the treatment. Personalized medicine, while offering tailored solutions, must also reconcile the practicality of such approaches, balancing the procedural demands with the expected therapeutic benefits. The development of standardized protocols for the harvesting, processing, and application of autologous SVF is therefore a critical step forward, ensuring the reproducibility and efficacy of regenerative therapies. As the field evolves, the continuous refinement of these techniques and a deeper understanding of the biological behavior of SVF will be instrumental in maximizing the potential of autologous cell therapies in regenerative medicine.

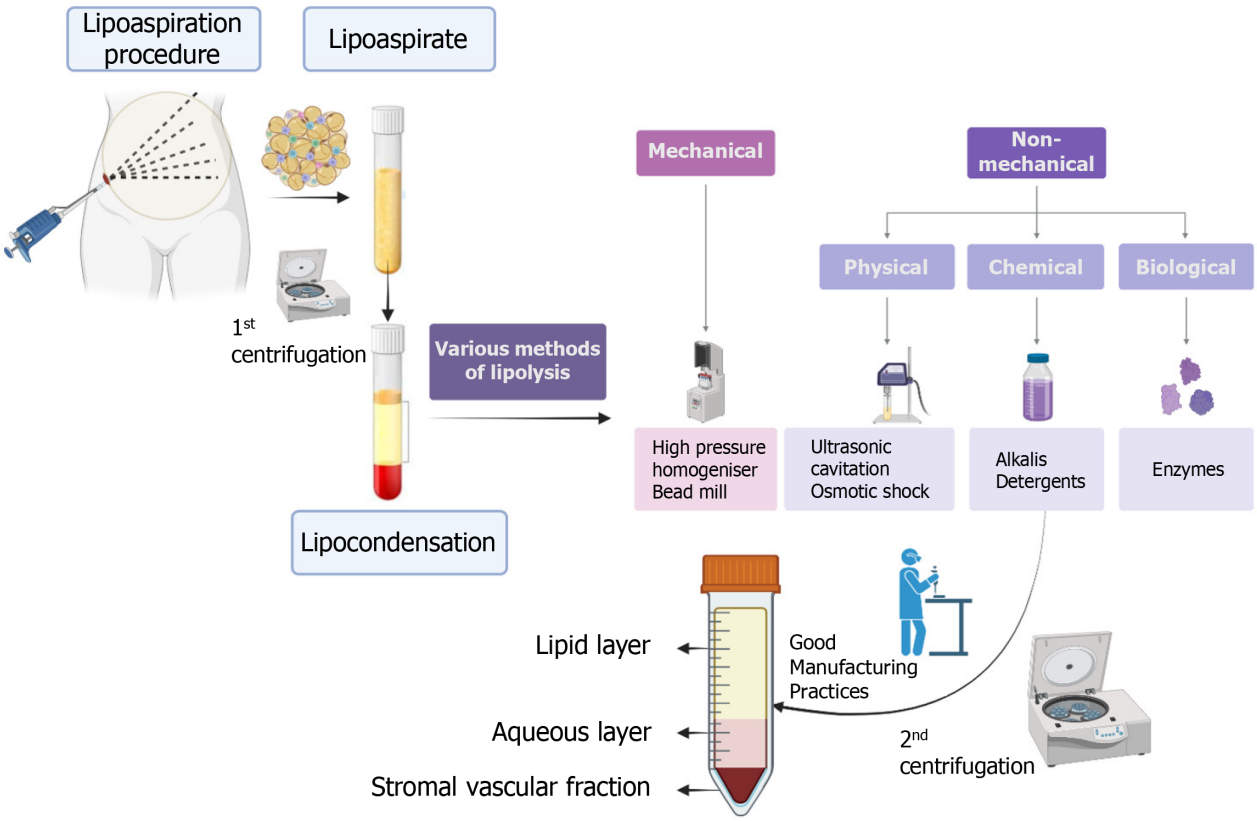

Figure 2 Various methods of lipolysis for mesenchymal stem cell isolation in stromal vascular fraction derived from adipose tissue.

ALLOGENIC SOURCE OF SVF

Allogeneic SVF represents a paradigm shift in the availability and application of regenerative cellular therapies, where SVF is sourced from donors rather than the recipients themselves. This approach leverages the adipose tissue of healthy individuals, which upon extraction and processing, yields SVF that can be administered therapeutically to patients. The allogeneic SVF, once isolated, can be cryopreserved, thus offering a stockpile of regenerative material that can be tapped into as needed[38]. This method stands out for its logistical efficiency, providing a uniform and on-demand supply of SVF, obviating the need for individualized tissue harvesting through surgical procedures.

The benefits of using allogeneic SVF are multifaceted. Primarily, it eliminates the necessity for each patient to undergo a surgical procedure for adipose tissue extraction, thereby reducing the overall invasiveness of the treatment and the associated risks of surgery. Furthermore, it assures a steady and consistent source of SVF, which is particularly advantageous when treating multiple patients or in scenarios where immediate treatment is required. Allogeneic SVF is also a vital resource for patients who may not have an adequate amount of adipose tissue for autologous SVF extraction, such as those with low body fat or certain medical conditions. Despite these advantages, allogeneic SVF is not without potential drawbacks. The risk of immune rejection is a concern, given the possible disparity in human leukocyte antigens between the donor and the recipient, which can provoke an immunological response. Moreover, the transmission of diseases remains a risk factor that necessitates rigorous donor screening, testing protocols, and adherence to stringent regulatory standards to mitigate potential complications.

When considering the use of autologous *vs* allogeneic SVF, the decision-making process must integrate an assessment of the risks and benefits tailored to the individual patient's condition and the intended therapeutic application. While autologous SVF is typically favored for its reduced risk of immune complications, allogeneic SVF offers practical benefits in contexts where autologous tissue is not viable or when there is a need for large-scale or immediate treatment regimens. The employment of allogeneic SVF, therefore, requires a comprehensive framework for donor selection, tissue processing, and quality control to maximize safety and therapeutic efficacy. This holistic approach ensures that the potential of allogeneic SVF can be harnessed effectively, expanding the scope of regenerative medicine. The comparative analysis of autologous *vs* allogenic SVF sources is tabulated in Table 2.

CHOICE OF ANESTHESIA

The management of anesthesia during the procurement of SVF for regenerative medical applications is a nuanced area that currently lacks uniform guidelines. The options of anesthesia for SVF isolation procedures are written in Table 3[39-43]. This absence of consensus leaves the choice of anesthesia to be tailored to the specifics of the SVF isolation procedure,

Table 2 Comparative analysis of autologous *vs* allogenic stromal vascular fraction sources

SVF source	Advantages	Disadvantages
Autologous SVF	Eliminates immune rejection. No donor disease transmission. Enables personalized treatments	Requires surgical adipose extraction. Variable SVF quality and quantity
Allogenic SVF	Avoids surgery for each patient. Steady, ready supply. Beneficial for patients with scant adipose tissue	Possible immune response. Risk of disease if donor screening is lax

SVF: Stromal vascular fraction.

Table 3 Anesthesia options for stromal vascular fraction isolation procedures

Anesthesia type	Description	Application in SVF isolation
Local anesthesia [39]	Involves injecting anesthetic agents like lidocaine at the harvest site. Patients remain conscious and have minimal systemic side effects	Preferred for small-scale SVF isolation; ensures patient comfort and cooperation
Tumescent anesthesia[40]	Diluted lidocaine with epinephrine and saline is injected to anesthetize, minimize bleeding, and ease tissue harvest	Common in liposuction to obtain adipose tissue; provides extended anesthesia and vasoconstriction
General anesthesia [41-43]	Induces complete sedation and unconsciousness, used in comprehensive surgical settings	Rarely used solely for SVF isolation; considered if combined with other surgical interventions

SVF: Stromal vascular fraction.

the patient's health profile, and the combined judgment of the patient and the medical team. During SVF isolation, adipose tissue is harvested primarily from subcutaneous fat depots. This process is essential for accessing regenerative cells such as ADSCs, which are integral to subsequent therapeutic applications. The anesthetic techniques adopted for this procedure vary, with local anesthesia being a preferred method due to its simplicity and direct action.

Local anesthesia involves injecting anesthetic agents into the tissue extraction site and numbing the specific area to be treated. This localized approach offers several advantages - it not only provides effective pain control during the procedure but also allows patients to remain awake, which can be critical for monitoring their well-being throughout the process. This method is associated with a favorable tolerance profile, as it minimizes the patient's exposure to systemic anesthetic agents and the related side effects. Tumescent anesthesia, a derivative of local anesthesia, expands upon its basic principle by infusing a larger volume of a dilute anesthetic solution into the subcutaneous tissue. This solution typically consists of lidocaine, epinephrine, and saline, and serves multiple functions. The lidocaine provides local anesthesia, the epinephrine induces vasoconstriction to minimize bleeding, and the saline facilitates the mechanical process of adipose tissue extraction. This technique is particularly valued in liposuction, a common method for harvesting adipose tissue, and by extension, is often employed in SVF isolation.

General anesthesia is seldom used for SVF isolation alone, primarily due to its comprehensive nature, which renders the patient completely unconscious. This type of anesthesia is generally reserved for procedures that are either too painful or too complex to perform under local or tumescent anesthesia. When SVF isolation is performed in conjunction with other, more invasive surgical procedures, general anesthesia may become necessary to ensure patient comfort and procedural efficiency. The selection of an anesthetic regimen is a multifaceted decision that hinges on several factors. The complexity of the procedure, the anticipated duration, the patient's medical and surgical history, and the presence of comorbidities all play critical roles in this decision-making process. Additionally, patient preference is paramount; some may favor local anesthesia to avoid the risks and recovery associated with general anesthesia, while others may opt for complete sedation due to anxiety or discomfort with being awake during the procedure.

In practice, local or tumescent anesthesia is often the method of choice for SVF isolation, striking a balance between minimizing procedural pain and reducing the risk of systemic complications associated with anesthesia. These approaches ensure that the patient remains comfortable and cooperative, which is especially important in outpatient or office settings where rapid recovery post-procedure is advantageous. The determination of the most suitable anesthetic approach for SVF isolation should be a collaborative endeavor, engaging the patient, the surgeon, and the anesthesiologist. This collaborative approach is essential for aligning the anesthetic plan with the patient's needs, and procedural requirements, and ensuring the highest standards of safety and care. As the field of regenerative medicine continues to evolve, so too will the strategies for anesthesia management, to optimize patient experiences and outcomes in SVF isolation and application.

SITE OF ASPIRATION

The aspiration of SVF is a medical procedure integral to regenerative medicine, wherein subcutaneous adipose tissue is the primary target for harvesting[44]. This layer of fat, located directly beneath the skin's surface, provides a readily

accessible and rich source of adipose tissue, making it ideal for SVF isolation. The process of SVF aspiration is routinely conducted at sites where subcutaneous fat is both abundant and easily accessible (Table 4).

The abdomen, particularly the lower abdominal area, is a prime site for adipose tissue harvesting. It is routinely selected for its accessibility and the substantial presence of subcutaneous fat. This region's predominance of fat deposits not only ensures a sufficient yield for SVF isolation but also contributes to the relative simplicity and lower risk profile of the procedure. Such characteristics render the abdomen an optimal site for various regenerative procedures, including those requiring liposuction or manual aspiration techniques.

The thighs are another common site for SVF aspiration, with both the inner and outer thighs offering ample subcutaneous fat. The choice between these sub-regions is typically informed by the patient's body composition and the quantity of adipose tissue needed. The thighs are comparable to the abdomen in terms of the volume of tissue available and are often used when additional adipose tissue is required or when the abdominal site is less suitable due to previous surgeries, scarring, or patient preference. Flanks are also a favored site for SVF aspiration. The flank area, which extends from the lower back around the side of the body, is another region where adipose tissue tends to accumulate. This site can be particularly beneficial for patients with less adipose deposition in the abdomen or thighs or when a larger quantity of SVF is necessary, as the flanks can provide an additional reservoir of adipose tissue.

In some scenarios, adipose tissue may also be harvested from the buttocks for SVF isolation. The buttocks may be chosen based on the patient's specific anatomical and fat distribution characteristics, and in cases where other sites are not viable due to anatomical constraints, prior procedures, or patient choice. The decision on the precise site for SVF aspiration is multifactorial, taking into account individual patient factors, the desired quantity of SVF, ease of access to the fat deposit, patient comfort, and any concurrent procedures that might be taking place. For example, if adipose tissue is being harvested for aesthetic purposes in addition to SVF isolation, the choice of site might be influenced by the goals of the aesthetic procedure.

Various techniques for SVF aspiration are available, with the choice often depending on the physician's expertise, the volume of tissue needed, and the specifics of the patient's case[23]. Methods can range from manual aspiration, which may be less technically demanding, to more technologically advanced approaches like liposuction, which can facilitate a more significant harvest of adipose tissue. Regardless of the method, the primary aim remains to procure an adequate quantity of viable adipose tissue for SVF isolation while maintaining the highest standards of patient safety and comfort.

Before undertaking SVF isolation, healthcare providers must engage patients in a comprehensive informed consent process. This conversation is vital to ensure that the patient is fully apprised of the procedural details, understands the rationale for site selection, and is aware of any potential risks or complications associated with the procedure. Such informed consent is a cornerstone of ethical medical practice, enabling patients to proceed with a clear understanding of the treatment plan and its implications for their care.

TECHNIQUES OF ADIPOSE TISSUE EXTRACTION

The isolation of SVF from adipose tissue is a critical step in regenerative medicine that requires the precise dissociation of the tissue to harvest a viable population of regenerative cells, including ADSCs and other stromal cells. The adipose tissue lyser, also referred to as a dissociator or homogenizer, is instrumental in this process, applying either mechanical, enzymatic or a combination of forces to efficiently disrupt the adipose tissue matrix[7,45].

The isolation of SVF from adipose tissue employs various techniques, predominantly categorized into enzymatic and mechanical methods. Enzymatic digestion is often considered the gold standard for SVF separation, utilizing collagenase to break down adipose tissue and release stromal cells[26,30,46]. In this approach, adipose tissue is digested with enzymes such as type I and VIII collagenase, followed by centrifugation and washing with phosphate-buffered saline to purify the SVF[27,30]. This method ensures a high yield of viable cells, including ADSCs, pericytes, fibroblasts, and other progenitor cells[28,47]. Mechanical isolation techniques, such as using rotating blades, vibrating shakers, and centrifuges, provide a rapid and cost-effective alternative to enzymatic digestion. These methods involve physically disrupting the adipose tissue to release stromal cells, followed by filtration and centrifugation to obtain the SVF[25,37,46]. Despite yielding lower quantities of cells compared to enzymatic methods, mechanical techniques can still produce clinically acceptable SVF with good viability and regenerative potential[31,46]. Additionally, approaches such as the use of Liberase Blendzyme and ultrasound for cell membrane permeabilization have been explored to enhance SVF yield and efficiency[29].

When selecting an adipose tissue lyser for SVF isolation, several factors must be carefully considered to ensure the optimal release of cells: (1) The volume of adipose tissue being processed is a determinant of the scale and type of lyser required. Larger volumes may necessitate the use of more robust systems capable of processing increased tissue amounts efficiently; (2) The desired level of tissue dissociation is crucial, as different dissociation techniques may yield varying degrees of cell liberation and viability, which in turn can influence the success of subsequent regenerative applications; (3) The throughput requirements of the procedure, including the number of samples to be processed and the time frame for processing, will influence the choice of lyser; (4) The resources available within the laboratory, including equipment and expertise, are also key considerations. Some dissociators may require additional infrastructure or specialized training to operate effectively; and (5) Safety and reproducibility are paramount. The chosen lyser must be reliable and consistent in performance, ensuring patient safety and the highest quality of SVF isolation.

Adhering to the manufacturer's instructions and laboratory best practices is essential when using an adipose tissue lyser. It is this adherence to the protocol that ensures the effective and reproducible dissociation of adipose tissue, which is a cornerstone for obtaining a high yield of viable and functional SVF. Such attention to detail is critical for translating

Table 4 Common sites for stromal vascular fraction aspiration from subcutaneous adipose tissue

Common SVF aspiration sites	Description
Abdomen[44]	Preferred due to accessibility and ample subcutaneous fat; less invasive for adequate tissue collection
Thighs[44]	Inner and outer thighs provide a high concentration of subcutaneous fat suitable for harvesting
Flanks[44]	Often chosen for excess adipose tissue, easily accessible for SVF aspiration
Buttocks[44]	Utilized when a significant amount of adipose tissue is needed, especially in regenerative procedures

SVF: Stromal vascular fraction.

the potential of SVF into successful clinical outcomes in the rapidly advancing field of regenerative medicine.

ADIPOSE TISSUE ASPIRATION CANNULA TYPE

The selection of an appropriate cannula type for the aspiration of adipose tissue during SVF isolation is a decision that hinges on a variety of procedural factors and desired outcomes (Table 5). Clinicians typically choose between two primary instruments: The hypodermic needle and the liposuction cannula (Table 6). Each tool has its advantages and limitations, which must be considered in the context of the SVF isolation process[48].

The hypodermic needle is a ubiquitous and cost-effective tool in medical procedures and is valued for its simplicity and ease of use, especially in manual aspiration techniques. It is available in a range of sizes, allowing for its application across different scales of adipose tissue aspiration. Despite its versatility, the hypodermic needle may not be the optimal choice for larger-scale harvesting or liposuction procedures due to its limited capacity for tissue collection, often necessitating multiple punctures to collect an adequate volume of adipose tissue.

In contrast, the liposuction cannula is specifically designed for the efficient harvest of larger volumes of adipose tissue, making it the instrument of choice for liposuction procedures. Its design minimizes trauma to surrounding tissues during the aspiration process. However, this efficiency comes at the cost of requiring specialized equipment and a higher level of expertise for safe and effective use. Additionally, for smaller-scale or manual aspiration procedures, the use of a liposuction cannula may not be as appropriate. There is a paucity of evidence that explores the variability of the SVF quality based on the choices of liposuction technique, anatomical location, or surgical approach selection.

When determining the most suitable cannula type for SVF isolation, several factors must be evaluated: (1) The volume of adipose tissue required is a decisive factor; larger quantities necessitate the use of a liposuction cannula for practical and efficient extraction; (2) The nature of the procedure is also critical. For manual aspirations, hypodermic needles are typically sufficient, whereas liposuction procedures are best served by cannulas; (3) Patient comfort and procedure tolerance must be considered, as smaller needles can be less invasive and more tolerable during manual aspirations; (4) The expertise of the physician is paramount. The clinician should possess proficiency in the use of the chosen instrument to ensure the procedure's success; and (5) Safety considerations must guide the selection process, ensuring that the chosen needle type aligns with safety protocols to minimize the risk of complications.

Ultimately, the needle type for adipose tissue aspiration should be selected based on a careful assessment of the SVF isolation requirements, the healthcare provider's skill set, and the highest standards of patient care. Adherence to sterile techniques and safety guidelines is essential to secure a successful outcome and the procurement of high-quality SVF for use in regenerative medicine.

CENTRIFUGATION

Centrifugation is a pivotal step in the isolation of SVF from adipose tissue, a process that enriches regenerative cells such as ADSCs by separating them from other cellular components[49]. The process begins with enzymatic digestion of the adipose tissue to break down the ECM and liberate the cells. Following digestion, the tissue is typically mixed with a buffer or saline to create a cell suspension suitable for centrifugation.

The goal of centrifugation in SVF isolation is to leverage the density differences between the various components of the digested adipose tissue to effectively segregate the SVF from adipocytes and blood cells[49-51]. To achieve this, centrifugation parameters are carefully selected based on the specific protocol and the equipment being utilized.

Centrifugation speeds are generally categorized as low, medium, or high, corresponding to the gravitational forces applied to the cell suspension (Table 7): (1) Low-speed centrifugation, ranging from 400 to 600 × *g*, is often employed for a preliminary separation, gently pelleting down the heavier fractions while leaving lighter components in the supernatant; (2) Medium-speed centrifugation, usually between 1200 and 1500 × *g*, can provide a balance between efficiency and cell viability, often used to refine the separation process; and (3) High-speed centrifugation, at 2000 to 2500 × *g*, is utilized to firmly pellet the SVF, ensuring maximal separation from the less dense lipid-rich adipocytes and other cellular debris. Building upon these speed considerations, the duration of centrifugation is equally critical in optimizing SVF isolation.

Table 5 Comparative analysis of adipose tissue harvesting tools: Hypodermic needle *vs* liposuction cannula

Instrument	Advantages	Disadvantages
Hypodermic needle	Widely available and cost-effective. Simple, easy for manual aspiration. Various sizes for different applications	Limited use for large-scale harvesting. Multiple punctures may be necessary
Liposuction cannula	Efficient for large-volume harvesting. Suitable for extensive liposuction needs. Reduces trauma to surrounding tissues	Requires special equipment and training. Less suitable for small-scale procedures

Table 6 Instrument selection criteria: Hypodermic needle *vs* liposuction cannula

Factor	Hypodermic needle	Liposuction cannula
Adipose tissue amount required	Suitable for smaller quantities	Preferred for larger volumes
Procedure type	Ideal for manual aspiration	Designed for liposuction procedures
Patient comfort	Potentially less invasive and more comfortable	Larger cannulas may be more discomforting
Physician expertise	Requires basic proficiency in aspiration techniques	Necessitates expertise in liposuction equipment and methods
Safety	Low-risk tool for manual procedures	Must be used in line with stringent safety protocols

Table 7 Centrifugation speed and time parameters for stromal vascular fraction isolation

Centrifugation parameters	Speed, × *g*	Time in min
Low-speed centrifugation	400-600	5-10
Medium-speed centrifugation	1200-1500	10-15
High-speed centrifugation	2000-2500	15-20

The duration of centrifugation is similarly tiered, with times typically extending from 5 min to 20 min depending on the speed. Shorter times are usually adequate for low-speed centrifugation, while longer periods are reserved for high-speed centrifugation to ensure a complete pellet formation. Some protocols may incorporate a two-step centrifugation process, initially applying a lower speed to remove larger adipocytes and conclude with a higher speed to consolidate the SVF into a pellet. This approach can be particularly beneficial for enhancing the purity and yield of the SVF. It is important to note that centrifugation parameters are not one-size-fits-all and may require optimization based on the volume of the suspension, the centrifuge model, and the intended use of the SVF. Variations in equipment, rotor types, and sample sizes necessitate adjustments to centrifugation speeds and times to achieve the desired outcome.

The key to successful SVF isolation lies in the careful optimization and validation of centrifugation steps to ensure the isolation of a high-quality SVF. This involves following validated protocols, ideally within the framework of Good Laboratory Practices or Good Manufacturing Practices, particularly when SVF isolation is conducted for clinical applications[52,53]. Consulting with experienced laboratory personnel and adhering to established best practices is recommended to achieve the best possible results in SVF isolation for regenerative medicine purposes.

VOLUME OF DELIVERY

Having established the methods for SVF isolation, the next critical consideration is determining the appropriate volume for therapeutic delivery, particularly in the context of treating knee osteoarthritis. The treatment protocol itself is a primary determinant of the volume of SVF to be delivered. Different protocols, which may be based on the latest research findings, expert consensus, or institutional practices, can vary in their recommendations. These protocols consider the concentration of the cellular components within the SVF, the expected volume of the knee joint space, and the therapeutic regimen's design, whether it be a single treatment or part of a series[54-57]. The severity of the OA condition directly impacts the volume of SVF required. A knee joint with extensive cartilage degeneration or significant synovial inflammation may require a higher volume of SVF, as the therapeutic goal is to reach a larger area of damaged tissue and to provide a sufficient number of cells capable of exerting a regenerative effect[57-60]. In establishing the appropriate SVF volume for knee OA, a thorough assessment of the patient's clinical presentation is essential. A careful evaluation of imaging results, such as magnetic resonance imaging or X-rays, can offer insights into the extent of joint damage and the volume of space available for SVF injection.

Individual patient characteristics are pivotal in tailoring the SVF volume. The patient's age can influence the regenerative capacity of their cells, while overall health and comorbidities may affect the tissue's receptivity to the treatment[61-64]. A few studies have reported a positive correlation between body mass index and SVF yield[62,63].

However, other studies have found no significant relationship between body mass index and SVF yield[64,65]. Moreover, the patient's history of responses to previous interventions can inform the likelihood of a favorable response to SVF therapy, potentially adjusting the volume needed to achieve clinical improvement. The intended goals of the treatment also guide the volume decision. For patients seeking relief from pain, a different volume may be employed compared to those whose primary objective is to enhance joint function or induce repair of the cartilage itself. The volume administered may be modulated to prioritize these distinct therapeutic endpoints. Furthermore, considerations of the joint's biomechanical function, previous surgical history, and individual anatomy will also play a role. Having noted suboptimal clinical response in single-dose treatment protocols, multiple dosing has also been implemented to compensate for the reduced primary volume of SVF delivered during the index procedure[66].

Given that SVF therapy for knee OA is still a developing area, it is subject to the dynamic flow of new research and clinical experience. The field is evolving with ongoing studies that seek to optimize dosage, frequency, and delivery methods to enhance the efficacy and safety of SVF treatments. Healthcare providers must therefore remain informed about the latest advancements and incorporate this evolving knowledge into their practice. It is also critical to adhere to high standards of clinical practice, including Good Laboratory Practices or Good Manufacturing Practices, especially when preparing SVF for clinical applications[67,68]. This ensures the quality and safety of the cellular product being administered. The determination of the SVF volume for knee OA treatment is a complex decision that integrates multiple clinical variables. The optimal volume must be carefully calculated to provide the greatest potential for joint repair and symptom alleviation while minimizing the risks of adverse effects. As the field advances, it is anticipated that more standardized protocols will emerge, enhancing the precision and predictability of SVF therapy for knee OA.

HETEROGENEITY OF MESENCHYMAL STEM CELLS

The heterogeneity of mesenchymal stem cells (MSCs) leads to discrepancies in therapeutic efficacies due to differences in donor sources, tissues, and intercellular variations, affecting proliferation, differentiation, and cytokine secretion[69]. MSCs from the same donor can show variability under identical culture conditions, impacting therapeutic outcomes. Age-related changes, such as telomere shortening and DNA damage, further contribute to this variability[69]. Subpopulations like differentiation-resistant MSCs upregulate YAP1, CDH6, and OCT4, promoting tumorigenic properties[70]. Similarly, osteogenesis-resistant MSCs retain stem cell characteristics and upregulate AJUBA, CDH4, and CTNNB1, facilitating head and neck squamous cell carcinoma[71]. This emphasizes the need for standardization to improve MSC-based therapies.

FUTURE PROSPECTS

SVF, with its rich diversity of cells including ADSCs, is lauded for its ability to differentiate into multiple cell types, supporting tissue repair and regeneration across a variety of medical conditions.

Another perspective frequently encountered in the literature pertains to the non-invasive nature of SVF isolation. Research scholars often advocate for the use of adipose tissue as a source of stem cells due to its abundance and accessibility. The minimally invasive methods employed for harvesting adipose tissue, such as liposuction, are considered advantageous over more invasive stem cell extraction techniques, with the potential for reduced patient discomfort and quicker post-procedure recovery.

Ethical and safety considerations also form a pivotal part of the dialogue surrounding SVF isolation. The autologous nature of SVF, derived from the patient's tissue, circumvents many of the ethical dilemmas associated with embryonic stem cells and alleviates concerns regarding immune compatibility and disease transmission. Researchers and clinicians alike may emphasize this aspect, noting the favorable safety profile of SVF therapies.

The clinical applications of SVF are a subject of extensive discussion among clinicians, with many exploring its role in managing OA, enhancing wound healing, and facilitating tissue repair. There is a keen interest in the anti-inflammatory and angiogenic properties of SVF and its utility in a range of therapeutic contexts. Additionally, the potential of SVF in novel medical applications continues to be a vibrant area of research, with research experts advocating for continued exploration into its full clinical potential.

Standardization and quality control in SVF isolation are points of emphasis for some researchers, who argue for the necessity of consistent and reproducible protocols. This perspective is grounded in the importance of ensuring patient safety and treatment efficacy, with a call for rigorous standards and practices to be established and maintained across the field.

The evolving nature of SVF research is a recurring theme in scholarly discussions. The research scientists acknowledge that while the therapeutic promise of SVF is considerable, there remains a need for further studies to elucidate its mechanisms of action and to refine its application in clinical practice. The consensus is that ongoing research and clinical trials will be critical in advancing the field and expanding the therapeutic repertoire of SVF. It is essential to recognize that the views expressed by researchers are influenced by their respective research focuses their interdisciplinary backgrounds, and the evolving body of evidence. As such, the perspectives on SVF isolation are as diverse as the scientific community itself, unified by a common pursuit of advancing regenerative medicine through robust evidence, well-designed clinical trials, and stringent adherence to ethical and regulatory standards (Table 8).

Table 8 Perspectives on stromal vascular fraction isolation in regenerative medicine

Perspective	Description
Regenerative potential	Highlights the diverse cells in SVF, like adipose derived stem cells, for tissue repair and potential in regenerative medicine
Non-invasive approach	Stresses minimally invasive adipose tissue harvest, leading to less discomfort and quicker recovery
Ethical and safety considerations	Notes the ethical advantage of autologous use and lower risk of rejection or disease transmission
Clinical applications	Discusses the role of SVF in osteoarthritis, wound healing, and other regenerative areas
Standardization and quality control	Urges for standardized protocols to ensure consistent results and safety in SVF therapies
Evolving research	Emphasizes the need for ongoing research to expand understanding and clinical use of SVF

SVF: Stromal vascular fraction.

CONCLUSION

SVF isolation and application represents a promising frontier in regenerative medicine, offering a rich source of ADSCs and regenerative factors. The field grapples with the complexities of autologous *versus* allogeneic sourcing, each presenting unique advantages and challenges. Isolation techniques, ranging from enzymatic to mechanical methods, require optimization to balance efficiency and cellular viability. The potential of SVF in treating conditions like osteoarthritis is significant, yet it demands rigorous standardization and quality control measures. Moving forward, large-scale clinical trials are essential to evaluate long-term efficacy and safety. Researchers must elucidate the mechanisms of action, explore combination therapies, and develop standardized protocols. Establishing robust regulatory frameworks is crucial for clinical translation. While SVF shows great promise, ongoing research is vital to fully realize its therapeutic potential and establish it as a mainstream regenerative medicine approach. The field's advancement hinges on addressing these multifaceted challenges.

FOOTNOTES

Author contributions: Jeyaraman N designed the research; Jeyaraman N and Nallakumarasamy A analyzed the articles for performing the review and wrote the manuscript; Shrivastava S, Ravi VR, Pundkar A, and Jeyaraman M finalized the manuscript.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Che XX

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World Journal of Stem Cells

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World J Stem Cells 2024 August 26; 16(8): 799-810

DOI: 10.4252/wjsc.v16.i8.799

ISSN 1948-0210 (online)

MINIREVIEWS

Bone marrow mesenchymal stem cells in treatment of peripheral nerve injury

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Specialty type: Cell and tissue engineering

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade B, Grade C, Grade C

Novelty: Grade B, Grade C

Creativity or Innovation: Grade B, Grade C

Scientific Significance: Grade B, Grade C

P-Reviewer: Amin A; Li SC; Wang X

Received: March 7, 2024

Revised: May 20, 2024

Accepted: August 5, 2024

Published online: August 26, 2024

Processing time: 171 Days and 22 Hours

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Abstract

Peripheral nerve injury (PNI) is a common neurological disorder and complete functional recovery is difficult to achieve. In recent years, bone marrow mesenchymal stem cells (BMSCs) have emerged as ideal seed cells for PNI treatment due to their strong differentiation potential and autologous transplantation ability. This review aims to summarize the molecular mechanisms by which BMSCs mediate nerve repair in PNI. The key mechanisms discussed include the differentiation of BMSCs into multiple types of nerve cells to promote repair of nerve injury. BMSCs also create a microenvironment suitable for neuronal survival and regeneration through the secretion of neurotrophic factors, extracellular matrix molecules, and adhesion molecules. Additionally, BMSCs release pro-angiogenic factors to promote the formation of new blood vessels. They modulate cytokine expression and regulate macrophage polarization, leading to immunomodulation. Furthermore, BMSCs synthesize and release proteins related to myelin sheath formation and axonal regeneration, thereby promoting neuronal repair and regeneration. Moreover, this review explores methods of applying BMSCs in PNI treatment, including direct cell transplantation into the injured neural tissue, implantation of BMSCs into nerve conduits providing support, and the application of genetically modified BMSCs, among others. These findings confirm the potential of BMSCs in treating PNI. However, with the development of this field, it is crucial to address issues related to BMSC therapy, including establishing standards for extracting, identifying, and cultivating BMSCs, as well as selecting application methods for BMSCs in PNI such as direct transplantation, tissue engineering, and genetic engineering. Addressing these issues will help translate current preclinical research results into clinical practice, providing new and effective treatment strategies for patients

with PNI.

Key Words: Bone marrow mesenchymal stem cells; Peripheral nerve injury; Schwann cells; Myelin sheath; Tissue engineering

Core Tip: Bone marrow mesenchymal stem cells (BMSCs) have become ideal seed cells for the treatment of peripheral nerve injury (PNI) due to their strong differentiation potential and the possibility of autologous transplantation. In this review, we introduce the biological characteristics of BMSCs related to PNI, outline the current mechanisms by which BMSCs promote the regeneration and repair of PNI, and summarize the various application methods of BMSCs in PNI, confirming the potential of BMSCs in the treatment of PNI and providing great support for the development of new treatment strategies for nerve regeneration and repair in PNI.

Citation: Zou XF, Zhang BZ, Qian WW, Cheng FM. Bone marrow mesenchymal stem cells in treatment of peripheral nerve injury. *World J Stem Cells* 2024; 16(8): 799-810
URL: https://www.wjgnet.com/1948-0210/full/v16/i8/799.htm
DOI: https://dx.doi.org/10.4252/wjsc.v16.i8.799

INTRODUCTION

Peripheral nerve injury (PNI) refers to damage that occurs to the peripheral nerve trunk or its branches due to direct or indirect trauma from external sources. It is characterized by sensory, motor, and autonomic dysfunction in the trunk or limbs, representing one of common neurological disorders in clinical practice[1]. PNI is a global issue, with an annual incidence rate of approximately 13/100000 to 23/100000 in developed countries[2-5]. While peripheral nerve axons can regenerate after injury, achieving complete functional recovery is often challenging in cases of proximal nerve injuries or large nerve defects[6]. Currently, autologous nerve transplantation is considered the gold standard for PNI repair[7]. However, even under ideal conditions, this approach does not fully restore impaired motor and sensory functions[8]. Additionally, it has significant drawbacks, such as prolonged surgical time, high economic costs, insufficient donor areas for reconstruction of long or multiple nerve defects, and potential donor site damage (painful neuroma, scarring, and sensory deficits)[9]. In recent years, several new methods for PNI repair have emerged, showing positive effects on restoring the continuity of injured neuroanatomy. However, their ability to restore nerve function is not ideal, and they all have varying degrees of limitations[10].

Tissue engineering is an emerging discipline in the field of biotechnology and has gained significant attention in PNI research. Previous studies have demonstrated that transplantation of Schwann cells (SCs) can promote nerve regeneration and accelerate nerve function recovery[11]. However, obtaining a large number of SCs in a short period is challenging, and it may cause irreversible damage to the donor area, thus limiting the clinical application of SCs transplantation[12]. Recent research has found that adult mesenchymal stem cells (MSCs) can also promote nerve regeneration and show potential for treating PNI, making them a more ideal alternative to SCs. Bone marrow MSCs (BMSCs) are one type of adult MSC with strong differentiation potential and advantages in autologous transplantation. Numerous studies have indicated that BMSCs can differentiate into nerve-like cells during the PNI treatment process and play a crucial role in nerve growth factor (NGF) secretion, endogenous stem cell migration and differentiation, and neovascularization[13-15]. These findings suggest that BMSCs effectively promote the repair of neurological deficits, which makes them ideal seed cells for PNI repair. Researchers are also striving to translate preclinical research findings into practical clinical applications for PNI patients. BMSCs can be applied to PNI therapy through a variety of techniques, such as cell transplantation, tissue engineering, gene engineering, and cell therapy, including the use of BMSC-derived exosomes. These approaches have the potential to improve the effectiveness of PNI regeneration and offer new hope for PNI patients.

Through literature search and analysis (Figure 1), in this review, we present the biological properties of BMSCs associated with PNI. We summarize the current mechanisms by which BMSCs promote nerve regeneration and repair in PNI, as well as various application methods in PNI. Moreover, based on these findings, we identify the existing problems and limitations in order to deepen our understanding of BMSCs, optimize treatment strategies, address their shortcomings in clinical application in PNI, and promote their use in PNI clinical practice.

BIOLOGICAL PROPERTIES OF BMSCS IN TREATMENT OF PNI

BMSCs are a type of pluripotent stem cell that, under specific conditions, can differentiate not only into tissue cells from the mesodermal lineage, such as osteocytes, chondrocytes, and cardiomyocytes[16,17], but also undergo transdifferentiation across germ layers to form neurons, glial-like cells from the ectoderm, and hepatocytes, among others[18]. Silva *et al*[19] discovered that BMSCs express genes associated with both epithelial tissues and mesenchymal tissues, providing a

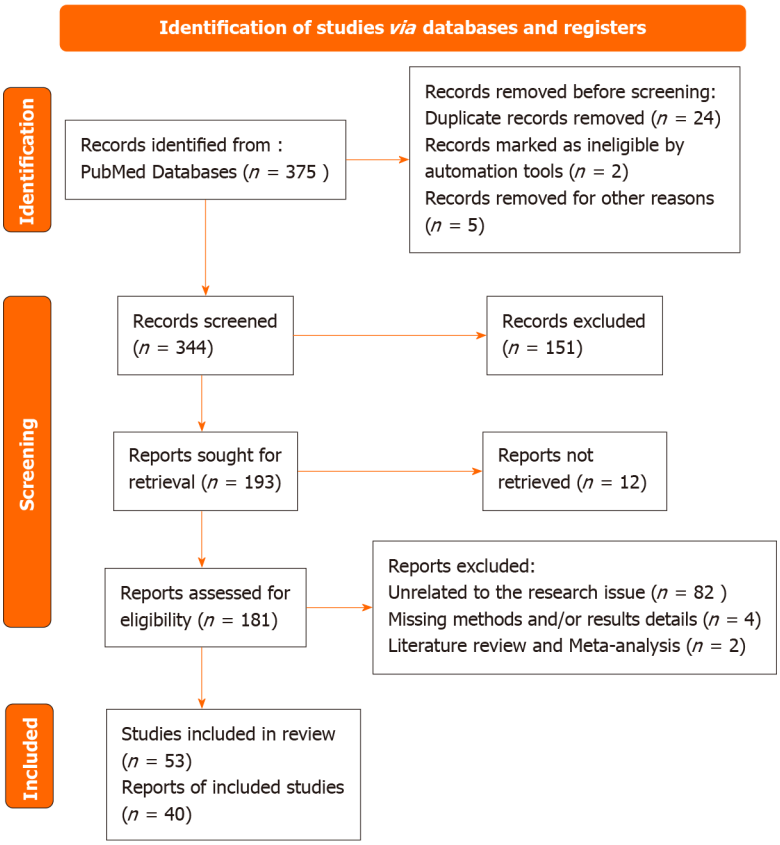

Figure 1 Flow chart of literature search and selection criteria. The initial search resulted in 344 articles. Out of 344 full-texts assessed, 251 articles were excluded. Thus, 93 articles that met the eligibility criteria were included.

theoretical basis for their multi-lineage differentiation potential at the gene level. Additionally, BMSCs possess self-renewal capacity. Tamir *et al*[20] found that approximately 90% of BMSCs are in the G0/G1 phase, which confirms their robust self-renewal capabilities.

BMSCs have no specific surface markers and generally exhibit low expression of major histocompatibility complex (MHC)-I molecules and do not express MHC-II molecules. They also do not express molecules required for T lymphocyte activation, such as Fas ligand and co-stimulatory molecules like B7-1, B7-2, and CD40 L[21]. This characteristic gives BMSCs low immunogenicity and strong immune-suppressive properties. Therefore, studies have shown that when co-cultured with allogeneic and xenogeneic T lymphocytes, BMSCs do not induce significant T cell proliferation but rather inhibit T cell proliferation[22]. In addition to being non-immunogenic, BMSCs are not targeted by CD8+ T cells, which allows them to evade cytotoxic T cell and natural killer cell killing, making them beneficial for successful autologous and allogeneic transplantations[23]. Furthermore, the antigenicity of BMSCs does not increase with their differentiation[24].

Indeed, it is evident that BMSCs possess the potential for multi-lineage differentiation and robust self-renewal capacity. Moreover, when transplanted into the body, they do not trigger significant rejection responses and can be allografted without causing immune rejection reactions[25,26]. The fact that BMSCs do not require the use of immunosuppressive drugs further adds to their appeal as seed cells for treating PNI, making them a promising candidate for potential applications in PNI therapy.

MECHANISMS OF BMSCS IN TREATMENT OF PNI

After PNI, if neurons have not died, their axons can undergo regeneration. SCs play a critical role in the repair of the peripheral nervous system. Following Wallerian degeneration of the peripheral nerve, SCs rapidly and massively proliferate, forming Büngner bands. They are involved not only in the formation, synthesis, and secretion of various NGFs but also in the synthesis and secretion of various extracellular matrix (ECM) components and other cell adhesion molecules. The above-mentioned NGFs, ECM, and cell adhesion molecules form gaps or tight junctions with adjacent axons, creating direct channels for the transfer of small molecules and information. These play an essential role in nerve injury regeneration and repair. Under specific conditions, BMSCs can differentiate into neural cells, including SC-like cells, and exert corresponding effects. In this section, we will explore the various functions of BMSCs in PNI repair and list the involved molecular mechanisms.

Differentiation into neural cells

BMSCs are one of the most widely used sources of cells for nerve regeneration. After transplantation, they can differentiate into different cells, such as neurons, astrocytes, and SC-like cells, under the influence of different physiological microenvironments and express corresponding antigen markers. *In vitro* studies have found that BMSCs can be induced to differentiate into neural-like cells by antioxidants (such as dimethyl sulfoxide and β-mercaptoethanol), cytokines [retinoic acid, basic fibroblast growth factor (bFGF), and epidermal growth factor], traditional Chinese medicine preparations (tetramethylpyrazine and baicalin), gene transfection, and other methods[27,28]. However, whether these induced neural-like cells possess the functional characteristics of normal neurons remains controversial. For instance, Hofstetter *et al*[29] successfully induced rat BMSCs to differentiate into neural cells using butylated hydroxyanisole but did not record the electrophysiological activity of mature neuronal cells. Some researchers believe that this phenomenon is not related to cell differentiation but rather cytotoxic changes[27]. On the other hand, other studies have shown successful induction of rat BMSCs into neural-like cells using a combination of bFGF, dimethyl sulfoxide, and butylated hydroxyanisole, with the capture of excitatory electrophysiological characteristics[27,28]. Wislet-Gendebien *et al*[30], through co-culturing, induced rat BMSCs to differentiate into neural cells that produced single action potentials and responded to neurotransmitters such as γ-aminobutyric acid, glycine, and glutamate. These findings suggest that BMSCs can differentiate into excitable neural-like cells *in vitro*.

In *in vivo* studies, Cuevas *et al*[31] injected 50000 bone MSCs (pre-labelled with bromodeoxyuridine BrdU) in 5 μL of culture medium solution into the distal stump of transected sciatic nerve of the rats, and found that after 33 d of implantation, almost 5% of BrdU cells express Schwann cell-like phenotype. Dezawa *et al*[32] obtained GFP-expressing BMSCs (GFP-MSCs) by retroviral vectors, adjusted the concentration of GFP-MSCs to (1-2) × 10^7 cells/mL, and then injected them into hollow fibres to make an artificial graft. The artificial graft was anastomosed to the cut end of the proximal nerve segment of the sciatic nerve in rats, and a large number of newly formed fibers were observed after 3 wk. They found that BMSCs had a myelination effect in regenerating nerve fibers through immunoelectron microscopy and confocal microscopy, indicating that BMSCs can differentiate into neuron-like cells and secrete a large amount of NGFs to induce axon growth. Additionally, BMSCs can directly transform into SCs to repair injured nerves, which has attracted considerable attention[33]. Furthermore, inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α) and interleukin (IL)-1β have been reported to affect the differentiation of MSCs, possibly driving MSCs toward specific cell phenotypes, such as astrocytes. Elevated levels of such pro-inflammatory cytokines can inhibit neuronal differentiation and promote the differentiation of BMSCs into astrocytes[34]. In conclusion, under specific conditions, BMSCs can differentiate into SCs and neural-like cells both *in vitro* and *in vivo*, facilitating nerve repair through cell replacement.

Improving neural regeneration microenvironment

Neurotrophic factors have the function of promoting nerve growth and inducing cell differentiation into neural cells, and they can be used to induce the differentiation of BMSCs into neural cells. BMSCs can secrete a variety of neurotrophic factors, such as brain-derived neurotrophic factor (BDNF), NGF, vascular endothelial growth factor (VEGF), bFGF, and insulin-like growth factor (IGF)[14]. They upregulate the expression of VEGF receptor (VEGFR) and IGF1 receptor (IGF-1R) and promote the secretion of endogenous neurotrophic factors in the central nervous system. These neurotrophic factors are synthesized and retrogradely transported to nerve cells, transmitting information or paracrine signals to proximal and distal nerves. They bind to their specific receptors, such as NGF with NGF receptor A, BDNF with tyrosine receptor kinase B, and neurotrophin-3 (NT-3) and neurotrophin-4/5 with neurotrophic tyrosine receptor kinase 3. Activation or inhibition of signaling pathways such as PI3K/Akt, Ras-ERK, cAMP/PKA, and PLC-γ-dependent pathways occurs, thereby promoting neuron survival, accelerating axonal and vascular growth, stimulating nerve fiber regeneration, preventing cell apoptosis, inducing SCs migration, proliferation, and myelination formation, and slowing down muscle atrophy, thus reversing the negative effects of PNI (such as preventing cell death caused by axonal injury) [5,35-37]. This improves the supportive microenvironment for neuron survival and regeneration[38] and exerts a neuroprotective effect on nerve cells[39]. Neuhuber *et al*[40] suggested that the neurotrophic factors produced by human BMSCs are essential for mediating axonal growth and functional recovery after spinal cord injury.

Wang *et al*[41] conducted a study and reported that using BMSCs transplantation in rats with PNI achieved results similar to autologous nerve transplantation, possibly due to the release of a large number of neurotrophic factors by BMSCs. Isele *et al*[42] found that the growth condition medium of BMSCs significantly reduced cross-cell-induced apoptosis in fetal rat hippocampal neurons, demonstrating a significant neuroprotective effect. During this process, they observed an increase in phosphorylation of MAPK/ERK and Akt. Blocking this protective effect occurred when using MAPK/ERK and PI3K/Akt specific inhibitors, suggesting that the neurotrophic factors secreted by BMSCs counteracted apoptosis stress response by activating these survival pathways and exerting a neuroprotective effect. They also discovered that stressed neuronal cells stimulated BMSCs to increase the secretion of trophic factors. In another study by Yang *et al*[43], they used BMSCs as support cells and injected them into a silk fibroin-based nerve conduit. This approach increased the expression of the SCs marker molecule S100 and enhanced the secretion of various neurotrophic growth factors such as BDNF, bFGF, and ciliary neurotrophic factor (CNTF). This, in turn, facilitated histological and functional recovery in rats with sciatic nerve injuries.

The ECM is a complex reticular structure composed of large molecules such as proteins and polysaccharides secreted by cells. It includes laminin, fibronectin, collagen, and other components. The ECM plays a crucial role in promoting cell proliferation and differentiation, supporting the transmission of important signals in the peripheral nervous system[44], which, together with neurotrophic factors and cell adhesion molecules, provides a favorable microenvironment for the survival of nerve cells and the formation of nerve connections[45-47]. Chen *et al*[48] mixed BMSCs cultured *in vitro* with gelatin and transplanted them into a 15 mm defect model of the rat sciatic nerve using silicone conduits. Compared to the

gelatin-only control group, the experimental group showed improved walking behavior in rats, reduced atrophy of the gastrocnemius muscle, and decreased reduction in compound motor action potential amplitude, with a significant amount of regenerated axons observed. Both *in vitro* and *in vivo*, BMSCs synthesize and secrete various ECM components, including NGF, CNTF, BDNF, glial cell-derived neurotrophic factor (GDNF), as well as type I and type IV collagen, fibronectin, laminin, and other ECM molecules. After transplantation, both early and late stages of nerve regeneration are accompanied by high expression of neurotrophic factors. Wright *et al*[49] reported that BMSCs can stimulate neuronal development and mediate nerve regeneration by modulating the expression of ECM components such as chondroitin sulfate proteoglycans, myelin-associated glycoproteins, and Nogo-A.

Cell adhesion molecules are also critical for axon guidance, including integrins, neural cell adhesion molecules, and calcium-binding proteins such as N-cadherin. Among them, neural cell adhesion molecules may preferentially promote the growth of sensory axons[50]. BMSCs can express various factors related to cell adhesion, such as Ninjurins 1 and 2, Netrin 4, Robo 1, and Robo 4[51-53]. These factors are recognized as neuroregenerative factors and effectively promote axonal growth and cell migration. In summary, BMSCs improve the microenvironment for neuron survival and regeneration through paracrine secretion of neurotrophic factors, ECM factors, adhesion molecules, and various other mechanisms. By promoting the regeneration of damaged neurons, BMSCs contribute to the repair of neural functions.

Promoting angiogenesis

After PNI occurs, the blood vessels within the nerves are damaged. Therefore, promoting vascular regeneration and restoring blood circulation are essential for the recovery of the normal neural tissue environment. Peripheral nerve regeneration is closely related to angiogenesis, which is a crucial process in the repair of peripheral nerves. VEGF is considered an effective factor for both angiogenesis and neuron generation, and it has long been recognized for its importance in promoting neuron survival and SCs proliferation. Popovich *et al*[54] reported that BMSCs can secrete various neuroprotective trophic factors such as BDNF, NGF, and VEGF in an autocrine and/or paracrine manner, which can upregulate the expression of these factors, thereby promoting local microvascular regeneration, nerve regeneration, and reconstruction, and ultimately facilitating the repair of injured cells. Induced SCs-like cells from BMSCs have been found to exhibit enhanced immunostaining for VEGF, suggesting that BMSCs may also promote blood vessel formation [55]. BMSCs can also increase the expression levels of endogenous VEGF and its receptor VEGFR2 in the ischemic penumbra, thereby promoting neovascularization[15]. Zurita and Vaquero[56] also observed that blood vessel wall cells in newly regenerated neural tissue at the site of spinal cord injury were differentiated from injected BMSCs. These studies indicate that BMSCs can promote angiogenesis through paracrine secretion of VEGF, and the newly formed blood vessels can, in turn, facilitate the repair of peripheral nerve injuries.

Promoting myelination and axon regeneration

Myelination is another essential process in the regeneration of PNI, determining the quality and functional recovery of nerve regeneration[5,35,47]. Typically, myelination can be achieved by promoting endogenous repair mechanisms or providing an exogenous source of myelinating cells, leading to subsequent nerve function restoration[47]. In a study conducted by Kizilay *et al*[57], the systemic application of BMSCs was explored in a PNI compression model. Wistar albino rats were used, and the sciatic nerve was compressed for 5 min to create the model. Approximately 5×10^5 BMSCs were injected intravenously. The results showed that animals treated with BMSCs exhibited higher nerve conduction velocity, compound action potential, and axon numbers compared to the control group. In addition, myelin damage was less severe in the BMSC-treated group, suggesting that systemic application of BMSCs has a positive impact on both myelination and axon survival in the peripheral nerve compression model.

SCs and various types of adult stem cells (in the form of SCs-like cells) have the ability to form myelinating neuronal cells and regenerate nerves. During the regeneration process after PNI, intracellular cAMP levels are elevated when SCs or SCs-like cells further differentiate into myelin-forming cells. This leads to the synthesis and secretion of abundant myelin proteins, such as myelin basic protein, myelin protein zero, peripheral myelin protein 22 (PMP22), and other proteins that are crucial for myelin structure and function. This promotes remyelination during and after regeneration[5, 47] and increased expression of IGF-1R and neurofilament type 1 and type 3 enhances axon alignment and myelination gene expression, resulting in increased myelin thickness and internodal length[35,50]. BMSCs also provide various cytokines and growth factors for nerve regeneration[58], including NGF, NT-3, VEGF, PMP22[59-62], and more. Zhao *et al* [63] also demonstrated that exosomes from BMSCs upregulate the expression of PMP22, VEGF, NGF receptors, and S100β protein, promoting increased neuronal length and axon diameter in the dorsal root ganglion. These protein factors play crucial roles in peripheral nerve regeneration. During the repair process, BMSCs not only directly affect SCs through their neurotrophic functions[64] but may also differentiate towards SCs directionally.

BMSCs, in addition to their ability to differentiate into neuron-like cells[65], also stimulate and induce axonal growth [66], and play an important role in maintaining the normal structure and function of myelin sheaths[67,68]. BMSCs can promote the repair of damaged nerves by regulating the expression of myelination-related genes. For instance, differentiation of BMSCs into SC-like cells can enhance the mRNA expression of myelin-associated factors, significantly increasing the number of myelinated axons, thereby promoting the functional recovery of the facial nerve[69]. In conclusion, MSCs promote myelination and axonal regeneration through various mechanisms, including the secretion of neurotrophic factors, direct interactions with neurons, and upregulation of genes involved in myelination. These combined effects contribute to enhanced axonal growth and improved functional recovery after PNI.

Immunomodulation

After PNI, various immune cells and cytokines are present, and the coordination of local inflammatory response is

essential for the recovery of PNI. BMSCs possess significant immunomodulatory properties, which can promote neural tissue regeneration and alleviate inflammation, therefore making them valuable in PNI treatment. BMSCs can exert immunomodulatory effects by regulating the expression of various cytokines. IL-6 is a multifunctional cytokine produced by macrophages and fibroblasts during PNI[70]. IL-17 is produced by activated CD4+ T cells and can increase the production of pro-inflammatory cytokines and neutrophil chemoattractants, showing elevated levels after PNI[71]. Studies by Ge *et al*[72] found that BMSCs can secrete high levels of IL-6 to modulate the balance of CD4+ T cell subgroups, promote the proliferation and differentiation of T helper type 17 (Th17) cells that secrete IL-17, and subsequently stimulate prostaglandin E2 secretion. Elevated prostaglandin E2 levels then inhibit Th17 cell secretion of IL-17, achieving therapeutic effects for facial nerve injury. The increased expression of IL-10 protein is associated with regeneration of myelin protein. Research by Cui *et al*[73] revealed that IL-10-stimulated BMSCs can inhibit the expression of the pro-inflammatory cytokines TNF-α and IL-1β. Fan *et al*[74] suggested that this may be achieved by reducing the release of the pro-inflammatory cytokines IL-2, interferon-γ, and TNF-α and increasing the secretion of IL-10 in lymphocyte supernatant and serum, thereby promoting neural regeneration.

BMSCs can modulate the polarization of macrophages, promoting their transition from the pro-inflammatory M1 phenotype to the anti-inflammatory M2 phenotype. This shift in macrophage polarization is crucial for controlling inflammation and establishing an environment for tissue repair and regeneration. Zhong *et al*[75] reported that BMSCs secrete GDNF, which converts the damaging M1 phenotype in microglia to the regenerative M2 phenotype, thereby suppressing neural inflammation. This process may be related to inhibiting the nuclear factor-kappaB signaling pathway and promoting the PI3K/AKT signaling pathway.

Another important aspect of MSC-mediated immune regulation is the release of extracellular vesicles (EVs), including apoptotic bodies, exosomes, microvesicles, *etc.*[76], which contain bioactive components. These EVs are considered an intriguing non-cellular therapy due to their low immunogenicity and ability to mediate cell-to-cell communication and modulate the function of recipient immune cells, contributing to the overall immunomodulatory effects of BMSCs. BMSCs' EVs may exhibit similar anti-inflammatory functions as BMSCs themselves by decreasing the levels of inflammatory cytokines and enhancing anti-inflammatory responses. For instance, Schäfer *et al*[77] found that BMSCs can release soluble mediators such as TNF-α and IL-1β to alleviate inflammation after PNI. It is evident that BMSCs can exert their immunomodulatory effects through various mechanisms, including regulating the expression of various cytokines, regulating macrophage polarization, releasing EVs, and secreting soluble factors. These effects can help control inflammation, prevent autoimmune reactions, and create a more favorable environment for nerve repair and regeneration following PNI.

In summary, BMSCs play a crucial role in promoting PNI repair and regeneration through various mechanisms (Table 1). First, BMSCs are able to differentiate into nerve cells (such as neurons and SCs) to replace damaged nerve cells and facilitate nerve regeneration. Second, they secrete neurotrophic factors, ECM molecules, and adhesion molecules, while also exerting immunomodulatory effects, creating a supportive microenvironment for the growth, differentiation, and survival of nerve cells. Third, BMSCs promote the formation of new blood vessels to ensure the necessary blood supply for the repair and accelerated regeneration of damaged nerves. Lastly, by synthesizing and releasing of proteins related to myelination and axon regeneration, BMSCs enhance the growth of myelinated axons and ultimately promote neuron regeneration. BMSCs utilize these different mechanisms to promote the repair and regeneration of damaged nerve cells and enhance the functional recovery after PNI. Utilizing these pathways can significantly enhance the therapeutic potential of BMSCs in PNI treatment.

APPLICATION METHODS OF BMSCS IN TREATMENT OF PNI

The unique mechanisms of action of BMSC make them promising candidates for the treatment of PNI. In this section, we will explore the various application methods of MSCs in PNI treatment (Figure 2), analyzing the advantages and disadvantages of each approach in order to comprehensively explore their potential in PNI treatment.

Direct transplantation

BMSCs have self-renewal and multi-lineage differentiation capabilities that make neuronal regeneration and nerve function recovery possible, rendering them one of the best choices for stem cell therapy in PNI treatment. Apart from their regenerative potential, BMSCs have been shown to migrate to the injury site and home to the injured area, exhibiting potential for targeted therapy[78,79]. Furthermore, BMSCs do not significantly stimulate the proliferation of T cells nor serve as a target for CD8+ T cells. Thus, when applied in autologous or allogeneic transplantation, they can evade the killing and clearance by immune cells in the body, further exerting their reparative effects. Cuevas *et al*[31] and Cuevas *et al*[80] cultured BMSCs from adult rats, labeled them with BrdU, and then injected them into the distal stump of the 5 mm-deficient sciatic nerve in rats. At 18 d and 33 d post-surgery, footprint analysis showed significant improvement in the motor function of the rat limbs compared to the control group injected with only culture medium. Immunofluorescence double-labeling showed that BrdU-labeled cells survived for at least 33 d after surgery, and nearly 5% of the cells expressed the S100 phenotype of SCs. In March 2004, they conducted a similar study on the long-term recovery of rat limbs 180 d after BMSC transplantation, finding that BMSCs continued to have a promoting effect on long-term recovery after surgery[80]. This experiment proves the great potential of BMSCs in peripheral nerve regeneration and lays the foundation for their application in the field of peripheral nerve regeneration. Wang *et al*[41] investigated the reparative effects of BMSCs by injecting them into the muscles after sciatic nerve injury in rats, and the results showed that the number of regenerating nerve fibers and spinal cord ventral horn neurons increased significantly, as well as a significant

Table 1 Mechanisms of bone marrow mesenchymal stem cell therapy for peripheral nerve injury

Mechanism	Key processes/factors	Description	Ref.
Differentiation into nerve cells	SCs, neurons	BMSCs differentiate into various nerve cells, including neurons, astrocytes, and Schwann cell-like cells, both *in vitro* and *in vivo*	[27-34]
Improvement of nerve regeneration microenvironment	Neurotrophic factors, ECM molecules, adhesion molecules, paracrine effects	BMSCs secrete neurotrophic factors, regulate ECM components, secrete adhesion molecules, and exert paracrine effects, creating a suitable microenvironment for nerve regeneration and functional recovery	[5,14,35-39,41-43,48,49,51-53]
Promotion of neovascularization	VEGF, endothelial cells	BMSCs secrete VEGF and differentiate into endothelial cells, participating in the process of vascular development and promoting the formation of new blood vessels	[15,54-56]
Enhancement of myelination and axon regeneration	Myelinating cells, myelin proteins, neurotrophic factors, Schwann cells	BMSCs promote myelination and axon regeneration by differentiating into myelinating cells or Schwann cells, secreting neurotrophic factors and regulating the expression of myelination-related genes, thereby improving nerve function	[5,47,57,63,64,66-69]
Immune modulation	Cytokine regulation, macrophage polarization, extracellular vesicles, soluble factors	BMSCs control inflammation, prevent autoimmune reactions, and create a favorable environment for nerve repair and regeneration by regulating the expression of various cytokines, modulating macrophage polarization, releasing EVs, and secreting soluble factors	[70-77]

SCs: Schwann cells; BMSC: Bone marrow mesenchymal stem cell; ECM: Extracellular matrix; VEGF: Vascular endothelial growth factor; EVs: Extracellular vesicles.

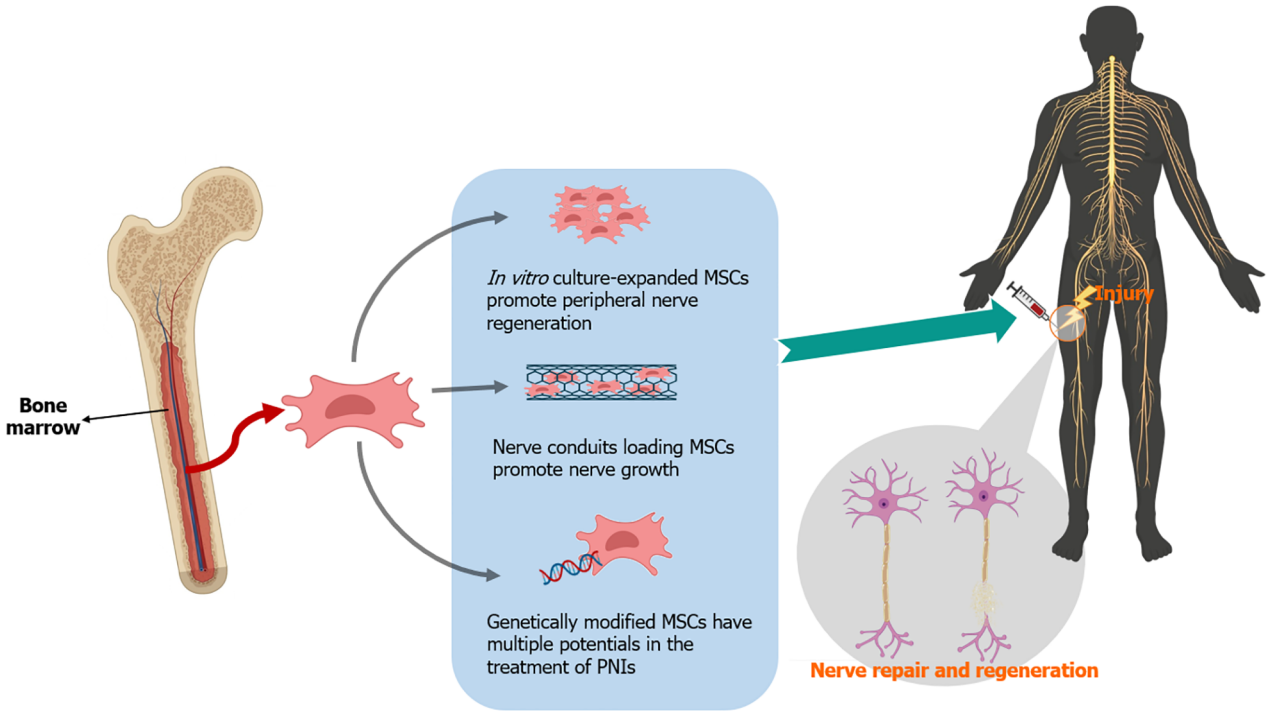

Figure 2 Application of bone marrow-derived mesenchymal stem cells in the treatment of peripheral nerve injury. Bone marrow-derived mesenchymal stem cells can be isolated from bone marrow, expanded *in vitro*, and directly transplanted into damaged nerve tissue. They can be loaded onto nerve conduits, which provide structural support, using tissue engineering techniques. Additionally, bone marrow-derived mesenchymal stem cells can be genetically modified with neurotrophic factors before being applied to the treatment of peripheral nerve injury to promote neuronal repair and regeneration. PNI: Peripheral nerve injury; MSC: Mesenchymal stem cell.

increase in regenerated myelin sheath thickness, which indicated that transplantation of BMSCs in PNI rats can achieve similar results as autologous nerve transplantation. Hu *et al*[81] transplanted BMSCs to repair a 50 mm midline nerve injury in monkeys and found that the healing process was similar to that of autologous transplantation, showing good functional and morphological outcomes. Another study found that when BMSCs were directly transplanted around the sciatic nerve stump, they induced axonal growth by differentiating into neuron-like cells and secreting neurotrophic factors[32]. They also differentiated into SCs to repair the injured nerves[33] and promoted remyelination of regenerating nerve fibers. From this, it can be seen that direct transplantation of BMSCs has played a positive role in repairing various PNI-damaged nerves. However, the invasive procedures required for obtaining BMSCs and the limited quantity of cells

obtained, as well as the reduced proliferative and differentiation abilities with increasing patient age, have restricted the research and application of BMSCs in clinical settings.

Tissue engineering

Scaffold technology has become a hot topic in tissue engineering research in recent years, and nerve conduits are a type of artificial tubular scaffold. BMSCs can simulate the structure and function of the human nervous system when loaded onto nerve conduits and connecting on both sides of the nerve stump. Nerve conduits can be made from natural materials such as chitosan and collagen or synthetic materials such as polyglycolic acid and polylactic acid. Each material has its own characteristics, generally inducing nerve axon regeneration and preventing infiltration of surrounding tissues to interfere with nerve repair. By loading BMSCs onto nerve conduits, not only does it achieve the neurotrophic guidance function of the nerve conduit, but it also provides a space for BMSCs and nerve axon regeneration induction, which helps to promote the effects of BMSCs in promoting nerve growth and regulating the microenvironment of the injury site[82]. In the process of repairing injured nerves using tissue engineering methods, comparing the transplantation effects of nerve conduits with and without BMSCs, it was found that the number and diameter of nerve axons in the experimental group significantly increased, and the improvement of nerve function was significantly better than that in the control group[83].

Costa *et al*[84] inplanted BMSCs into poly(L-lactic acid) nerve conduit scaffolds for repairing facial nerve defects in rats. The results showed that BMSCs could successfully integrate into the conduit, survive within the nerve tissue, and maintain their phenotype for up to 6 wk. In another study, researchers loaded BMSCs into chitosan nerve conduits and observed cell survival and proliferation within the conduit for 8-16 wk, which effectively promoted the repair of an 8 mm nerve defect[85]. Subsequent research by this team demonstrated that BMSC-loaded chitosan nerve conduits not only accelerated the efficiency of nerve repair but also improved the quantity and quality of regenerated nerve fibers, achieving therapeutic effects comparable to autologous nerve transplantation[86]. The degradation products of nerve conduit materials often trigger local immune reactions, leading to an inflammatory state at the site of injury, which can affect the repair outcome. However, in a study by Hsu *et al*[87], researchers modified chitosan nerve conduits with laminin to enhance the adhesion capability of BMSCs within the conduit. They observed that BMSCs successfully inhibited the local inflammatory response caused by chitosan degradation, resulting in improved promotion of nerve repair. Other experimental studies have also used BMSCs implanted in nerve conduits made of different materials, such as fibroin gel conduits[88], polylactic-co-glycolic acid conduits with ECM gel[89], and polyglycolic acid conduits[90], to intervene in PNI animal models, and all achieved favorable results.

Although encouraging results have been obtained in animal experiments, further research is still needed to optimize the design of nerve conduits, determine the optimal combination of BMSCs and biomaterials[91], and assess the long-term safety and efficacy of nerve conduits in clinical settings[92]. By addressing these issues, the use of BMSCs in tissue engineering approaches may have a more significant impact on PNI treatment, providing new strategies to promote neural functional recovery and improve the quality of life for patients.

Gene engineering

Gene-modified BMSCs have also gained increasing attention in tissue engineering research. In the field of neural repair tissue engineering, the main purpose of gene modification is to design target cells to overexpress growth factors, migration molecules, and adhesion molecules, as well as to inhibit the expression of defective genes. NT-3, NT-4, BDNF, NGF, CNTF, bFGF, and others are major neural growth factors suitable for peripheral nerve gene delivery, as they can provide a suitable microenvironment for the survival and axonal growth of BMSCs. In a study by Zhang *et al*[93] in 2015, BMSCs transfected with BDNF and CNTF were used for the treatment of rat sciatic nerve injuries. The results showed that BDNF- and CNTF-transfected BMSCs combined with nerve transplantation significantly improved the sciatic nerve function index, promoted the recovery of muscle activity, and increased the thickness of regenerating nerve myelin sheaths. This indicates that this approach is effective in promoting axonal growth and facilitates nerve repair in PNI. In another study[94], BDNF was successfully transfected into BMSCs using gene engineering technology, and the transfected BMSCs were combined with decellularized allogeneic nerve grafts to repair peripheral nerve defects. The results showed a significant improvement in the repair effectiveness of the nerve grafts and the morphology of the injured nerves. Gene-modified MSCs have multiple potentials in the treatment of PNI. However, since gene therapy is still in the experimental stage, its application in clinical settings requires addressing numerous challenges, such as the selection of diverse target genes, stable expression of target genes in the host, combination therapy with multiple genes, and ethical considerations.

CONCLUSION

Unlike the central nervous system, the peripheral nervous system has the ability for self-regeneration and repair after injury. However, this endogenous repair is limited, and extensive nerve damage cannot be fully repaired. Cell therapy is considered to be an important direction for future medical development, and in recent years, the field of PNI neural regeneration and repair has made vigorous progress, with enormous market potential and clinical application value. BMSCs have the advantages of abundant sources, easy and simple procurement, being easy to isolate and cultivate, and the potential for rapid expansion under certain conditions. Additionally, autologous BMSCs transplantation avoids ethical issues and immune rejection, offering broad prospects for PNI treatment. In this paper, we have reviewed the current biological characteristics of BMSCs related to PNI, summarized the mechanisms by which BMSCs promote PNI

neural regeneration and repair, and explored various application methods of BMSCs in PNI, confirming the potential of BMSCs in treating PNI.

However, most research on BMSCs transplantation for PNI intervention is still in the pre-clinical stage and has not yet had significant implications for clinical practice, and there are also certain limitations, such as the lack of specific surface markers on BMSCs[21], which poses some difficulties in identifying cultured BMSCs, and the lack of standardized treatment regimens, where many times after BMSC transplantation, the survival rate is not high, and the proportion of differentiation into neurons is low, resulting in unsatisfactory nerve repair effects. There are also safety issues with BMSC transplantation, where inducers transplanted into the human body along with BMSCs can cause varying degrees of damage to the human body, and there is a possibility of BMSCs transforming into malignant tumors[95]. These issues that need to be resolved point to a certain direction for future research, such as establishing standardized procedures for the extraction, identification, and cultivation of BMSCs; further clarifying the therapeutic mechanisms of BMSCs; and observing the safety of BMSCs applications. The choice of BMSCs application methods in PNI, such as direct transplantation, tissue engineering, and gene engineering, also requires further investigation. In conclusion, BMSCs transplantation offers broad prospects for PNI treatment, but significant theoretical and experimental research are needed before its clinical application can be fully developed and perfected.

ACKNOWLEDGEMENTS

We would like to thank all the professionals who contributed to the discussion and elaboration of this minireview.

FOOTNOTES

Author contributions: Zou XF, Zhang BZ, and Qian WW proposed the research topic and designed the research method; Zou XF organized and analyzed the literature and wrote and revised the manuscript; Zhang BZ and Qian WW provided critical revisions for the article; Cheng FM polished the language of the article. All authors have read and approved the final manuscript. Zhang BZ and Qian WW contributed equally to this paper and should be considered as co-corresponding authors.

Supported by CAMS Innovation Fund for Medical Sciences, No. 2022-I2M-C&T-B-034.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Wang JJ
L-Editor: Wang TQ
P-Editor: Yu HG

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World Journal of Stem Cells

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World J Stem Cells 2024 August 26; 16(8): 811-823

DOI: 10.4252/wjsc.v16.i8.811

ISSN 1948-0210 (online)

ORIGINAL ARTICLE

Basic Study

Microvesicles derived from mesenchymal stem cells inhibit acute respiratory distress syndrome-related pulmonary fibrosis in mouse partly through hepatocyte growth factor

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Specialty type: Cell and tissue engineering

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade B, Grade C, Grade C, Grade E

Novelty: Grade A, Grade B, Grade B, Grade C

Creativity or Innovation: Grade A, Grade B, Grade B, Grade D

Scientific Significance: Grade A, Grade A, Grade C, Grade C

P-Reviewer: Brody AR; Cui W; Li SC; Ricci AD

Received: March 19, 2024

Revised: July 4, 2024

Accepted: August 15, 2024

Published online: August 26, 2024

Processing time: 159 Days and 22.9 Hours

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Abstract

BACKGROUND

Pulmonary fibrosis is one of the main reasons for the high mortality rate among acute respiratory distress syndrome (ARDS) patients. Mesenchymal stromal cell-derived microvesicles (MSC-MVs) have been shown to exert antifibrotic effects in lung diseases.

AIM

To investigate the effects and mechanisms of MSC-MVs on pulmonary fibrosis in ARDS mouse models.

METHODS

MSC-MVs with low hepatocyte growth factor (HGF) expression (siHGF-MSC-MVs) were obtained *via* lentivirus transfection and used to establish the ARDS pulmonary fibrosis mouse model. Following intubation, respiratory mechanics-related indicators were measured *via* an experimental small animal lung function tester. Homing of MSC-MVs in lung tissues was investigated by near-infrared live imaging. Immunohistochemical, western blotting, ELISA and other methods were used to detect expression of pulmonary fibrosis-related proteins and to compare effects on pulmonary fibrosis and fibrosis-related indicators.

RESULTS
The MSC-MVs gradually migrated and homed to damaged lung tissues in the ARDS model mice. Treatment with MSC-MVs significantly reduced lung injury and pulmonary fibrosis scores. However, low expression of HGF (siHGF-MSC-MVs) significantly inhibited the effects of MSC-MVs ($P < 0.05$). Compared with the ARDS pulmonary fibrosis group, the MSC-MVs group exhibited suppressed expression of type I collagen antigen, type III collagen antigen, and the proteins transforming growth factor-β and α-smooth muscle actin, whereas the siHGF-MVs group exhibited significantly increased expression of these proteins. In addition, pulmonary compliance and the pressure of oxygen/oxygen inhalation ratio were significantly lower in the MSC-MVs group, and the effects of the MSC-MVs were significantly inhibited by low HGF expression (all $P < 0.05$).

CONCLUSION
MSC-MVs improved lung ventilation functions and inhibited pulmonary fibrosis in ARDS mice partly *via HGF* mRNA transfer.

Key Words: Microvesicles derived from mesenchymal stem cells; Acute respiratory distress syndrome; Pulmonary fibrosis; Hepatocyte growth factor; Mesenchymal stromal cells

Core Tip: Pulmonary fibrosis is strongly associated with poor outcomes in acute respiratory distress syndrome (ARDS) patients. Currently, there are no effective measures for treating pulmonary fibrosis. Microvesicles derived from mesenchymal stromal cells (MSC-MVs) have anti-pulmonary fibrosis effects; however, their specific effects and mechanisms in ARDS-related pulmonary fibrosis have not been fully established. This study revealed that MSC-MVs inhibited ARDS-related pulmonary fibrosis and fibrosis-related factors partly through hepatocyte growth factor, improved lung compliance and ventilation functions, and increased oxygenation indices. This study provides a new direction for the treatment of ARDS-related pulmonary fibrosis.

Citation: Chen QH, Zhang Y, Gu X, Yang PL, Yuan J, Yu LN, Chen JM. Microvesicles derived from mesenchymal stem cells inhibit acute respiratory distress syndrome-related pulmonary fibrosis in mouse partly through hepatocyte growth factor. *World J Stem Cells* 2024; 16(8): 811-823
URL: https://www.wjgnet.com/1948-0210/full/v16/i8/811.htm
DOI: https://dx.doi.org/10.4252/wjsc.v16.i8.811

INTRODUCTION

Acute respiratory distress syndrome (ARDS) is an important cause of respiratory failure in critically ill patients, with a mortality rate of 30%-40%[1,2]. The course of pulmonary fibrosis is also associated with the occurrence of ARDS, exhibiting lung injury, inflammation, and fibrosis. Pulmonary fibrosis is one of the main reasons for the high mortality rate among ARDS patients; however, there are no effective treatment options for pulmonary fibrosis[3-5]. There is an urgent need to identify safer and more effective pulmonary fibrosis treatment options to improve the outcomes of patients with ARDS.

Bone marrow mesenchymal stromal cells (MSCs) have antifibrotic effects; however, their clinical applications are limited by tumorigenicity and ethical issues[6-8]. MSC-derived microvesicles (MSC-MVs) are heterogeneous subcellular structures secreted by MSCs that play important roles in tissue and organ damage repair. Research has shown that MSC-MVs partially inhibit the apoptosis of renal tubular epithelial cells and alleviate renal ischemia-reperfusion injury by transferring miR-21[9]. Du *et al*[10] reported that MSC-MVs alleviate renal fibrosis after partial nephrectomy through M_2 macrophage polarization. MSC-MVs not only have strong tissue repair capabilities but also exert antitumor effects[11]. MSC-MVs derived from adipose tissue inhibit ovarian cancer cell proliferation, apoptosis, and necrosis through different signaling pathways[12]. MSC-MVs have been shown to exert antifibrotic effects in idiopathic pulmonary fibrosis mouse models[13]. Moreover, MSC-MVs have been shown to inhibit ARDS-related pulmonary fibrosis[14]; however, the specific effects and mechanisms have yet to be fully established.

Hepatocyte growth factor (HGF) is an important antifibrotic factor[15-17]. In an ARDS animal model, HGF significantly inhibited lung fibrous tissue proliferation in a dose-dependent manner, reduced hydroxyproline (PcNA) levels in lung tissues, and reversed pulmonary fibrosis[18]. Previously, we reported that MSC-MVSs inhibited pulmonary vascular endothelial fibrous tissue proliferation through HGF, thereby maintaining pulmonary vascular endothelial barrier functions[19]. Therefore, HGF may be an important factor in the inhibition of pulmonary fibrosis by MSC-MVs.

In this study, we investigated the effects of MSC-MVs on pulmonary fibrosis and lung functions in an ARDS model mice. Moreover, we investigated the significance of HGF in the MSC-MV-mediated inhibition of ARDS-related pulmonary fibrosis. Our findings elucidate the role and mechanisms of MSC-MVs in ARDS-induced pulmonary fibrosis

and provide insight into new treatment strategies.

MATERIALS AND METHODS

Culture of MSCs and gene interference

Mouse bone marrow-derived MSCs were purchased from Guangzhou Saiye Company (Guangzhou, China), cultured in a specialized medium (Guangzhou Saiye Company) and incubated at 37 °C in a 5% CO_2 atmosphere. The cell culture medium was changed every 3 days, and the cells were used in experiments after they had passed through 3-7 generations. On the basis of our previous method[19], we used lentivirus-mediated gene transfection technology to obtain MSCs with low expression of the *HGF* gene (siHGF-MSCs).

Isolation and identification of MSC-MVs

In accordance with our previous method[19], the MSC-MVs were separated from the MSCs by ultracentrifugation. The culture bottles containing 1 × 10^6 MSCs were incubated overnight with serum-free DMEM, followed by 0.5% fetal bovine serum. MSC-MVs were obtained by centrifugation at 2000 × *g* for 20 minutes, followed by centrifugation at 100000 × *g* and 4 °C for 1 hour. The pellets were washed with serum-free DMEM and ultracentrifuged to obtain the MSC-MVs, which were stored at -80 °C for further assays. In accordance with Teresa's method[20], flow cytometry was performed to detect the MSC-MVs markers [positive expression of surface molecules (CD29, CD44, α5 integrins, HLA-I antigen) and negative expression of CD34 and CD73 (Figure 1)]. The morphologies of the MSC-MVs were analyzed with electron microscopy and imaging.

Study ethics declarations

Animal care and interventions were performed in accordance with the National Institute of Health Guide for the Care and Use of Experimental Animals, and the animal protocol (YXYLL-2022-103) was approved by the Institutional Animal Care and Use Committees of the Yangzhou University School of Medicine. This study was carried out in compliance with the ARRIVE guidelines.

Animals and groups

Male C57BL/6 mice (aged 6-8 weeks and weighing 18-20 g) were purchased from Changzhou Cavens Experimental Animal Co., Ltd (Changzhou, China). The C57BL/6 mice were subjected to adaptive care for 2 days under controlled relative humidity (55% ± 10%) and temperature (25 ± 2 °C) conditions. Great care was taken to minimize their suffering. This protocol was prepared before the study. Briefly, 36 mice were randomly divided into six groups (6 mice in each group), as follows: Normal saline control group; ARDS group; ARDS pulmonary fibrosis group; MSC group; MSC-MVs group; and siHGF-MSC-MVs group. Lipopolysaccharide (LPS) (2 mg/kg) was instilled into the airways of all groups except the control group. The blank control group was injected with the same volume of normal saline. The ARDS mouse fibrosis model was generated *via* three LPS injections. Briefly, after weighing the 6-8-week-old C57BL/6 mice, they were intraperitoneally injected with 2% pentobarbital sodium (50 mg/kg) for anesthesia and fixed in the supine position; their airways were opened and LPS (1.5 mg/kg) was dropped into the airway. Twenty-four hours later, they were intraperitoneally injected with LPS (3 mg/kg). After 48 hours, the ARDS pulmonary fibrosis models were finally established by injecting 3 mg/kg LPS into the airways of the mice in the ARDS pulmonary fibrosis, MSC, MSC-MVs and siHGF-MSC groups. After 24 hours, MSCs (5 × 10^5/100 μL), MSC-MVs (100 μg/100 μL), or siHGF-MSC-MVs (100 μg/100 μL), respectively, were injected into the tail vein in the MSC group, MSC-MVs group and siHGF-MSC group.

MSC-MVs homing detection

The MSCs and MSC-MVs were dyed with a near-infrared dye (NIR815; Thermo Fisher Scientific, Waltham, MA, United States), as instructed by the manufacturer. After the ARDS pulmonary fibrosis models were established, they were injected with labeled MSCs or MSC-MVs *via* the tail vein. *In vivo* imaging was performed at 24 hours and 72 hours using the CRi Maestro Fluorescence Imaging System (Cambridge Research & Instrumentation, Inc, Woburn, MA, United States). The distributions of MSC-MVs and MSCs in the mouse bodies were determined at wavelengths ranging from 640-660 nm.

Assessment of pulmonary fibrosis and lung injury

Lung tissues were paraffin-embedded, sliced, stained with hematoxylin and eosin (referred to as HE), and evaluated for lung injury using the Smith scoring method to assess the pathological damage and repair status. The degree of pulmonary fibrosis was analyzed *via* Masson-stained sections. After the animals were euthanized, their left lungs were resected, weighed (wet weight), and placed in a 70 °C constant temperature oven for 72 hours to achieve a constant weight. After reweighing (dry weight), net weight of the two lungs was calculated as the wet/dry (W/D) ratio. Each group of mice was administered 20 g/L Evans blue through the tail vein. After the mice were sacrificed, their left atria were sliced open and uniformly injected with 10 mL of normal saline from the right ventricle to flush the lung tissues thoroughly. The lungs were resected, and the surface water of the lungs was removed. The wet weights were measured. The lung tissues were homogenized with 4 mL formamide and the Evans blue was extracted and centrifuged. The absorbance values of the supernatants were read at 620 nm, after which the amounts of extracted dye were determined using a standard curve. The permeability of the alveolar vascular barriers was determined by the amount of extracted dye

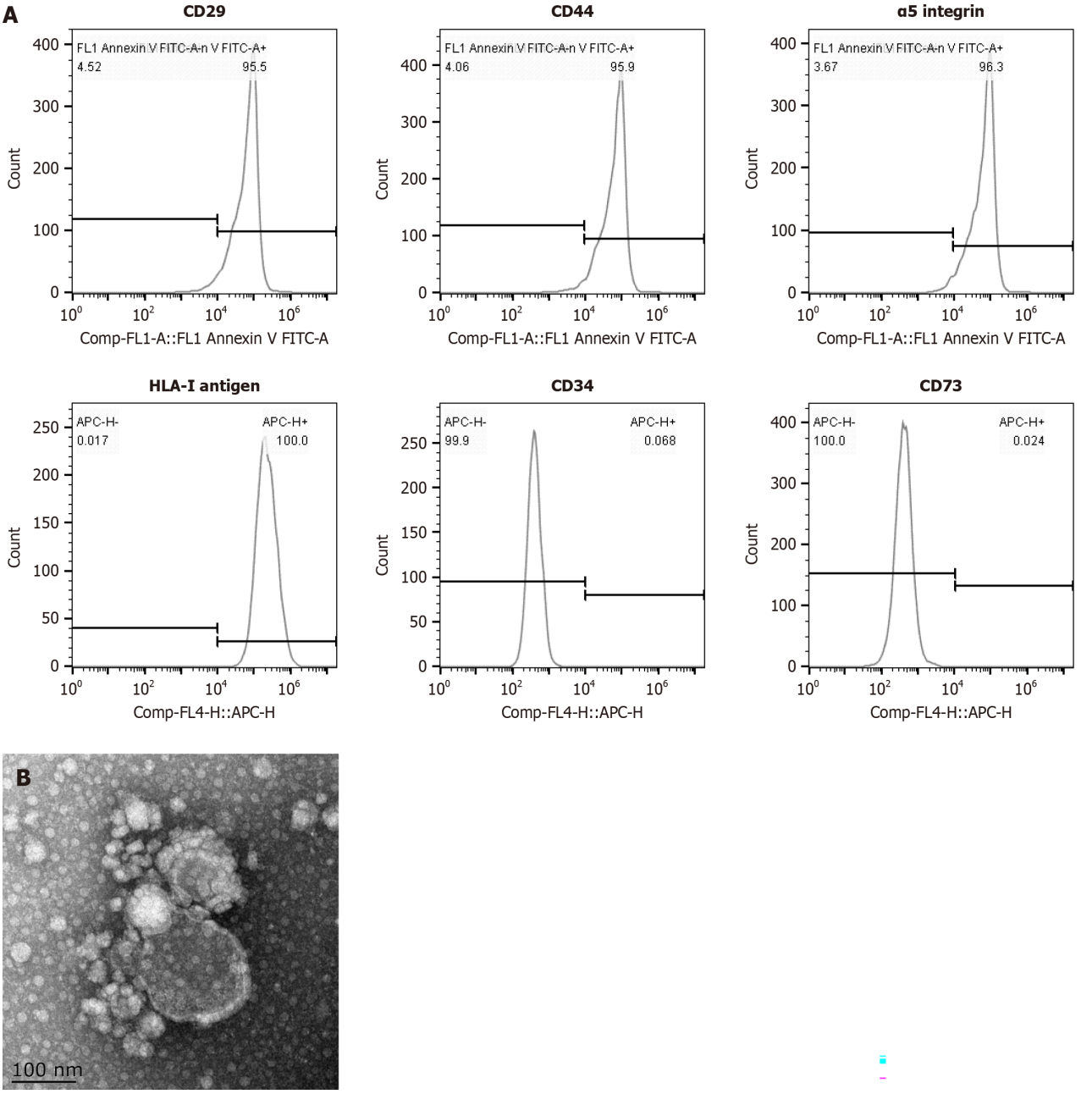


Figure 1 Detection of mesenchymal stromal cell-derived microvesicle markers by flow cytometry and transmission and scanning electron microscopy. A: The expression of surface molecules (CD29, CD44, α5 integrins and the HLA-I antigen) was positive, whereas the expression of CD34 and CD73 was negative; B: Transmission and scanning electron microscopy were performed on purified mesenchymal stromal cell-derived microvesicles to reveal their spheroid morphologies and confirm their sizes.

per unit of tissue.

Respiratory mechanics and functional detection

After the intervention in each group, the mice were intubated *via* the trachea, and respiratory mechanics-related indicators of the mice in each group were measured *via* an experimental small animal lung function tester. The total expiratory resistance, lung compliance, forced vital capacity (FVC) and forced expiratory volume at 0.1 seconds (Fev0.1) were measured in each group. After treatment, arterial blood samples were obtained *via* left ventricular puncture, and the arterial partial pressure of oxygen (PO_2) was measured using an automated blood gas analyzer. The oxygenation indices [PO_2/oxygen inhalation (FiO_2)] were calculated on the basis of the concentration of FiO_2.

Immunohistochemical detection of pulmonary fibrosis-related indicators

After the experiments, the mice were euthanized and their right lower lung tissues were excised and fixed in 10% neutral formaldehyde. The tissues were paraffin-embedded and subjected to immunohistochemistry to assess type I collagen, type III collagen, transforming growth factor-β (TGF-β) and α-smooth muscle actin (α-SMA) protein expression. Under low magnification, the cells were classified as strongly positive (brown), moderately positive (brown yellow), or weakly

positive (light yellow) on the basis of the degree of positive staining. The protein expression levels of type I and III collagens and TGF-β and α-SMA were quantified *via* the use of an immunohistochemical average absorbance tester.

Western blot analysis of pulmonary fibrosis-related indicators

The mice were euthanized, and their right lower lungs were excised for protein extraction. The proteins were incubated with antibodies (against HGF, α-SMA, type I collagen, type III collagen, and GADPH) and secondary antibodies. Finally, the target protein bands were visualized with a chemiluminescence imager for quantitative image analysis.

Determination of hydroxyproline and fibrosis-related inflammatory factor levels

Lung tissues were obtained from the middle lobe of the right lung to measure hydroxyproline levels using an alkaline hydrolysis kit. Briefly, the lungs were mixed with 2 mL of buffered saline (PBS) supplemented with 1 mL of 6 mol/L HCl for hydrolysis. Then, 25 μL of normal saline was added to 1 mL of chloramine (1.4%). After 20 minutes at room temperature, 1 mL of Erlich solution was added to the mixture and incubated at 65 °C for 15 minutes. The concentrations of hydroxyproline in the lung tissues were measured *via* a spectrophotometer. Mouse serum samples were removed from the -80 °C freezer for analysis of serum TGF-β, HGF, type III procollagen N-terminal aminopeptide (PIIIP), interleukin (IL)-1β and IL-10 expression *via* ELISA.

Statistical analysis

The data are presented as mean ± standard deviation. Statistical analyses were performed using the SPSS 21.0 software package (IBM Corp., Armonk, NY, United States). For group comparisons, one-way analysis of variance (*i.e.* ANOVA) followed by Bonferroni's post hoc correction was used. Statistical significance was considered at $P < 0.05$.

RESULTS

MSC-MVs homed to the lungs of ARDS pulmonary fibrosis model mice

Near-infrared live imaging was performed to detect homing in the ARDS model mice after injection of MSCs or MSC-MVs. MSCs and MSC-MVs gradually migrated and homed to damaged lung tissues after tail vein injection. After 24 hours, the MSCs and MSC-MVs initially migrated into the damaged lung tissues. After 72 hours, the MSC-MVs distinctly homed to the lung tissues, with significantly increased fluorescence intensity (Figure 2). In summary, the MSC-MVs that were injected into the tail veins of ARDS pulmonary fibrosis model mice were mainly associated with injured lung tissues.

Effects of MSC-MVs on lung injury and pulmonary fibrosis in ARDS pulmonary fibrosis model mice

We compared the effects of MSC-MVs on lung injury and fibrosis in ARDS pulmonary fibrosis model mice *via* HE and Masson staining, respectively. Compared with those in the control group, the ARDS group showed significantly greater lung injury and pulmonary fibrosis scores. After the injection of the MSC-MVs, there were significant reductions in lung injury and pulmonary fibrosis scores. However, suppression of HGF expression markedly inhibited the above effects of MSC-MVs (Figure 3). These findings suggest that MSC-MVs inhibit ARDS-related pulmonary fibrosis partly through HGFs.

Effects of MSC-MVs on pulmonary fibrosis-related indicators in ARDS pulmonary fibrosis model mice

We further performed immunohistochemistry and western blot assays to investigate the effects of MSC-MVs on expression of fibrosis-related protein markers in the ARDS pulmonary fibrosis model mice. Compared with the ARDS pulmonary fibrosis group, the MSC-MVs group exhibited suppressed expression of type I collagen antigen, type III collagen antigen, and the proteins TGF-β and α-SMA, whereas the siHGF-MVs group exhibited significantly elevated expression of those proteins (Figures 4 and 5). Thus, MSC-MVs inhibit the expression of pulmonary fibrosis-related proteins in ARDS pulmonary fibrosis model mice partly through HGFs.

Effects of MSC-MVs on hydroxyproline in ARDS pulmonary fibrosis model mice

Hydroxyproline levels in lung tissues are correlated with pulmonary fibrosis severity. In this study, we found that the hydroxyproline levels in the pulmonary fibrosis group were significantly greater than those in the control group; those in the MSC-MVs treatment group were significantly lower than those in the pulmonary fibrosis group; whereas those in the siHGF-MSC-MVs group were significantly greater than those in the MSC-MVs group. Therefore, MSC-MVs suppressed hydroxyproline levels in the lung tissues of ARDS pulmonary fibrosis model mice partly through HGFs (Figure 6A).

Effects of MSC-MVs on pulmonary vascular permeability and inflammatory indicators in ARDS pulmonary fibrosis model mice

Lung vascular permeability was assessed by measuring W/D ratios and Evans blue leakage. The lung W/D ratio and Evans blue leakage in the ARDS pulmonary fibrosis group were greater than those in the control group and were significantly lower after MSC-MVs injection. However, the above effects of MSC-MVs were significantly inhibited by HGF suppression (Figure 6B and C). These findings indicate that MSC-MVs inhibited pulmonary vascular permeability in ARDS pulmonary fibrosis partly through HGFs. ELISA was subsequently performed to detect the expression of inflam-

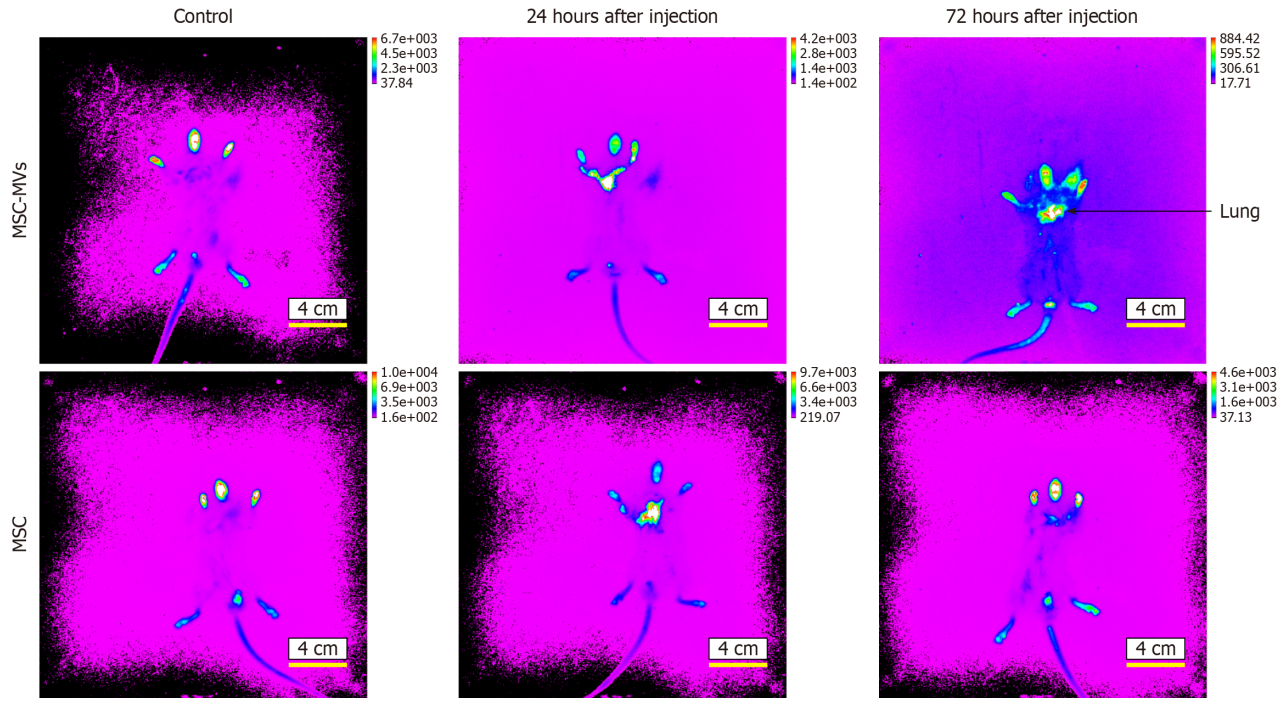


Figure 2 Microvesicles derived from mesenchymal stromal cells homing detection by the near-infrared dye NIR815. At 24 hours after injection with mesenchymal stromal cell-derived microvesicles (MSC-MVs), the fluorescence appears mainly yellow in lung, and at 72 hours after injection, the fluorescence appears mainly yellow and red with significantly increased fluorescence intensity.

matory factors related to pulmonary fibrosis. Compared with those in the control group, the expression levels of TGF-β, PIIIP, IL-1β, and IL-10 in the pulmonary fibrosis group were significantly greater, whereas the HGF levels were lower. However, TGF-β, PIIIP, IL-1β, and IL-10 expression in the MSC-MVs group were significantly lower while HGF levels were higher than those of the pulmonary fibrosis group. The above effects of MSC-MVs were significantly inhibited by the suppression of HGF expression (Figure 6D). Therefore, MSC-MVs inhibited the expression of fibrosis-related inflammatory factors through HGFs.

Effects of MSC-MVs on respiratory mechanics and lung functions in ARDS pulmonary fibrosis model mice

Pulmonary compliance, Fev0.1, FVC and PO_2/FiO_2 in the ARDS pulmonary fibrosis model group were significantly lower than those in the control group, and MSC-MVs significantly improved these indicators after tail vein injection. However, the above effects of MSC-MVs were significantly inhibited by HGF suppression. There were no significant differences in respiratory resistance or the Fev0.1/FVC among the groups (Figure 7). Therefore, MSC-MVs improved lung ventilation functions and oxygenation indices in ARDS pulmonary fibrosis mouse models partly through HGFs.

DISCUSSION

Pulmonary fibrosis is strongly associated with poor prognostic outcomes in ARDS patients[21-23]. Currently, there are no effective measures for treating pulmonary fibrosis[24,25]. MSC-MVs have anti-pulmonary fibrosis effects; however, their specific effects and mechanisms on ARDS-related pulmonary fibrosis have not been fully established[26]. We found that after MSC-MVs were injected into the tail vein of ARDS pulmonary fibrosis model mice, they first migrated and homed to damaged lung tissues, inhibited the expression of ARDS pulmonary fibrosis-related factors and fibrosis-related factors partly through HGFs, improved lung compliance and ventilation functions, and increased oxygenation indices. The findings of this study provide a new direction for the treatment of ARDS-related pulmonary fibrosis.

Pulmonary fibrosis develops in the early stages of ARDS and occurs throughout the entire process of ARDS[27]. We established ARDS model mice by instilling LPS into their airways. On the 7th day of ARDS model establishment, the lung fibrosis score and fibrosis-related protein expression in the mice, including type I collagen antigen, type III collagen antigen, TGF-β and α-SMA, were significantly increased; therefore, significant pulmonary fibrosis occurred on the 7th day of ARDS. Previous studies on pulmonary fibrosis have focused mostly on bleomycin-induced pulmonary fibrosis models [28,29]. On the basis of a previous study[30], we successfully established ARDS pulmonary fibrosis models *via* three LPS injections. After pulmonary fibrosis was induced, pulmonary fibrosis scores and the expression of type I collagen antigen, type III collagen antigen, TGF-β and α-SMA significantly increased, whereas lung compliance Fev0.1, FVC and ventilation functions significantly decreased, which was consistent with the pathological and pathophysiological changes in pulmonary fibrosis.

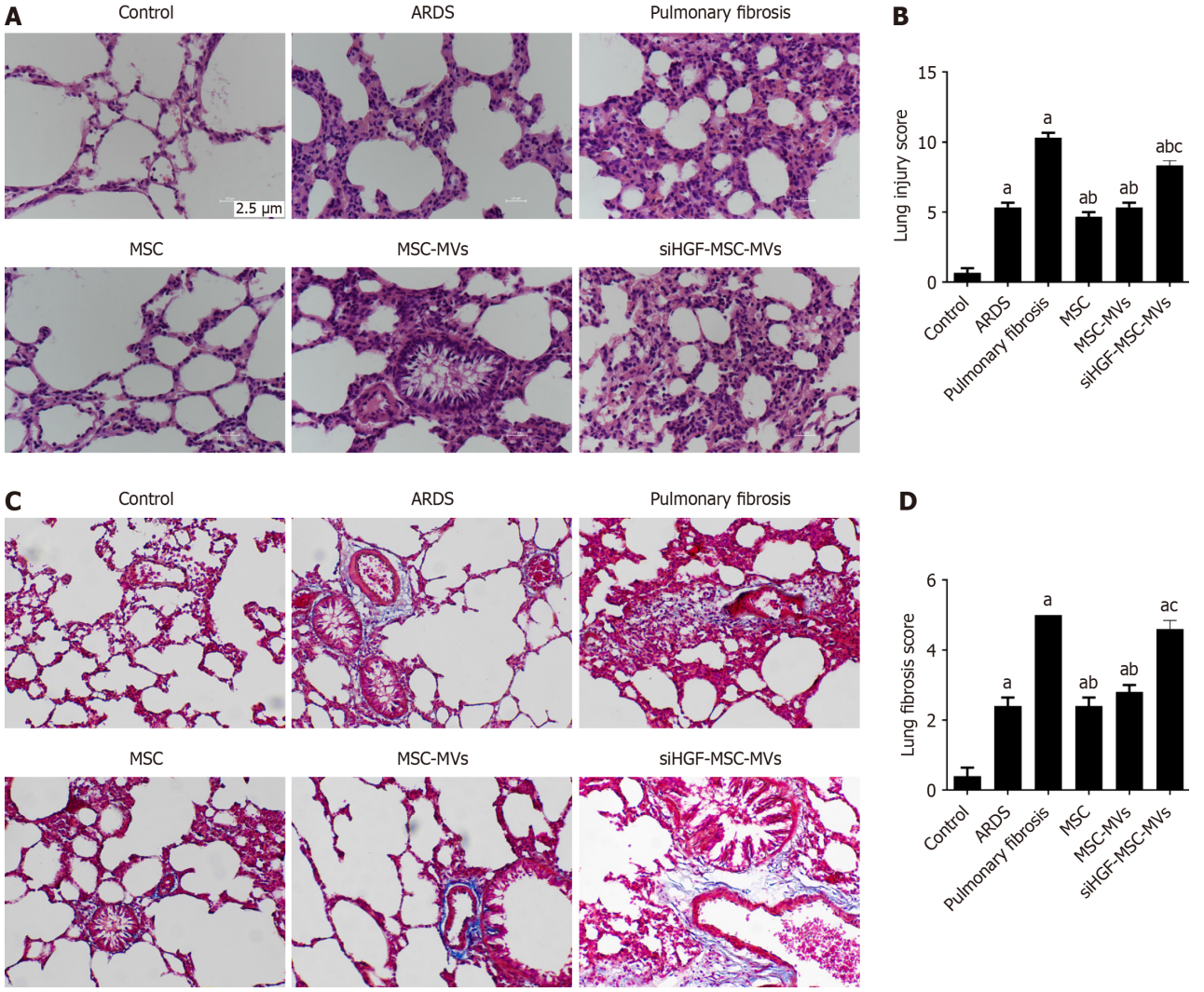
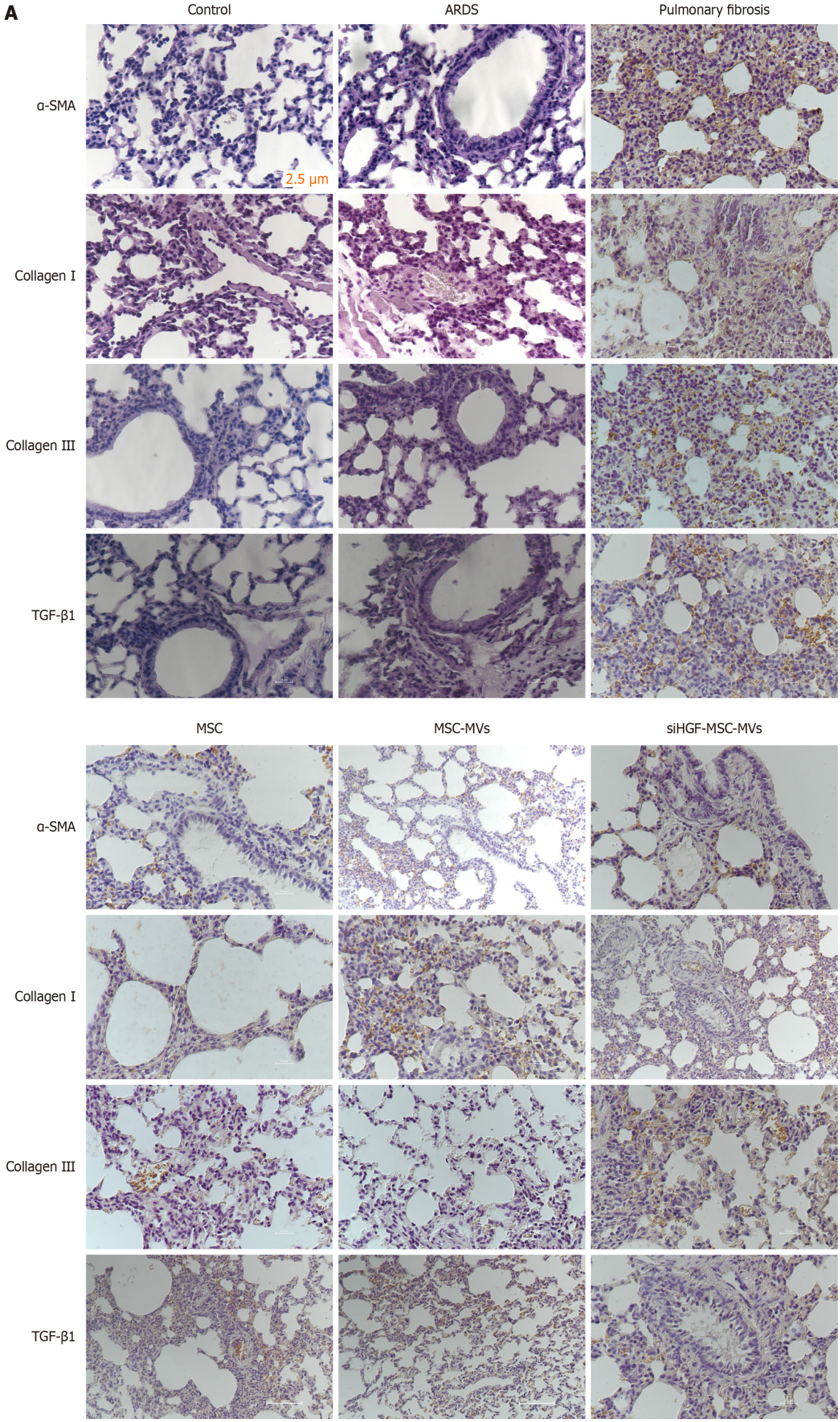


Figure 3 Immunohistochemical detection of the effects of mesenchymal stromal cell-derived microvesicles on pulmonary and fibrosis-related indicators in acute respiratory distress syndrome pulmonary fibrosis mouse models. A: Hematoxylin and eosin staining of lung tissues from each group; B: Lung injury scores of pathological sections from each group; C: Masson staining of lung tissues from each group; D: Lung fibrosis scores of pathological sections from each group. $^{a}P < 0.05$, *vs* the control group; $^{b}P < 0.05$, *vs* the pulmonary fibrosis group; $^{c}P < 0.05$, *vs* the mesenchymal stromal cell-derived microvesicles (MSC-MVs) group ($n = 6$). Control, acute respiratory distress syndrome (ARDS), pulmonary fibrosis, MSC, MSC-MVs and low hepatocyte growth factor (HGF)-MSC-MVs represent the control group, ARDS group, pulmonary fibrosis group, MSC group, MSC-MVs group and low HGF-MSC-MVs group, respectively.

MSCs have good anti-pulmonary fibrosis effects[31], but their applications are limited by their tumorigenicity and ethical considerations[32]. MSC-MVs can avoid these challenges and are convenient for storage, which is conducive to large-scale production[33]. MSC-MVs exhibited good anti-pulmonary fibrosis effects in bleomycin-induced pulmonary fibrosis mouse models; however, their specific effects and the mechanisms involved in alleviating ARDS-related pulmonary fibrosis have yet to be established[34,35]. In this study, we found that most of the MSC-MVs and MSCs homed to the injured lung tissues and significantly inhibited the expression of type I collagen antigen, type III collagen antigen, TGF-β and α-SMA in the ARDS pulmonary fibrosis model mice, with reduced pulmonary fibrosis scores, improved lung compliance, Fev0.1 and FVC, and increased oxygenation indices. Like MSCs, MSC-MVs homed toward the site of lung injury, and there were no significant differences in the inhibition of pulmonary fibrosis between the two. Therefore, MSC-MVs can replace MSCs for anti-ARDS pulmonary fibrosis treatment.

HGF is a cell repair factor that promotes epithelial cell proliferation and collagen degradation and inhibits epithelial cell apoptosis. It is one of the few growth factors that can inhibit fibrosis[36-38]. MSC-MVs contain abundant mRNAs, such as *HGF* mRNA[39]; RNA transfer is an important way by which MSC-MVs exert damage-repair effects[40]. Previously, we reported that MSC-MVs alleviate pulmonary vascular endothelial injury and reduce pulmonary vascular endothelial permeability through *HGF* mRNA transfer[19]. In this study, lentivirus transfection was used to suppress *HGF* mRNA expression in MSC-MVs. The ability of MSC-MVs to inhibit ARDS-related pulmonary fibrosis score, affect the expression of type I collagen antigen, type III collagen antigen, TGF-β and α-SMA, and improve lung compliance and lung ventilation functions such as Fev0.1, FVC, and oxygenation indices, which were significantly inhibited after suppression of *HGF* mRNA in MSC-MVs. These findings imply that *HGF* mRNA transfer is one of the main mechanisms through which MSC-MVs exert their anti-ARDS pulmonary fibrosis effects. During ARDS-induced pulmonary fibrosis, the MSC-MVs administered through the tail vein first homed to the injured lung, after which the HGF transcribed from


A
Control
ARDS
Pulmonary fibrosis
α-SMA
2.5 μm
Collagen I
Collagen III
TGF-β1
MSC
MSC-MVs
siHGF-MSC-MVs
α-SMA
Collagen I
Collagen III
TGF-β1

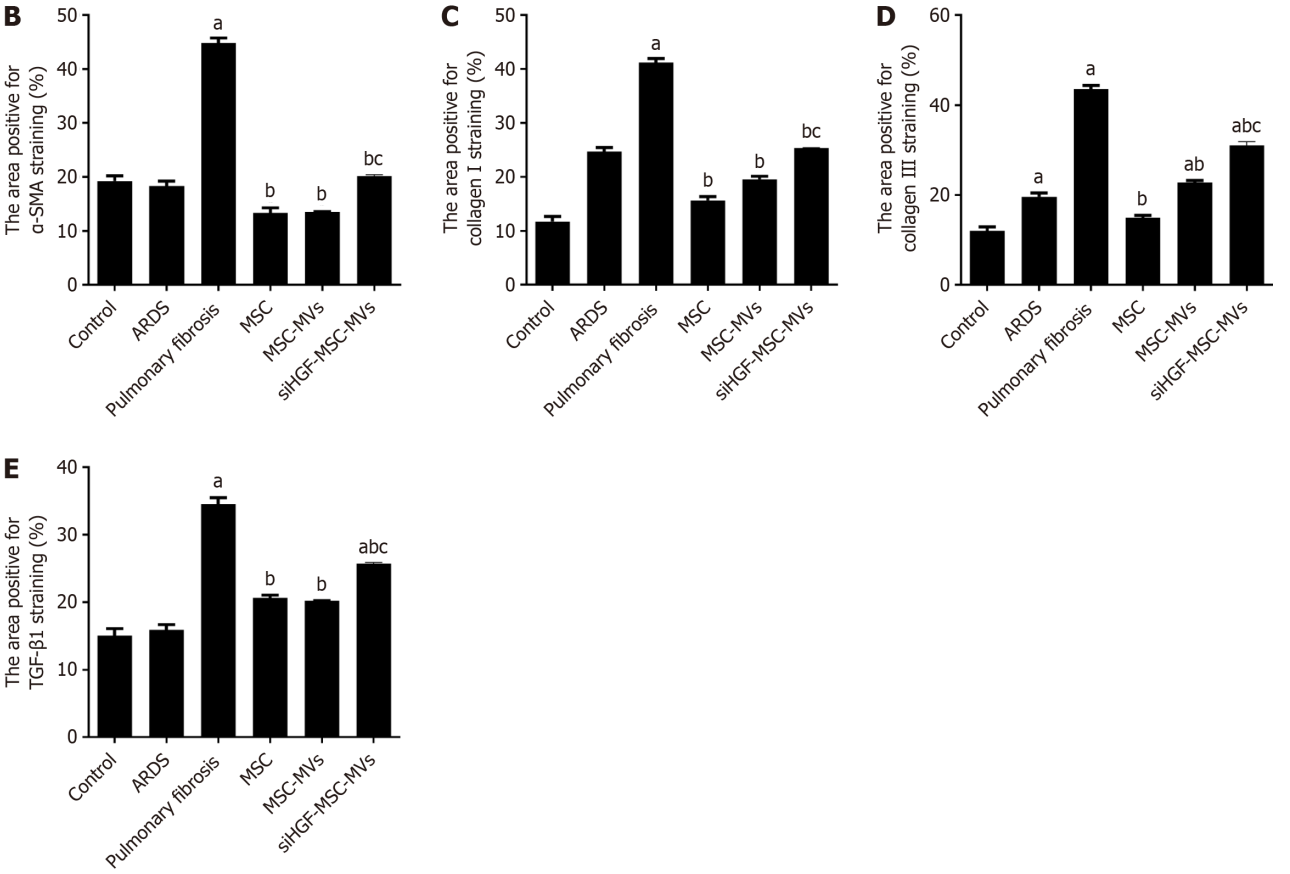


Figure 4 Effects of mesenchymal stromal cell-derived microvesicles on acute respiratory distress syndrome-related pulmonary fibrosis and lung injury. A: Immunohistochemical images of pathological sections of lung tissues from each group; B: α-smooth muscle actin (α-SMA) protein expression; C: Type I collagen protein expression; D: Type III collagen protein expression; E: Transforming growth factor-β1 (TGF-β1) protein expression. $^{a}P < 0.05$, *vs* the control group; $^{b}P < 0.05$ *vs* the pulmonary fibrosis group; $^{c}P < 0.05$ *vs* the mesenchymal stromal cell-derived microvesicles (MSC-MVs) group ($n = 6$). Control, acute respiratory distress syndrome (ARDS), pulmonary fibrosis, MSC, MSC-MVs and low hepatocyte growth factor (HGF)-MSC-MVs represent the control group, ARDS group, pulmonary fibrosis group, MSC group, MSC-MVs group and low HGF-MSC-MVs group, respectively.

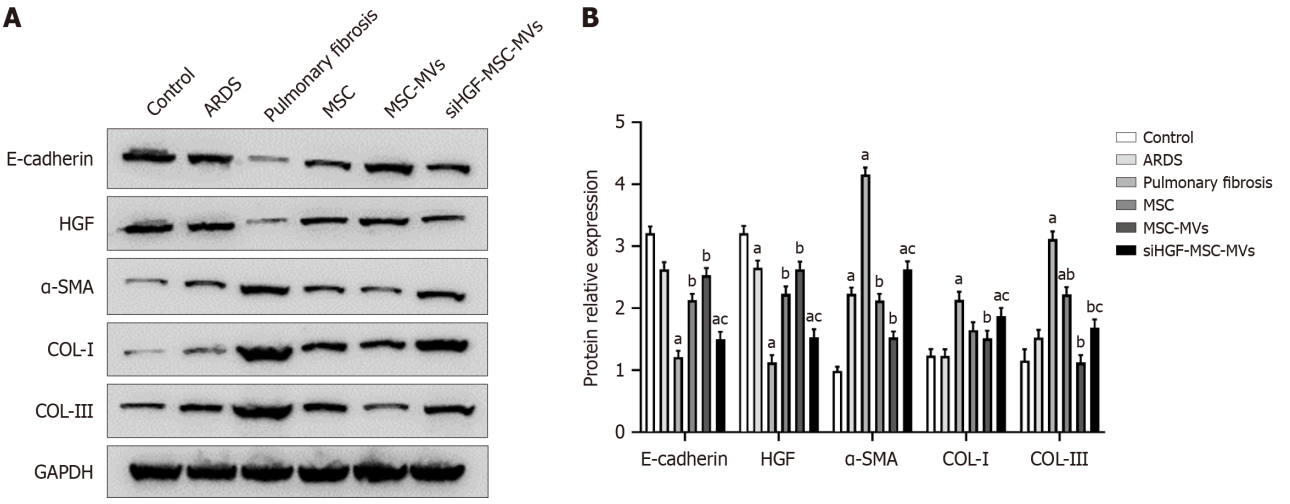


Figure 5 Western blot analysis of the effects of mesenchymal stromal cell-derived microvesicles on pulmonary fibrosis-related proteins in acute respiratory distress syndrome pulmonary fibrosis model mice. A: Western blot bands of fibrosis-related proteins in the lung tissues of each group; B: Relative expression of fibrosis-related proteins. $^{a}P < 0.05$, *vs* the control group; $^{b}P < 0.05$, *vs* the pulmonary fibrosis group; $^{c}P < 0.05$, *vs* the mesenchymal stromal cell-derived microvesicles (MSC-MVs) group ($n = 6$). Control, acute respiratory distress syndrome (ARDS), pulmonary fibrosis, MSC, MSC-MVs and low hepatocyte growth factor (HGF)-MSC-MVs represent the control group, ARDS group, pulmonary fibrosis group, MSC group, MSC-MVs group and low HGF-MSC-MVs group, respectively. α-SMA: α-smooth muscle actin; COL: Collagen.

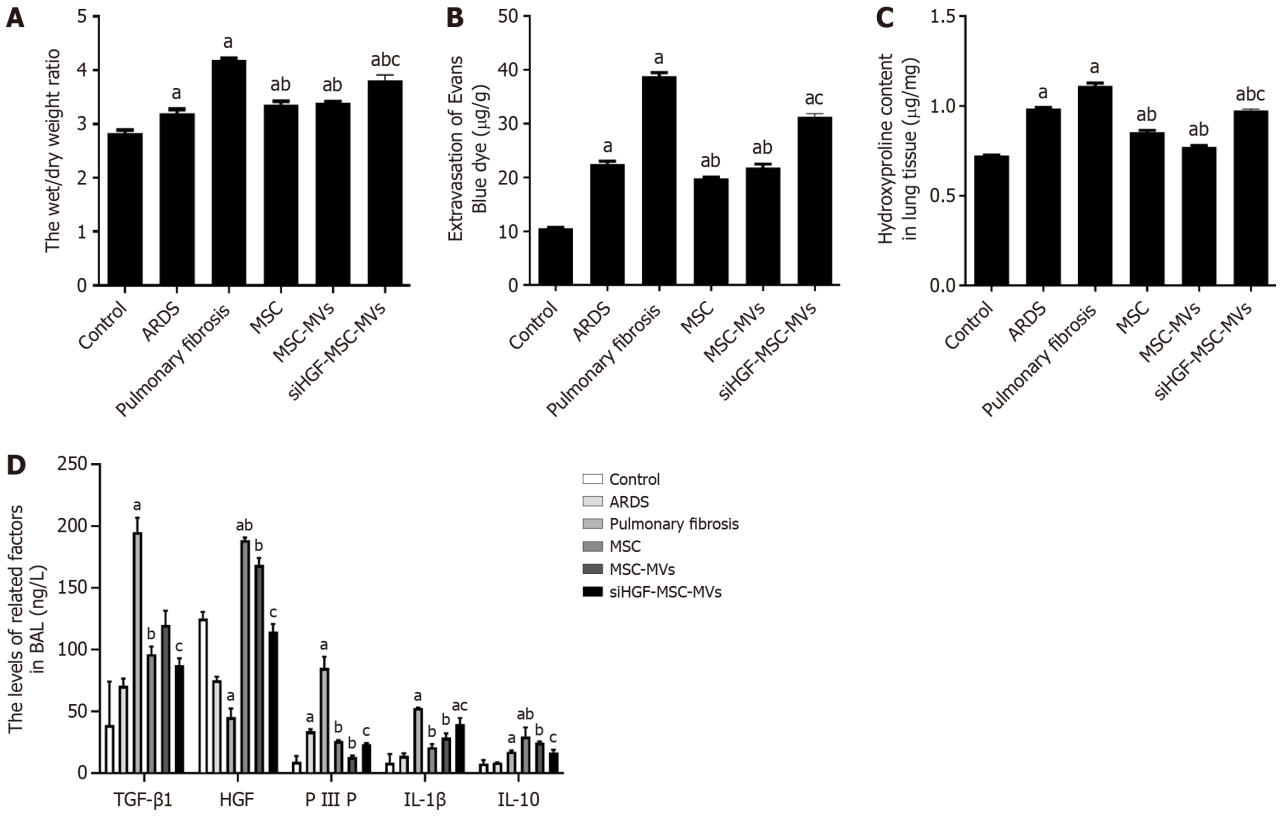


Figure 6 Effects of mesenchymal stromal cell-derived microvesicles on pulmonary vascular endothelial permeability and related proteins in acute respiratory distress syndrome pulmonary fibrosis mouse models. A: Lung wet/dry weight ratios for each group; B: Evans blue detection of vascular endothelial permeability in each group; C: Hydroxyproline levels in each group; D: Expression of inflammatory factors in each group detected by ELISA. $^{a}P < 0.05$, *vs* the control group; $^{b}P < 0.05$, *vs* the pulmonary fibrosis group; $^{c}P < 0.05$, *vs* the mesenchymal stromal cell-derived microvesicles (MSC-MVs) group ($n = 6$). Control, acute respiratory distress syndrome (ARDS), pulmonary fibrosis, MSC, MSC-MVs and low hepatocyte growth factor (HGF)-MSC-MVs represent the control group, ARDS group, pulmonary fibrosis group, MSC group, MSC-MVs group and low HGF-MSC-MVs group, respectively. IL: Interleukin; PIIIP: Type III procollagen N-terminal aminopeptide; TGF-β1: Transforming growth factor-β1.

the mRNA in the MSC-MVs bound to the c-Met receptors in pulmonary vascular epithelial cells, thereby exerting anti-pulmonary fibrosis effects. Therefore, *HGF* mRNA transfer is one of the mechanisms by which MSC-MVs resist ARDS-induced pulmonary fibrosis.

MSC-MVs have advantages over MSCs, such as easy mass production, easy storage, no immunogenicity, and no ethical issues[41]. Therefore, MSC-MVs have broader therapeutic prospects than do MSCs in the treatment of ARDS-related pulmonary fibrosis. However, the treatment of pulmonary fibrosis caused by ARDS with MSC-MVs is still in the preliminary research stage, and the following problems remain: (1) There is still a lack of standard methods for the extraction, purification, and identification of MSC-MVs; (2) The timing, dosage, and administration methods of MSC-MV treatment need to be clarified; and (3) The mechanism by which MSC-MVs combat pulmonary fibrosis is not very clear. Future research should focus on how to obtain MSC-MVs in a standardized manner, clarify the timing, dosage and administration of MSC-MVs treatment, explore the mechanism of MSC-MVs treatment for pulmonary fibrosis, and carry out further clinical research[42,43].

This study has several limitations. First, researchers have not determined whether the same or better effects can be obtained by administering MSC-MVs intratracheally instead of intravenously. Second, MSC-MVs were administered 24 hours after the establishment of the pulmonary fibrosis model. However, the antifibrotic effects of MSC-MVs administered at different times after pulmonary fibrosis may vary. In addition, finally, only a single dose of MSC-MVs was administered, and two or more doses after the development of pulmonary fibrosis may have improved the anti-fibrosis effects. These limitations should be addressed in future studies.

CONCLUSION

Intravenously administered MSC-MVs homed to the site of lung injury and inhibited ARDS-related pulmonary fibrosis. In addition, *HGF* mRNA transfer is an important mechanism by which MSC-MVs inhibit ARDS-related pulmonary fibrosis. This study provides insight into new treatment approaches for ARDS-related pulmonary fibrosis.

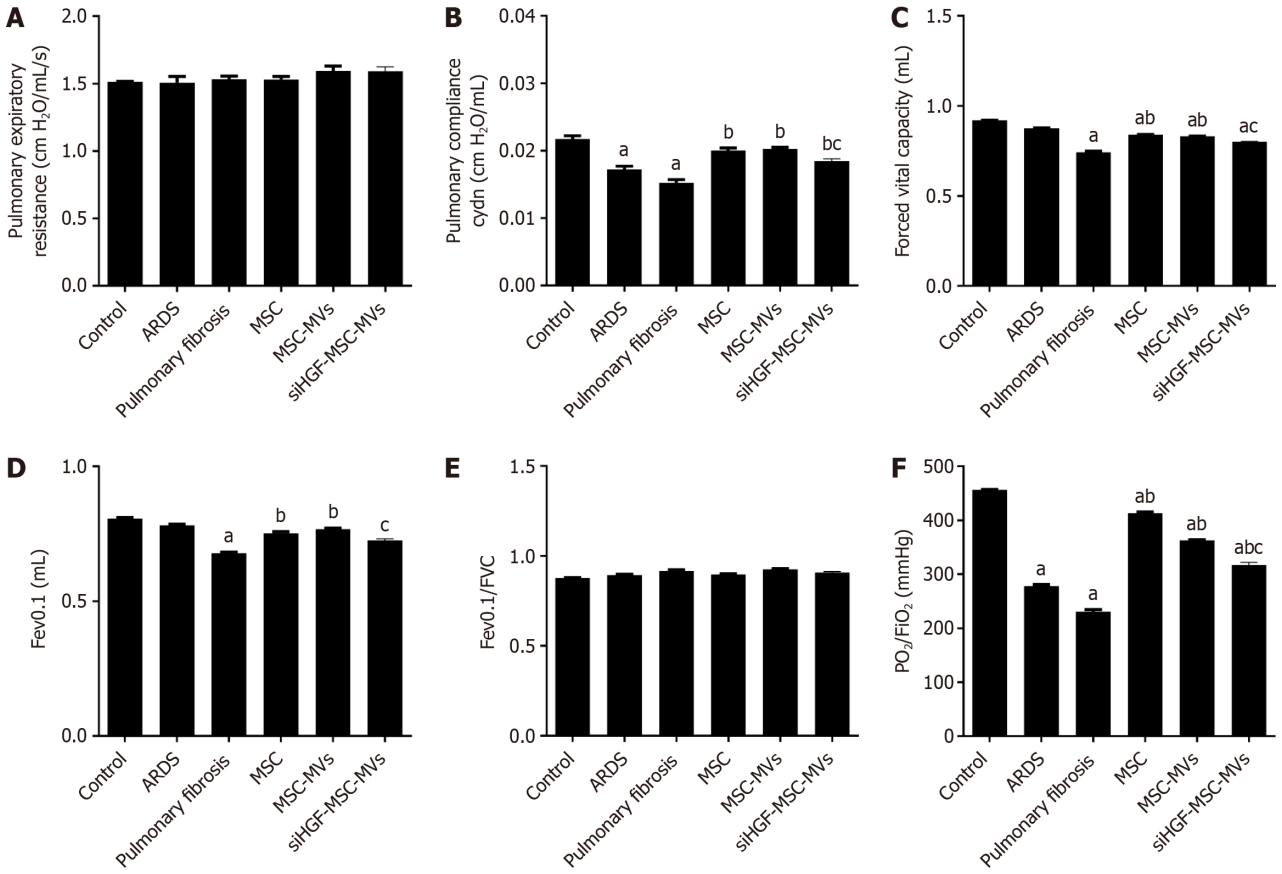


Figure 7 Effects of mesenchymal stromal cell-derived microvesicles on respiratory mechanics and lung functions in acute respiratory distress syndrome pulmonary fibrosis mouse models. A: Pulmonary expiratory resistance; B: Pulmonary compliance; C: Forced expiratory volume in 0.1 seconds (Fev0.1); D: Forced vital capacity (FVC), E: Fev0.1/FVC; F: Pressure of oxygen (PO_2)/oxygen inhalation (FiO_2); G: Transmission and scanning electron microscopy were performed on purified mesenchymal stromal cell-derived microvesicles (MSC-MVs) to reveal their spheroid morphologies and confirm their sizes. $^aP < 0.05$, *vs* the control group; $^bP < 0.05$, *vs* the pulmonary fibrosis group; $^cP < 0.05$, *vs* the MSC-MVs group ($n = 6$). Control, acute respiratory distress syndrome (ARDS), pulmonary fibrosis, MSC, MSC-MVs and low hepatocyte growth factor (HGF)-MSC-MVs represent the control group, ARDS group, pulmonary fibrosis group, MSC group, MSC-MVs group and low HGF-MSC-MVs group, respectively.

FOOTNOTES

Author contributions: Chen QH designed the study and wrote the manuscript; Zhang Y participated in the cell culture and molecular biology experiments; Gu X performed the animal experiments and data acquisition; Yang PL contributed to the data acquisition and statistical analysis; Yuan J helped with the animal experiments and data acquisition; Yu LN performed the molecular biology experiments and statistical analyses; Chen JM contributed to research quality control and manuscript revision; All authors approved the final manuscript.

Supported by Research Project of Jiangsu Provincial Health Commission, No. Z2022008; and Research Project of Yangzhou Health Commission, No. 2023-2-27.

Institutional animal care and use committee statement: Animal care and interventions were performed in accordance with the National Institute of Health Guide for the Care and Use of Experimental Animals, and the animal protocol (YXYLL-2022-103) was approved by the Institutional Animal Care and Use Committees of the Yangzhou University School of Medicine.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

Data sharing statement: Technical appendix, statistical code, and dataset available upon reasonable request from the corresponding author at 18051063567@163.com.

ARRIVE guidelines statement: The authors have read the ARRIVE guidelines, and the manuscript was prepared and revised according to the ARRIVE guidelines.

Country of origin: China

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S-Editor: Wang JJ
L-Editor: Filipodia
P-Editor: Zheng XM

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World Journal of Stem Cells

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World J Stem Cells 2024 August 26; 16(8): 824-826

DOI: 10.4252/wjsc.v16.i8.824

ISSN 1948-0210 (online)

LETTER TO THE EDITOR

Potential plausible role of Wharton's jelly mesenchymal stem cells for diabetic bone regeneration

Sheng Zheng, Guan-Yu Hu, Jun-Hua Li, Yi-Kai Li

Specialty type: Cell and tissue engineering

Provenance and peer review: Unsolicited article; Externally peer reviewed.

Peer-review model: Single blind

Peer-review report's classification

Scientific Quality: Grade A, Grade A

Novelty: Grade A, Grade B

Creativity or Innovation: Grade A, Grade A

Scientific Significance: Grade A, Grade A

P-Reviewer: Mizukami T; Zheng Y

Received: August 1, 2024

Revised: August 11, 2024

Accepted: August 22, 2024

Published online: August 26, 2024

Processing time: 24 Days and 22.8 Hours

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Abstract

This letter addresses the review titled "Wharton's jelly mesenchymal stem cells: Future regenerative medicine for clinical applications in mitigation of radiation injury". The review highlights the regenerative potential of Wharton's jelly mesenchymal stem cells (WJ-MSCs) and describes why WJ-MSCs will become one of the most probable stem cells for future regenerative medicine. The potential plausible role of WJ-MSCs for diabetic bone regeneration should be noticeable, which will provide a new strategy for improving bone regeneration under diabetic conditions.

Key Words: Wharton's jelly mesenchymal stem cells; Vascular endothelial growth factor; Osteogenesis; Angiogenesis; Diabetic bone regeneration

Core Tip: Both osteogenesis and angiogenesis are closely related to bone regeneration. Diabetes mellitus normally impairs angiogenesis, which leads to diabetic bone regeneration deficiency. Wharton's jelly mesenchymal stem cells not only possess the ability to differentiation into osteoblasts, but also produce a crucial secretory factor (vascular endothelial growth factor) to promote angiogenesis. Thus, Wharton's jelly mesenchymal stem cell is expected to exert more vital role in improving diabetic bone regeneration.

Citation: Zheng S, Hu GY, Li JH, Li YK. Potential plausible role of Wharton's jelly mesenchymal stem cells for diabetic bone regeneration. *World J Stem Cells* 2024; 16(8): 824-826
URL: https://www.wjgnet.com/1948-0210/full/v16/i8/824.htm
DOI: https://dx.doi.org/10.4252/wjsc.v16.i8.824

TO THE EDITOR

Recently, we read an insightful review entitled, "Wharton's jelly mesenchymal stem cells: Future regenerative medicine for clinical applications in mitigation of radiation injury" by Sharma and Maurya[1], published in the *World Journal of Stem Cells*. This review highlights the regenerative potential of Wharton's jelly mesenchymal stem cells (WJ-MSCs) and explains the reason that WJ-MSCs are among the most promising stem cells for future regenerative medicine. This letter is a pivotal addition to the role of WJ-MSCs in regenerative medicine, highlighting the potential of WJ-MSCs to improve diabetic bone regeneration.

MSCs have great potential in regenerative medicine because of their ability for self-renewal and multilineage differentiation. Recently, increasing evidence has indicated that MSCs produce secretory factors that are crucial in regenerative medicine[2]. WJ-MSCs of the umbilical cord produce abundant secretory factors, including vascular endothelial growth factor (VEGF)[3]. VEGF is crucial in promoting angiogenesis. Therefore, the important role of VEGF is highly valued in tissue regeneration.

Bone is a highly vascularized tissue[4]. Osteogenesis and angiogenesis are closely associated with bone regeneration [5]. Therefore, angiogenesis should be studied during bone regeneration. Diabetes mellitus impairs angiogenesis, leading to deficient diabetic bone regeneration[6]. WJ-MSCs not only possess the ability to differentiate into osteoblasts but also produce a crucial secretory factor (VEGF) to promote angiogenesis. Additionally, WJ-MSCs have several advantages, such as no ethical concerns, shorter population doubling time, and broad differentiation potential, which make them superior to other sources of MSCs[7]. Therefore, WJ-MSCs may be vital in improving diabetic bone regeneration.

Knowingly, no previous studies have discussed the use of WJ-MSCs therapy for diabetic bone regeneration. A previous study reported that special AT-rich sequence-binding protein 1 promotes the osteogenic differentiation of diabetic rat bone marrow-derived MSCs through mitogen-activated protein kinases signaling activation[8]; however, no study has focused on the role of WJ-MSCs in improving diabetic bone regeneration. This letter provides a new strategy for improving bone regeneration under diabetic conditions.

FOOTNOTES

Author contributions: Zheng S and Li YK wrote the original draft; Zheng S, Hu GY, Li JH, and Li YK contributed to conceptualization, writing, reviewing and editing. All authors have read and approved the final version of the manuscript.

Supported by China Postdoctoral Science Foundation, No. 2024M751344; and the Postdoctoral Fellowship Program of China Postdoctoral Science Foundation, No. GZC20231088.

Conflict-of-interest statement: All the authors report no relevant conflicts of interest for this article.

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S-Editor: Wang JJ
L-Editor: A
P-Editor: Zhang XD

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