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REVIEW

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# Progression of mesenchymal stem cell regulation on imbalanced microenvironment after spinal cord injury

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## Abstract

Spinal cord injury (SCI) results in significant neural damage and inhibition of axonal regeneration due to an imbalanced microenvironment. Extensive evidence supports the efficacy of mesenchymal stem cell (MSC) transplantation as a therapeutic approach for SCI. This review aims to present an overview of MSC regulation on the imbalanced microenvironment following SCI, specifically focusing on inflammation, neurotrophy and axonal regeneration. The application, limitations and future prospects of MSC transplantation are discussed as well. Generally, a comprehensive perspective is provided for the clinical translation of MSC transplantation for SCI.

**Keywords** Spinal cord injury, Mesenchymal stem cells, Microenvironment imbalance, Paracrine

## Introduction

SCI is a catastrophic event that results in extensive cellular death and significant damage to the central nervous system (CNS), due to primary injury and subsequent secondary cascades [1–3]. Given its low cure rate and high mortality rate, SCI imposes a substantial burden on a global scale [4]. The initial mechanical trauma directly harms the tissue and triggers an inflammatory amplification. Additionally, the deficiency of neurotrophic factors and disruption of vascular integrity compromise the nutrient microenvironment. The formation of inhibitory scar tissue, coupled with the insufficient intrinsic mechanisms, ultimately hinders axonal regeneration, leading to failure in the recovery process [5, 6].

MSCs are pluripotent stem cells derived from multiple tissues with self-renew and multiple differentiation ability, serving promising candidates for cell transplantation therapy in many diseases, particularly in the treatment of SCI [7]. (i) The powerful paracrine ability comprehensively improves the imbalanced microenvironment [8, 9]. (ii) MSCs can be separated and cultured easily while maintain active after multiple passages [10]. (iii) Numerous chemokine receptors (CXCR1, CXCR2 and CCR2 etc.) expressed on the surface of MSCs allow them to target the lesion site precisely without additional modification [11]. (iv) The low immunogenicity and tumorigenicity due to un-prominent surface antigen, makes it a safe choice for clinical therapy [12, 13]. Nevertheless, due to variations in different experimental focus, the existing literature on MSC regulation for the imbalanced

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microenvironment following SCI lacks a comprehensive understanding.

This review mainly elucidates the comprehensive mechanism by which MSCs regulate the imbalanced microenvironment following SCI, especially in inflammation, neurotrophy and axonal regeneration. A summary of the application of MSCs is also discussed, highlighting its limitations and future directions.

## Microenvironment imbalance after SCI

Following SCI, the destruction of neurons, glial cells and other cells, as well as the disruption of the surrounding environment, have been observed [1, 14]. Previous studies extensively examined the phases (acute, subacute, intermediate and chronic) following SCI and established an international consensus [1, 5, 15]. Therefore, a novel perspective on the imbalance of the microenvironment after SCI is aimed to be offered (Fig. 1).

### Activation and cascade amplification of inflammation

In the context of SCI, inflammation assumes a pivotal role as the initial stress response. It functions as a dual-edged sword, wherein appropriate inflammation safeguards the tissue and hampers the propagation of damage. Conversely, excessive inflammation and the subsequent cascade of injuries contribute to additional neuronal demise and impede the regeneration of axons [5, 6].

The pattern recognition receptor (PRR) is recognized as the primary responder [16]. Following SCI, deceased cells discharge their contents, including the damage-associated molecular pattern (DAMP), which binds to PRR (Toll-like receptors (TLRs), Nod-like receptors (NLRs) etc.) expressed on microglial cells, astrocytes

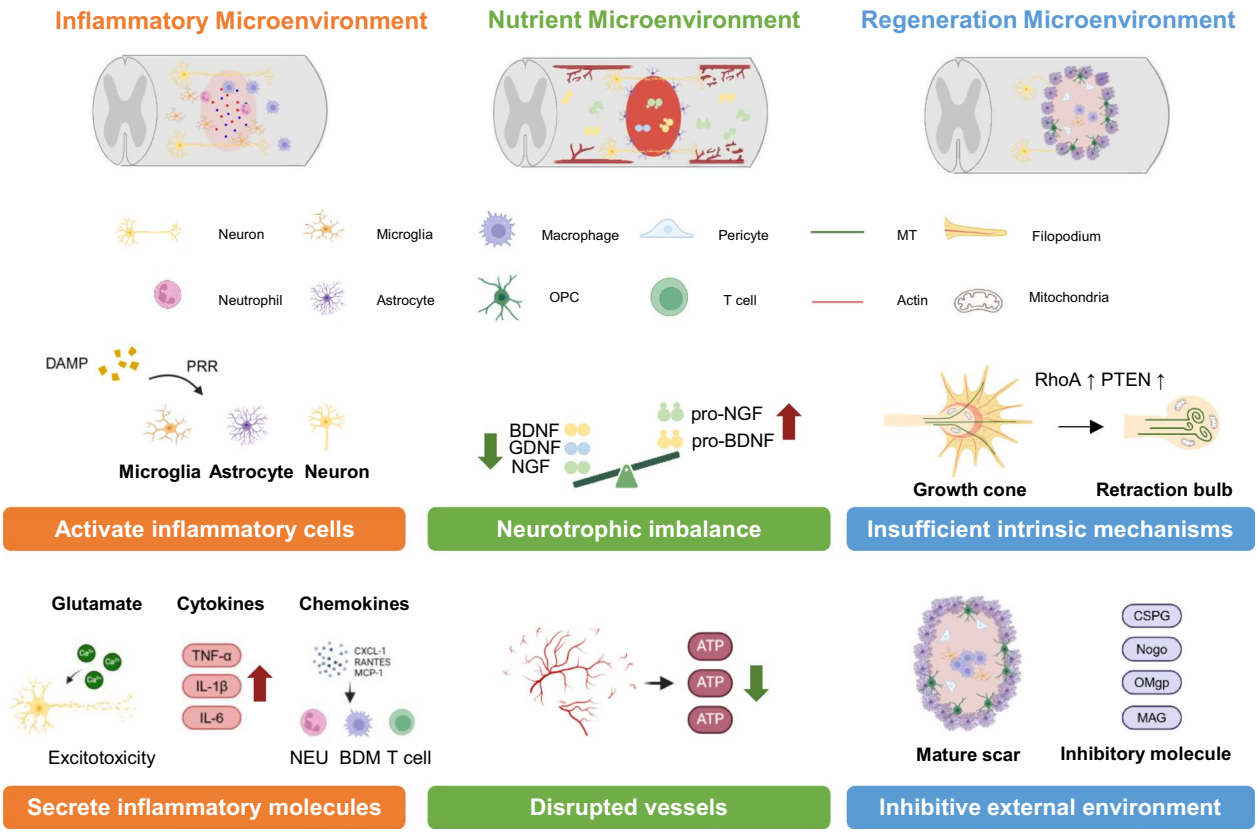


**Fig. 1** The imbalanced microenvironment after SCI. In inflammatory microenvironment, DAMP released by dead cells binds to PRRs on microglia, astrocytes and neurons to activate the initial inflammatory response. Glutamate released due to massive neuronal death leads to excitotoxicity. Cytokines such as TNF-α, IL-1β and IL-6 secreted by microglia and astrocytes are upregulated rapidly. Chemokines attract immune cells to the lesion site leading to cascade amplification. In neurotrophic microenvironment. The upregulation of NF and downregulation of pro-NF lead to a neurotrophic imbalance. Disrupted vessels result in an insufficient energy supply, culminating in cytotoxic edema and demyelination. In regeneration microenvironment, the silence of intrinsic regenerative mechanism (upregulation of RhoA and PTEN) transforms the growth cone into a retraction bulb. The inhibitive external microenvironment, including mature scar and inhibitory molecules further impedes axonal regeneration

and neurons, thereby initiating the initial inflammatory response [16]. In the early stage, pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6) and IL-1β, primarily secreted by astrocytes and microglia cells, experience a significant surge, resulting in heightened cellular demise [17–22]. Extensive neuronal apoptosis triggers massive release of glutamate, which induces excessive influx of calcium on neurons and glial cells called excitotoxicity, leading to mitochondrial dysfunction and cellular demise [23–26].

Chemokines such as CXCL-1, RANTES and MCP-1 are increasingly expressed after SCI to attract immune cells from blood to the lesion site, infiltrating the broken blood spinal cord barrier (BSCB) [27–30]. In mice models, neutrophils reach their peak at 24 h after SCI and subsequently release reactive oxygen species (ROS) and reactive nitrogen species (RNS), which induces lipid peroxidation to clear cellular debris. [31, 32]. Blood-derived macrophage (BDM) and microglial cells reach their peak at 7 days [33, 34]. The pro-inflammatory cytokines and chemokines secreted by them not only clear debris but also induce axon retraction and dieback, which exacerbates inflammatory response [35]. T cells reach their peak at 14 days [36]. After SCI, the balance between pro- and anti-inflammatory phenotype disrupts, for cytotoxic T-cells (Th1 and Th17) more than 90% while regulatory T-cells (Th2 and Treg) less than 10% [37], leading to an increased release of IFN-γ, TNF-β and IL-17 [38, 39]. The perforin produced by CD8 T cells exacerbates secondary injury by disrupting the BSCB [40].

Regardless of the initial cause of inflammation or the subsequent cascade resulting from various aforementioned factors, it will give rise to a positive feedback loop, ultimately leading to an overexpression of inflammation and excessive tissue damage.

#### Damaged neurotrophic environment

The neurotrophic factor (NF) is comprised of nerve growth factor (NGF), brain derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3) and neurotrophin-4/5 (NT-4/5), binding to tropomyosin-related kinase (Trk) receptors and non-specific receptor p75 [41] to promote neuronal survival, modify glia phenotype and enhance axonal plasticity [42]. NF is initially synthesized as a precursor, some of which can function as distinct ligands by binding to the p75 receptor and sortilin to induce cell death [41, 42]. Following SCI, neurotrophic factor precursor (pro-NF) increases. It leads to a relative deficiency of NF, which disrupts the balance of the neurotrophic microenvironment. This disruption in the balance between NF and pro-NF serves as the pathological foundation for the neurotrophic microenvironment. Pro-BDNF increases within 1 to 3 days after SCI, which acts as a suppressor for macrophage migration and infiltration [43]. Pro-NGF induces apoptosis at nanomolar concentrations. It can mediate oligodendrocyte death after SCI and break the integrity of myelin sheath [44–46]. Several studies have demonstrated the efficiency of reducing pro-NF in treatment of SCI, which provides evidence of the detrimental impact of pro-NF [47–49].

Another manifestation of the imbalanced neurotrophic microenvironment is the damaged vasculature. The initial injury directly impairs the blood vessels, and the ensuing cascade damage further injures the vascular endothelial cells. This results in a lack of energy supply, impairing ATP-dependent ion pumps on the cell membrane. Consequently, the permeability of the membrane increases, causing an imbalance of ions across it [14]. Excessive intracellular sodium leads to cytotoxic edema and exacerbates acidosis [50], while the potassium imbalance hinders the transmission of active potentials and leads to demyelination [51].

#### Inhibition of axonal regeneration

The achievement of favorable axonal regeneration continues to be of utmost importance, serving as the structural basis for functional recovery. Nevertheless, the inadequate activation of intrinsic mechanisms and the presence of inhibitory external factors pose significant challenges in attaining the desired regenerative outcomes.

##### *Insufficient intrinsic regeneration mechanisms*

The cytoskeleton regulates the extension of the axon. It is mainly composed of actin and microtubules (MT). During the elongation, actin extends to form filopodia, along which MT extends outward to form the cytoskeleton of axon. However, the orderly structure is disrupted after SCI, forming a malnourished structure called retraction bulb, in which MT and actin highly overlap. This is considered as the intrinsic structure basis for the failure of axonal regeneration [52].

The RhoA/ROCK pathway has been extensively investigated for regulating the cytoskeleton and is commonly regarded detrimental for axonal regeneration [52]. After SCI, Rock activates LIMK1, which phosphorylates cofilin at Ser-3, inhibiting its depolymerization function for actin [53]. Rock also phosphorylates collapsin response mediator protein-2 (CRMP-2), thereby impeding the interaction between CRMP-2 and MTs [54]. These result in the inhibition of MT extension and axon growth.

The PI3K-mTOR pathway plays a crucial role in regulating cell growth and its inhibition after SCI leads to unsuccessful axonal regeneration. PTEN is an upstream component, which can diminish mTOR activity and

reduce its mRNA translation. Inhibiting PTEN while activating mTOR show great axonal regeneration after SCI [55–57].

The inadequate understanding of the intrinsic regeneration mechanism necessitates further investigation. It is emphasized for the significance of comprehending the intrinsic pathway accurately, rather than relying on imprecise adjustments. A precise understanding of the upstream and downstream components is essential to identify the pivotal element which can stimulate intrinsic regeneration effectively.

#### *Inhibitive external microenvironment*

Traditionally, the glial scar has been viewed as an inhibitory barrier impeding axonal regeneration. However, it also plays a crucial role in limiting inflammation extension and preventing additional tissue damage. Given its dual nature and indispensable function, a comprehensive analysis will be presented on cellular and molecular levels.

*Cellular behavior* After SCI, peripheral-derived macrophage (PDM) and microglia near the lesion site rapidly infiltrate the core through the mediation of Plexin-B2 (PB-2) [58, 59]. PDM occupies a central position while microglial cells surround it. They clear debris, limit inflammation and compact extracellular matrix [59]. However, it is important to note that they also secrete pro-inflammatory cytokines and chemokines, which can contribute to further tissue damage.

Astrocytes transform into a reactive state characterized by hypertrophy and elongated processes in the first week after SCI, activated by neuroinflammation and ischemia probably through TNF-STAT3-a1ACT signaling axis [60]. They are divided into A1 and A2 phenotype. A1 exhibits neurotoxic properties and promotes inflammation while A2 possess neuroprotective qualities and facilitates axonal regeneration [61]. YAP pathway (bFGF-RhoA-YAP-p27Kip1) and PI3K/AKT pathway mediate astrocyte proliferation [62, 63] and Type I collagen triggers its maturation into a scar-forming morphology with thicker processes through the integrin-N-cadherin pathway, which creates an impermeable barrier to inhibit axonal regeneration [64].

It is worth noting, though, that the binary divisions of reactive astrocytes into A1/A2 is easily comprehensible and memorable, it is vague and cannot represent the complex reality [65]. Multidimensional methods should be used to indicate different characteristics of subtypes of astrocytes [66]. A comprehensive working model to define diverse astrocyte responses to CNS disorders has been proposed, including morphology, proliferation, molecular expression pattern through transcriptome and proteome, cellular functions and interactions [67]. Based on a comprehensive review in SCI, the reactive astrocytes are non-proliferative with variable degrees of cellular hypertrophy, marked by CD44, C3, Hsbp1, Vim and GFAP to seclude inflammatory cells at early phase. While scar-forming astrocytes are proliferative with thicker processes, marked by Cdh2, Sox9, Xylt1 and Chst11 to form astrocytic scar [61, 68, 69]. A more accurate and detailed definition is needed for different subtypes of astrocytes to characterize their subtle and diverse functions.

NG2 cells, also referred to oligodendrocyte precursor cell (OPC) has lineage plasticity to differentiate into oligodendrocytes, astrocytes, and Schwann cells. Nevertheless, a significant proportion of NG2 cells remain undifferentiated and secrete chondroitin sulfate proteoglycan (CSPG) to form glial scar to impede axonal regeneration [70].

Pericytes can differentiate into fibroblasts to secrete collagen and fibronectin in the lesion site, constituting connective tissue of the scar [36, 71]. Additionally, they show exert vasoconstrictive effects and regulate blood flow [72].

In summary, a comprehensive analysis of the cellular composition of the glial scar is presented. The pericenter of the scar is occupied by PDM with microglia surrounding it. Pericytes are dispersed around the microglia and secrete stromal components. Astrocytes form a barrier and encapsulate the scar along with NG2 cells. The initial formation of the glial scar is believed to have beneficial effects by limiting neuroinflammation and preventing further tissue damage. However, the mature scar sets an insurmountable obstacle for axonal regeneration. It is imperative to gain a deeper understanding of this complex phenomenon and implement appropriate adjustments at different stages, such as improving tissue contraction and containment of damaged tissue during the acute phase, while inhibiting its maturation during the chronic phase.

*Inhibitive molecule* After SCI, reactive astrocytes are primarily responsible for secreting CSPG, the primary inhibitory matrix in CNS, mainly comprising a glycosaminoglycan (GAG) [73, 74]. CSPG has two important protein tyrosine phosphatases receptors (RPTPs), protein tyrosine phosphatase σ (PTPσ) and leukocyte antigen-related (LAR) subfamily in adult mammals [75–77], through which it inhibits axonal regeneration by RhoA/ROCK and PKC pathway [78]. CSPG can inhibit lysosome and autophagosome fusion during autophagy process, thereby impacting autophagy regulation for the growth cone [79]. Besides, CSPG hinders transformation of immune cells from a pro-inflammatory to a pro-repair

state via the TLR4-dependent pathway, which offers a novel perspective [80].

Another inhibitory molecule is myelin-associated inhibitors (MAI), which is a consequence of demyelination. Accumulating myelin debris after SCI, due to destroyed oligodendrocytes, are referred to as Nogo, oligodendrocyte myelin glycoprotein (OMgp) and myelin-associated glycoprotein (MAG). Nogo-A is the main inhibitor and has a synergistic effect on MAG and OMgp [1, 81]. The clearance of MAI becomes challenging due to the significant reduction in macrophage phagocytic ability after SCI. This is attributed to the excessive consumption of lipids, which transforms macrophages into a 'foamy' phenotype [82]. MAI share common receptors, NgR1 (Nogo receptor 1) and paired immunoglobulin-like receptor B (PirB) [83], while NgR2 (Nogo receptor 2) is specific for MAG signaling pathway [84], which can inhibit neuron regeneration and upregulate NG2/CSPG4 in macrophages, diminishing its phagocytic capacity [85].

### The dual effects of imbalanced microenvironment on MSC transplantation after SCI

The unfavorable microenvironment poses challenges for the viability of transplanted MSCs, making it imperative to enhance its survival and target rate to reduce costs. Simultaneously, the powerful adaptive capacity of MSCs makes adjustments to the microenvironment to facilitate tissue repair. Therefore, it is reasonable to investigate the objective effects of the microenvironment on MSCs.

#### *Inflammatory microenvironment threatens the survival of MSCs in acute phase*

Inflammation persists throughout the entire process, yet it is most pronounced during the initial phase. The primary inflammatory response, along with subsequent cascades, undoubtedly impairs the survival and function of MSCs upon transplantation. Pro-inflammatory cytokines potentially induce cellular apoptosis. Additionally, the secretion of ROS and RNS by infiltrated neutrophils may lead to lipid peroxidation and cellular membrane damage. The subsequent cascade amplifies this adverse effect.

#### *Inflammatory microenvironment activates the immunomodulatory effects of MSCs in acute phase*

MSCs interact with immune system actively, through which they adopt both anti-inflammatory and pro-inflammatory capacity [86–88]. MSCs sensor inflammatory signals to make corresponding phenotype changes to modulate immune cells for better tissue repair [89–91]. MSCs pretreated with inflammatory cytokines in vitro, mimicking injury environment, show enhanced immunomodulatory effect to treat CNS injury, partly due to the metabolic reprogramming by inflammatory cytokines to increase glycolysis of MSCs [92, 93].

Therefore, the double-edged role of the immune system is also reflected in its impact on MSCs. To mitigate the adverse consequences of the microenvironment while improve the adaptability of MSCs, employing modified MSCs capable of secreting anti-inflammatory factors could potentially serve as a viable remedy. This approach may confer MSCs resistance to inflammation, thereby enabling them to survive and assume diverse advantageous functions in acute phase.

#### *Mature scar formation and declined axonal plasticity limit the effectiveness of MSCs in chronic phase*

The presence of a mature scar hinders the therapeutic efficacy of MSCs in promoting axonal regeneration, as it creates an impassable barrier and diminishes axonal plasticity. The body naturally attempts to minimize the size of the scar, aiming to limit tissue destruction at the lowest possible expense. However, it becomes pathological basis to inhibit axonal regeneration.

#### *Would scar formation help MSCs to achieve a better regeneration in chronic phase?*

The common sense is to inhibit scar formation for its harmful effect discussed above. However, it is interesting to find an unfavorable outcome when scar formation is prevented [94]. The scar-forming astrocytes support robust axonal regeneration through upregulating growth supportive molecules. It is worth exploring whether it plays a synergistic role with MSC in promoting nerve regeneration. Another advantage is the significantly reduced inflammation accompanied by massive neovascularization in chronic phase, making a stable environment for therapeutic effect of MSCs.

In conclusion, a precise regulation is sought to achieve that neither hampers the restriction for further tissue damage in early stage nor impedes future axonal regeneration. This inquiry may offer a novel perspective for MSC-based treatments. Further investigation is required to fully elucidate the underlying mechanism.

## Transplanted MSCs regulate the imbalanced microenvironment after SCI

The therapeutic effects of MSCs on SCI can be mainly attributed to the paracrine capacity. MSCs secrete cytokines and extracellular vesicles (EVs) including micro-vesicles and exosomes [8, 9]. These EVs contain numerous proteins, mRNA and micro-RNA, thereby influencing cell metabolism. Direct cell-to-cell communication also plays a role in the regulation. However, the differentiation potential of MSCs exhibits limited efficiency, as the majority remain undifferentiated [95].

Consequently, the impact and mechanism of microenvironment regulation by MSCs, due to their paracrine ability and direct cell-to-cell communication, are aimed to be comprehensively examined to enhance the understanding of MSC treatment for SCI (Fig. 2).

## Inflammation microenvironment

As one of the main targets, Inflammation emerges promptly following injury and persists over the whole process. The immunomodulatory function of MSCs has been comprehensively characterized through intricate interactions with immune cells, mediated either by the soluble secretions or direct cell communication [96]. The impact of MSCs on various inflammatory components at the molecular and cellular levels after SCI is aimed to be elucidated.

### *MSCs inhibit inflammatory cell activation*

Numerous cells actively engage in the initial inflammatory response and subsequent cascade after SCI, as discussed above. MSCs modulate the metabolic activities of diverse inflammatory cells either via secretome or

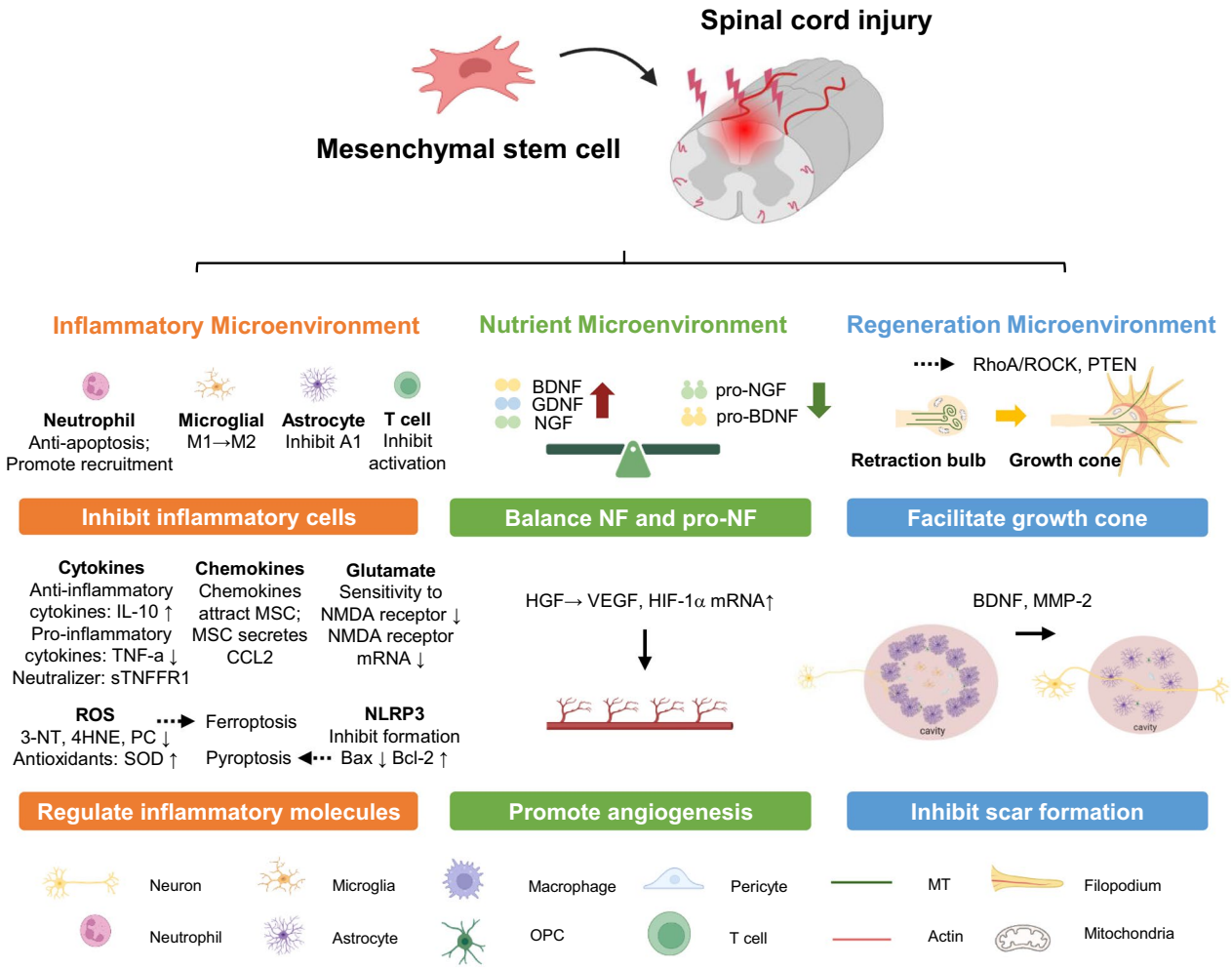


**Fig. 2** Regulation of MSCs on different imbalanced microenvironment. In inflammatory microenvironment, there is a significant infiltration of inflammatory cells accompanied by an increase of cytokines, chemokines, and other factors. MSCs have the ability to inhibit the activation of inflammatory cells, transform them into the anti-inflammatory phenotype and decrease the levels of inflammatory molecules. In nutrient microenvironment, the balance between NF and pro-NF is disrupted with damaged blood vessels. MSCs can secrete NF and counteract pro-NF to restore the balance. Additionally, MSCs can regulate key genes such as VEGF and HIF-1a to promote angiogenesis. In regeneration microenvironment, the intrinsic mechanism operates silently and insufficiently, leading to the formation of retraction bulb. The reactive astrocytes form a dense glial scar thereby impeding axonal regeneration. MSCs possess the ability to activate the intrinsic mechanism and secrete MMP-2 and BDNF, which effectively reduces the expression of GFAP and inhibits the formation of the glial scar, consequently resulting in a decrease of the cavity size

direct cellular communication, thereby influence their function within the inflammatory microenvironment.

*Neutrophils* MSCs reduce infiltration of neutrophils to modulate inflammatory response through downregulating CXCL2 in traumatic brain injury [97]. However, there is a lack of specific mechanistic studies related in SCI and CNS injury currently. Based on experiments in vitro and other disease models, it can be generalized for the dual regulation of MSCs on neutrophils. MSCs inhibit apoptosis of neutrophils through IL-6, IFN-β and granulocyte–macrophage colony-stimulating factor (GM-CSF) [87, 98, 99], which preserves its cellular pool, thereby facilitating the clearance of debris in early inflammation. Conversely, MSCs inhibit the infiltration of neutrophils and impedes the formation of neutrophil extracellular through superoxide dismutase-3 [100, 101], thereby inhibiting the amplification of inflammation.

Hence, it is of academic interest to investigate the precise regulation mechanism of MSCs on neutrophil in SCI. Given the observed surge of neutrophils at 1 day after SCI, this endeavor will provide a novel perspective in understanding the early inflammatory response.

*Microglia and macrophages* MSCs regulate the phenotype transform of microglia and macrophages from M1 to M2 in order to reduce inflammation after SCI [102, 103]. M1 (classically activated macrophage) and M2 (alternatively activated macrophage) represent two terminals of the full spectrum of macrophage activation [104]. M1 is a pro-inflammation phenotype, marked by iNOS, CD80 and CD86, with enhanced expression of TNF-α, IL-1 and IL-6 while M2 is characterized by anti-inflammation ability, marked by Arg1, CD206 and CD163, with increased secretion of IL-10 and transforming growth factor-β (TGF-β) [104–109].

For macrophages, IL-4 and IL-13 are critical medium secreted by MSCs to regulate the polarization, through activating JNK, JAK/STAT6 and PI3K pathway to promote M2 related genes expression after SCI [108, 110–113]. MSCs secrete CCL5, which induces enhanced production of IL-4, accelerating M2 polarization [108, 114]. In microglial cells, MSCs can activate A2bR/cAMP/PKA pathway and counteract the downregulation of Zbtb16, Per3, and Hif3a genes after SCI, thereby facilitating M2 polarization [115, 116].

*Astrocytes* Astrocytes induce inflammation and form glial scar after SCI. In this section, the MSC regulation of inflammation in astrocytes will be reviewed. The scar formation content will be discussed in the subsequent section. A1 astrocytes (reactive astrocytes) primarily contribute to inflammation, known to be neurotoxic [117]. MSCs secrete tumor necrosis factor-stimulated gene-6 (TSG-6) to decrease cyclooxygenase-2 (COX-2) and IL-6 levels [118, 119], which downregulates NF-κB pathway to inhibit the formation of A1 astrocytes [120–122]. Extracellular vesicles secreted by MSCs promote A2 phenotype transformation for SCI recovery [123]. The Jagged1/Notch pathway in astrocytes is inhibited by MSCs which induces a reduction in JAK/STAT3 phosphorylation, thereby exhibiting a therapeutic impact [124]. The activation of astrocytes is known to be dependent on microglial activity. It remains to be investigated whether the regulation of astrocytes is directly on itself or MSCs affect the crosstalk between microglia and astrocytes.

As discussed above, it is inappropriate and imprecise to define astrocytes in terms of binary divisions (A1, A2). Phenotype switch and function changes of astrocytes regulated by MSCs can similarly be characterized by multidimensional methods. More comprehensive studies are needed to elucidate the intricate interactions between MSCs and astrocytes in the future.

*T cells* MSCs can inhibit and escape from T cell mediated inflammation. (i) Lack of CD40, CD80, and CD86 on the surface of MSCs makes it hard to activate T cells [125]. (ii) MSCs can secrete nitric oxide (NO), indoleamine 2,3-dioxygenase (IDO) and TGF-β to inhibit T cell proliferation and activation [126, 127]. (iii) MSCs can escape from T cell recognition, due to the absence of major histocompatibility complex II (MHC-II) and the limited expression of major histocompatibility complex I (MHC-I) on the surface [125].

#### *MSCs reduce inflammatory molecule levels*

*Inflammatory cytokines* Inflammatory cytokines can be categorized as pro-inflammatory (such as TNF-α, IL-1β, IFN-γ) and anti-inflammatory (such as IL-10, TNF-β). MSCs can increase IL-4, IL-10, IL13 and TGF-β levels [128–130] and decrease IL-6 and TNF-α expression [102, 129] after transplantation. Besides, many studies also report the improvement of inflammatory microenvironment after MSC administration, leading to functional recovery of SCI [124, 131].

The potential mechanism can be summarized as two points. MSCs can directly secrete anti-inflammatory cytokines and neutralizers for pro-inflammatory molecules [132]. Furthermore, MSCs can modulate the metabolism and polarization of inflammatory cells as discussed above, thereby transforming them into an anti-inflammatory phenotype.

In conclusion, the phenomenon that inflammatory cytokines undergo a shift towards an anti-inflammatory orientation after MSCs transplantation serves as the basis

for the treatment of SCI. It is essential to pay attention to the intricate interplay between inflammatory cells and molecules to achieve a comprehensive understanding.

*Chemokines* Chemokines recruit inflammatory cells and induce amplification of subsequent cascade. Chemokines interact with MSCs. Stromal cell-derived factor 1 (SDF-1) and MCP-1 are up-regulated after SCI [133–135], which attracts MSCs to the lesion site through CXCR4 and CCR2 expressed on the surface [136, 137]. MSCs secrete C–C motif ligand 2 (CCL2) to attract macrophages and induce their transformation into M2 phenotype, thereby protecting neurons and myelin sheaths [138].

It is interesting to identify an optimal timing for inhibiting the recruitment function of chemokines. This timing should neither impede the early clearance of debris after inflammation, nor exacerbate the tissue damage caused by the cascade.

*ROS* The peroxide background after SCI leads to cellular death and hinders the regeneration of axons, with ROS serving as the primary mediator of oxidative harm. MSCs secrete superoxide dismutase to decrease oxidative metabolites (3-NT, 4HNE and PC) in SCI and other neurodegenerative diseases [139].

Ferroptosis is a distinct form of programmed cell death, which differs from apoptosis, autophagy and pyroptosis. It is closely associated with the peroxidation microenvironment, specifically involving lipid peroxidation of the cell membrane and impairment of the intracellular antioxidant system [140]. The important role of ferroptosis in the serious consequences of secondary injury following SCI is reviewed [141]. Neurons mainly suffer from ferroptosis after SCI and MSCs mediate mitochondria transfer to inhibit mitochondrial quality control and ferroptosis, which promotes neuronal survival after SCI [142]. Besides, the antioxidative capacity of MSCs can inhibit neuronal ferroptosis after SCI to attenuate neuronal dysfunction through miR-5627-5p/FSP1 axis [143].

*NLRP3 inflammasome* Nucleotide-binding oligomerization domain-like receptor protein 3 (NLRP3) inflammasome involves in the secondary inflammatory cascade after SCI. MSCs can impede the formation of NLRP3 inflammasome, which decreases the pro-apoptotic protein Bax and increases the anti-apoptotic protein Bcl-2, showing a neurological functional recovery after SCI [144]. MSCs inhibit activity of caspase-1 and decrease levels of IL-1β, IL-18 and TNF-α, resulting in a notable improvement in motor function [145, 146].

Furthermore, the NLRP3 inflammasome is significant in pyroptosis, a form of cell death closely linked to proinflammatory responses, which primarily occurs in myeloid lineage cells, specifically professional phagocytes such as macrophages and neutrophils. After SCI, DAMP recruit pro-caspase-1 through activating apoptosis-associated speck-like protein containing a CARD (ASC), leading to formation of NLRP3 inflmmasome [147–149]. It mediates pyroptosis, which triggers downstream neuroinflammation [150]. MSCs inhibit pyroptosis by increasing autophagy through PELI1 axis, which reduces inflammation after SCI [151]. Besides, this inhibition also mitigates tissue damage and maintains integrity of BSCB, resulting in functional recovery [152, 153].

*Glutamate and excitotoxicity* Inflammation-induced neuronal cell death results in the release of glutamate (Glu) and subsequent excitotoxicity. MSCs reduce the mRNA expression of glutamate N-methyl-D-aspartate (NMDA) receptor subunits and down-regulates neuronal sensitivity to the ligand of the NMDA receptor [154]. This modulation impedes the calcium influx triggered by glutamate, ultimately inhibiting excitotoxicity.

### Nutrient microenvironment

A stable and advantageous nutrient microenvironment serves a neuroprotective function by inhibiting neuron apoptosis and increasing neuron counts. It facilitates axon regrowth and extensive sprouting, resulting in improved functional recovery. The regulation of MSCs in the nutrient microenvironment can be attributed to the impact on the balance between NF and pro-NF and pro-angiogenesis function.

#### *MSCs regulate the balance between NF and pro-NF*

MSCs can secrete NF to ameliorate the imbalance. An increase of BDNF, NGF and glial cell-derived neurotrophic factor (GDNF) has been observed after transplantation, which results in an increase in the area of grey matter and white matter and functional improvement [155–157]. A reduction of pro-NGF and pro-BDNF after administration of MSCs also accounts for neuroprotection effect [158, 159]. These results demonstrate the equilibrium capacity of MSCs for NF and pro-NF. However, their findings are limited to a descriptive analysis and lack a comprehensive investigation of the underlying mechanisms. Except for direct secretion, it is imperative to further explore whether MSCs influence the synthesis process of NF in other cells, secretes neutralizing antagonists or employs other mechanisms.

The conventional mechanism demonstrates NF binds to respective receptors and exerts a neuroprotective effect. However, it is reported that MSCs secrete BDNF to enhance the balance between excitation and inhibition, which is achieved through the upregulation of g-aminobutyric acid type A receptor ($GABA_AR$) subunits

β3 & γ2 and $K^+/Cl^-$ cotransporter 2 (KCC2) in injured neurons [160]. These findings present a novel angle for MSC-mediated recovery in SCI and contribute to our understanding of the role of NF.

The present investigation into the secretion of NF by MSCs is overly broad. It should be asserted that a more precise and specific regulatory mechanism pertaining to a particular lineage of neurons is important. For instance, IGF-1 facilitates axonal growth in cortical spinal tract (CST) neurons [161], NT-3 induces sprouting of CST neurons [162] and BDNF enhances their branching and arborization [163], as well as promoting connections with spared descending interneurons [164]. Different NFs exhibit distinct neuroprotective priorities for CST. Instead of making generalizations, it is advisable to identify the most appropriate NF for specific stages and subsequently enhance its expression through MSC transplantation to achieve the optimal regulation.

#### *Pro-angiogenesis effect of MSCs*

Angiogenesis plays a crucial role in tissue regeneration after SCI, offering benefits of enhanced blood supply, metabolic regulation and cell transportation. MSCs show pro-angiogenic effect after transplantation for treating SCI. This phenomenon is primarily attributed to the MSC regulation of key genes in endothelial cell vasculature. MSCs secrete hepatocyte growth factor (HGF), leading to increased expression of vascular endothelial growth factor (VEGF) and hypoxia-inducible factor-1α (HIF-1α) mRNA, thereby promoting angiogenesis [165].

### Regeneration microenvironment

Axonal regeneration serves as the fundamental mechanism for achieving functional recovery after SCI, making it a crucial focus for therapeutic interventions. MSCs possess the ability to modulate the intrinsic regenerative microenvironment at the molecular level, while also regulate the extrinsic inhibitory microenvironment at the cellular and tissue levels.

#### *MSCs activate intrinsic regeneration mechanism*

MSCs have the ability to restore the disrupted cytoskeleton and modulate the development of growth cone. Following transplantation of MSCs, a significant presence of growth cone-like structures occurs [166], which can be partially attributed to the regulation of RhoA/ROCK and PTEN/mTOR pathway discussed above.

There is a decrease of RhoA level after MSC transplantation, with the most pronounced effect one week after SCI [167], which suggests that MSCs have the potential to alleviate the inhibitory effects of the RhoA/ROCK pathway on MT extension, thereby promoting a more organized MT structure that facilitates growth cone formation. MSCs secrete EVs containing miR-29b-3p targeting PTEN gene to suppress its expression [168]. Consequently, the inhibition exerted by PTEN on the mTOR pathway is relieved, resulting in the activation of the mTOR pathway and subsequent promotion of axonal growth.

In summary, MSCs inhibit retraction bulb formation and make an orderly growth cone by modulating RhoA/ROCK and PTEN/mTOR pathways, thereby influencing MT assembly and extension. However, the current research on the interaction between MSCs and the intrinsic pathway is insufficient and further investigation is necessary to gain a comprehensive understanding on the regulation of the intrinsic regeneration microenvironment.

#### *MSCs improve inhibitive external microenvironment*

The glial scar has been extensively investigated and serves as a significant evaluation of the effectiveness of MSC therapy. After MSC transplantation, it demonstrates a reduction in cavity size and tissue loss from the epicenter to rostral and caudal levels, accompanied by a decrease in GFAP [169], which suggests that MSCs inhibit the formation of glial scar. Several factors contribute to this phenomenon. MSCs inhibit reactive astrocytes, secretes BDNF and enhances Matrix metalloproteinases-2 (MMP-2) expression, resulting in a reduction in the expression and immunoreactivity of CSPG to prevent scar formation, which effectively mitigates cavity size [166, 170, 171].

The inhibitory effects of MSCs on glial scar formation on different levels are concluded. On the molecular level, MSCs secrete BDNF and upregulates MMP-2 expression. On the cellular level, MSCs exert inhibitory regulation on reactive astrocytes. On the tissue level, MSCs contribute to a reduction in cavity size and tissue loss. It is highlighted for the importance of investigating the impact of MSCs on other cellular components of the glial scar, such as macrophages, microglia and pericytes. Currently, research primarily focuses on the inflammatory aspects of these cells, as discussed above, rather than their role in scar formation and regeneration inhibition.

It is worth mentioning that even though the scar has already formed, MSCs have the ability to facilitate axonal regeneration through mechanisms known as 'cell bridge' and 'cell towing'. MSCs can migrate on inhibitory molecules such as CSPG or MAG/Nogo-A, acting as a cell bridge to facilitate the growth of dorsal root ganglion (DRG) across these inhibitory environments [172]. Additionally, MSCs can also tow the growth of co-located DRG. The elucidation of the mechanism behind MSC migration on inhibitory molecules and its

role in promoting DRG growth presents an intriguing area of investigation. Several research have demonstrated that the secretome by MSCs including BDNF, NGF, VEGF and SDF-1 can effectively enhance the outgrowth of motor neurites in the presence of glial scar inhibitors. It is plausible to hypothesize that MSCs not only serve as a physical substrate but also actively releases cytokines to facilitate neuronal growth in the inhibitory environment.

In summary, MSCs have demonstrated the ability to not only impede scar formation at different levels, but also facilitate axonal regeneration on a formed glial scar through paracrine effects and 'cell bridge'. Consequently, MSCs hold significant potential for the treatment of SCI, particularly in the chronic phase.

The regulation of the imbalanced microenvironment after SCI by MSCs covers the entire period, including inflammatory, nutrient and regeneration microenvironments. A brief overview has been provided to facilitate a more comprehensive understanding of the regulatory mechanisms of MSCs (Table 1).

## Application of MSCs in SCI

### Clinical trials demonstrate the effectiveness of MSCs in treating SCI

In addition to the numerous basic studies and animal experiments which proved the recovery of SCI after MSC administration, many clinical trials also show promising improvement. Most MSCs used clinically are derived from bone marrow or umbilical cord. The patients enrolled are complete or incomplete injury grade, with American Spinal Injury Association (ASIA) Impairment

**Table 1** Imbalanced microenvironment regulation of different sources of MSCs

| Source of MSCs | Mechanism of MSCs | Effect | Refs. |
|---|---|---|---|
| *Inflammatory microenvironment* | | | |
| UCMSCs | Zbtb16, Per3, and Hif3a genes↑ | Promote M1 to M2 of microglial | [115, 116] |
| | TSG-6↑ | Inhibit A1 astrocyte formation | [118–122] |
| | sTNFR1 ↑ | Counteract TNF-α | [132] |
| | Inhibit NLRP3 formation | IL-1β, IL-18 ↓ | [145] |
| BMSCs | Secrete EVs (NO, IDO, TGF-β) | Inhibit T cell proliferation and activation | [126, 127] |
| | IL-6, IFN-1β, GM-CSF ↑ | Inhibit neutrophil apoptosis | [87, 98, 99] |
| | Secrete EVs (miR-21) | Transform the phenotype of astrocytes to A2 | [123] |
| | Increase autophagy through PELI1 axis | Inhibit pyroptosis | [151] |
| | NMDA receptor subunit mRNA↓ | Inhibit excitotoxicity of neurons | [154] |
| BMSCs, ADMSCs | Anti-inflammatory cytokines↑<br>Pro-inflammatory cytokines↓ | IL-4, IL-10, IL13 and TGF-β ↑<br>IL-6 and TNF-α ↓ | [102, 129] |
| ADMSCs | Inhibiting the Jagged1/Notch pathway | Reduce JAK/STAT3 phosphorylation in astrocytes | [124] |
| | 3-NT, 4HNE, and PC↓ | Anti-oxidation | [139] |
| CBMSCs | CCL2↑ | attract macrophages, induce into M2 | [138] |
| EFMSCs | Inhibit NLRP3 formation | Bax↓ Bcl-2↑ | [144] |
| *Nutrient microenvironment* | | | |
| UCMSCs | GDNF↑ | Promote tissue repair | [156] |
| | BDNF↑, upregulate β3 & γ2 and KCC2 | balance excitation and inhibition in injured neurons | [159] |
| BMSCs | BDNF, NGF↑ | Stimulate recovery of SCI | [155] |
| | NGF↑ | increase the area of grey matter and white matter | [157] |
| | pro-NGF↓ | Improve nutrient microenvironment | [158] |
| AFMSCs | HGF↑ | VEGF and HIF-1α↑, promote angiogenesis | [165] |
| *Regeneration microenvironment* | | | |
| UCMSCs | Secrete miR-29b-3p | PTEN↓, promote axon growth | [168] |
| | Inhibit reactive astrocytes | Inhibit scar formation | [166] |
| | MMP-2↑ | CSPG↓, inhibit scar formation | [170] |
| UCMSCs, ADMSCs | GFAP↓ | Decrease cavity size and tissue loss | [169] |
| BMSCs | 'Cell bridge' and 'cell towing' | Facilitate neuronal growth | [172] |
| CMSCs | RhoA↓ | Promote growth cone formation | [167] |
| UCBMSCs | BDNF↑ | CSPG↓, mitigate cavity formation | [171] |

Scale scoring from A-C. After treatment for different periods, there are varying degrees of improvement in sensation function, motor function, electrophysiology, imaging and urodynamics [173–180]. Table 2 summarizes some clinical trials of MSC therapy for SCI, including cell type, dose, transplantation route, therapeutic phase and treatment effect. Taken together, MSCs bring functional recovery, which show promising prospect (Table 2).

However, the recovery in clinical trials is far less significant and effective than that in preclinical experiments. This can be attributed to different designs between preclinical and clinical trials. A good paradigm for discussing this issue is proposed [181]. Based on this, a brief overview is provided to illustrate the design differences (Table 3). For the disease model, species differences should be prioritized, emphasizing the need to select more representative animals, such as non-human primates. Animal models are typically young and healthy, exhibiting homogeneity degrees of injury, whereas actual patients often present with comorbidities and varying injury severities. Individual differences should also be considered. For transplanted MSCs, immunogenicity and source should be considered substantially. Besides, the preservation, dose and time of transplantation are also critical. For evaluation metrics, preclinical experiments typically observe effects over a short period. Conversely, clinical trials require long-term follow-up to comprehensively evaluate efficacy and safety. Differences in evaluation methods and ethical considerations further limit clinical translation.

## The optimal transplantation strategy of MSCs

### *Timing*

The effectiveness of different transplantation time points of MSCs has been assessed through experimental models and clinical trials. In experimental models, the subacute phase (2 weeks after SCI) is often considered the optimal choice [182, 183]. Because the intense inflammation and a detrimental environment in the acute phase hinders MSC survival and the formation of a mature scar in the chronic phase limits the efficacy [184–187]. Meta-analysis related proves subacute phase as the most effective time window in preclinical experiments [183, 188]. However, clinical trials have not yet determined an optimal time point. Due to practical considerations, most patients included in the trials are in the subacute or chronic phase as discussed above. Therefore, more comprehensive research should be conducted in the future to determine the optimal time window for transplantation.

### *Dose*

The therapeutic effect of MSC transplantation for SCI is dose-dependent. In rodent animals, high dose ($>1\times10^6$) reports better functional recovery than low dose ($<1\times10^6$) in umbilical cord derived mesenchymal stem cells (UCMSCs) and adipose derived mesenchymal stem cells (ADMSCs) [182, 183]. However, an over-dose transplantation may trigger inflammation, which harms repair effect [189]. Consequently, $10^6$ is utilized as an effective dose in most rodent models [190]. In large animal models, canines and pigs are administrated a dose of $10^7$ [139, 191, 192]. There is currently no research on MSC transplantation for SCI therapy in non-human primates. However, one study involving the transplantation of MSC-derived neurons into rhesus monkeys, with a dosage of $2.5\times10^6$, can be referenced for further research [193]. In humans, there are no systematic clinical trials comparing the optimal transplantation dose. Currently, the dose range is $10^7$–$10^8$ [174, 175, 177, 179, 180, 188]. A more precise criterion is $1.2\times10^6$/kg or $5\times10^6/cm^3$ (per lesion volume which was performed by MRI analysis) [176, 178] The allometric dose translation approach should be investigated in the future to advance clinical translation.

### *Route*

Despite being a non-invasive method, intravenous injection lacks specificity, with the majority of the cells predominantly accumulating in the lungs, spleen and kidneys [194], showing inferior efficacy compared to local transplantation [182, 183]. in situ injection has been documented as a direct means to target the lesion site. However, it may potentially cause secondary damage. Given the practical circumstances in clinical settings, intrathecal injection is considered as a relatively safe and valid method because it makes MSCs circulate in CSF to target lesion site [195].

Notably, the powerful homing and migration capabilities of MSCs have been systematically documented. After entering into blood flow, MSCs extravasate at the injury site crossing endothelial barrier through 'leukocyte-like process', finally targeting lesion site precisely [196]. SDF-1/CXCR4 plays a crucial role in recruiting MSCs in SCI [197]. However, due to massive capture of MSCs by lungs, spleen and kidneys when administrated through intravenous injection, strategies to improve homing capacity require urgent development. For instance, Integrin α4 overexpression enhances trans-endothelial migration of MSCs [198]. This is highly promising. It may promote intravenous injection as a superior administration route in the future, which is a non-invasive transplantation method to completely eliminate the risk of secondary injury to the spinal cord.

**Table 2** Clinical trials of MSC treatment for SCI

| # | Registration number | Status | Country | Participants | Cell type | Dose | Implantation route | Immunogenicity | Phase | ASIA Scoring | | Electrophysiology | Imaging | Urodynamics | Refs. |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | Sensation | Motor | | | | |
| 1 | NCT02510365 | Finished | China | 40 | UCMSCs | $4\times10^7$ | in situ transplantation | allogeneic | acute | ↑ | ↑ | ↑ | ↑ | ↑ | [175] |
| 2 | NCT03003364 | Finished | Spain | 10 | UCMSCs | $10\times10^6$ | Intrathecal transplantation | allogeneic | chronic | ↑ | | | | ↑ | [173] |
| 3 | NCT01393977 | Finished | China | 34 | UCMSCs | $4\times10^7$ | in situ transplantation | allogeneic | chronic | | ↑ | | | ↑ | [174] |
| 4 | NCT02352077 | Finished | China | 8 | UCMSCs | $4\times10^7$ | in situ transplantation | allogeneic | chronic | ↑ | | ↑ | | | [188] |
| 5 | NCT01909154 | Finished | Spain | 8 | BMSCs | $1.2\times10^6$/kg | in situ + intra spinal canal + intravenous | autologous | acute, chronic | ↑ | ↑ | | ↑ | ↑ | [176] |
| 6 | NCT01325103 | Finished | Brazil | 14 | BMSCs | $5\times10^6$/$cm^3$ | in situ transplantation | autologous | chronic | ↑ | ↑ | | | | [178] |
| 7 | NCT01676441 | Finished | Korea | 10 | BMSCs | $9\times10^7$ | in situ + intradural injection + lumbar tapping | autologous | chronic | | ↑ | ↑ | ↑ | | [179] |
| 8 | NCT05018793 | On going | Greece | 14 | ADMSCs | $9\times10^7$ | Intrathecal transplantation | autologous | subacute, chronic | ↑ | ↑ | | | | [177] |
| 9 | NCT03308565 | Finished | America | 10 | ADMSCs | $1\times10^8$ | Intrathecal transplantation | autologous | subacute, chronic | ↑ | ↑ | ↑ | | | [180] |

**Table 3** Design differences between preclinical experiments and clinical trials

| | Preclinical experiments | Clinical trials |
|---|---|---|
| *Disease model* | | |
| Species | Rodents | Human |
| Degree of injury | Relative homogeneity | Varying degree of severity |
| Comorbidity | Healthy | Different degree |
| Individual differences (age, gender…) | Relative homogeneity | Considerable variability |
| *MSCs* | | |
| Immunogenicity | Allogeneic & xenogeneic | Autologous & allogeneic |
| Source | Various sources | BM, UC, AT |
| Preservation | Cryopreserved | Fresh |
| Transplantation dose | Relatively small quantity | Large quantity |
| Time window | Mainly subacute | Mainly chronic |
| *Evaluation Metrics* | | |
| Fllow-up duration | Short-term | Long-term |
| Evaluation method | BMS, BBB | ASIA, Imaging |
| Ethics | Less consideration | Thorough consideration |

#### *Source*

Bone marrow (BM), umbilical cord (UC) and adipose tissue (AT) are three main sources utilized in experiments and clinical trials [199]. In spite of different characters, they all show effective therapy in different phases of SCI. As pluripotent stem cells, bone marrow derived mesenchymal stem cells (BMSCs) can differentiate into neurons and glial cells [200, 201]. However, the therapeutic effect mainly relies on the paracrine and transdifferentiation capacity, which can reduce inflammation, recover BSCB integrity, secrete neurotrophic factor and promote axonal regeneration [201–204]. UCMSCs can be easily obtained in a non-invasive manner. Besides, the low immunogenicity and rapid proliferation capacity make it a safer and more economical candidate [205]. They can inhibit reactive astrocytes activity, neuron apoptosis and scar formation [166, 171, 206]. ADMSCs contain more somatic stem cells with higher proliferation activity [207, 208]. They may activate angiogenesis more significantly due to enhanced expression of IGF-1, IL-8 and VEGF-D [209].

#### *Immunogenicity*

The low immunogenicity of MSCs minimizes the risk of immune rejection. However, it is still a nonnegligible issue to be discussed in the future clinical transformation. Autologous MSCs are isolated from self-body, expanded in vitro and then administrated back to self. It is considered the safest without the risk of immune rejection, which is widely used in clinical trials [176–180]. Although allogeneic MSC are not derived from the patients themselves, they achieve immune escape due to lack of CD40, CD80, CD86 and low expression of MHC complex as well as ability to inhibit T cell activation [125–127, 205]. UCMSCs are classical examples utilized clinically, which show effective restoration [173–175]. Xenogeneic MSC transplantation is limited to animal experiments with effective functional recovery while it is rare clinically because of species difference [210, 211]. It is promising if applied clinically because it holds the potential to solve the problem of insufficient clinical donors, provided it is ethical.

#### *Survival*

The adverse environment following SCI reduces the survival rate of transplanted MSCs, thereby limiting the effectiveness. Despite the presence of MSCs being detectable 6–8 weeks post-transplantation, their survival rate is less than 1% after 8 weeks [212, 213]. There are two methods to improve the survival rate and quantity of transplanted MSCs, including preconditioning of MSCs to enhance survival capacity (such as chemical factors or hypoxia) and administering multiple injections at different time points [214–217].

In conclusion, developing the optimal transplantation strategy to construct a comprehensive transplantation system is a complex yet essential task, particularly for future clinical translation. Critical factors including timing, dose, route, sources, immunogenicity and cell survival have been summarized, aiming to provide insightful directions for subsequent research.

### Exosomes derived from MSCs show promising effects

Due to certain constraints associated with MSCs, exosomes have emerged as an alternative therapeutic option, which is attributed to their distinct advantages, such as the absence of ethical concerns, the ability to evade capture by the liver and lungs and smaller size that facilitates easier infiltration of the BSCB [218].

Exosomes demonstrate similar effects to MSCs by means of their secretory activity, which can be summarized as anti-inflammatory, regulation of macrophage polarization, reduction of A1 astrocytes and protection of BSCB integrity [219]. Exosomes derived from hUCMSCs can induce a phenotype switch toward anti-inflammation of macrophage and decrease the levels of TNF-α, MIP-1α, IL-6 and IFN-γ [119].

Moreover, exosomes have the ability to modulate signal pathways via miRNA. Currently, miRNA-21, miRNA-133 and miRNA-126 have been identified as potential targets. These miRNAs specifically target crucial genes which are

involved in the process of injury repair. Exosome-derived miRNA-21 targets PTEN, thereby facilitating functional recovery [220]. miRNA-133b suppresses RhoA, leading to the activation of PI3K/AKT and MEK/ERK pathways, ultimately promoting regeneration [221]. In addition to inhibitory function, miRNA-133b promotes the phosphorylation of CREB and STAT3, which is associated with axonal regeneration [221].

However, there remain certain limitations that require resolution. It is imperative to attain the standardization in the isolation method, purification technology and source of MSCs for exosomes. Additionally, further investigation is necessary to discern harmful components in exosomes.

#### Modification of MSCs to enhance effectiveness

Different imbalanced microenvironments emphasize different therapeutic priorities. The modification of MSCs in vitro can potentially alter the properties. Therefore, it is advantageous to enhance the performance of MSCs through modification to adapt to various environment. In general, there are two approaches called preconditioning and genetic modifications [222].

##### *Preconditioning*

Preconditioning can be achieved through hypoxia, cytokines and physical factors. Hypoxia preconditioning has been shown to enhance the proliferation and migration of MSCs [223]. Additionally, the expression of pro-survival signals and trophic factors is increased after hypoxia/reoxygenation [224]. Cytokine preconditioning has also demonstrated beneficial effects, as evidenced by the upregulation of VEGF and activation of the pro-growth pathways AKT and ERK in MSCs treated with SDF-1 [225]. Additionally, physical factors and materials also show favorable effect. Pretreatment with pulsed electromagnetic fields (PEMF) can effectively enhance the AKT and RAS signaling pathways, thereby inhibiting the apoptosis of MSCs [226].

Different primed conditions show various effect. The optimal and suitable preconditioning method should be identified for different microenvironments. Particularly, whether preconditioning is harmful remains to be explored.

##### *Genetic modification*

MSCs can undergo genetic modification through trophic factors, cytokines and anti-apoptosis factors. MSCs genetically modified by NT-3 develop larger spare myelin sheaths and reduce the capsular area, which leads to significant improvement in motor function after SCI [227]. Similarly, BDNF, GDNF and NGF have also been employed, yielding notable effects [228].

Genetic modification presents a promising approach for regulating the properties of MSCs at different stages following injury. The selection of an appropriate gene for specific modifications of MSCs is crucial to enable their adaptation to diverse microenvironments. Additionally, understanding the intricate interplay between genes and achieving multi-gene modifications is of utmost importance.

#### Biomaterials and scaffold combination with MSCs

The direct administration of MSCs yields a diminished rate of cell survival and the migration of MSCs results in a reduced targeting efficiency. The utilization of degradable biomaterials can facilitate the retention of MSCs at the site of injury, ensure a sustained supply of nutrients and offer a structural substrate for axonal regeneration.

Currently, four primary materials, including collagen, fibrin, chitosan and poly lactic-co-glycolic acid (PLGA), are predominantly utilized. Each material possesses distinct characteristics [229]. After transplantation of MSC-scaffold, there is an increase in neurofilaments 200 (NF-200) and CD31 as well as a decrease in CD11b, indicating a reduction in inflammation, ultimately promoting angiogenesis, axonal regeneration and motor ability restoration of SCI [229, 230].

In addition to experimental evidence, clinical trials have also demonstrated potential value. In the clinical trial NCT02352077, Zhao et al. conducted a transplantation of hUCMSC-biodegradable collagen scaffolds to patients with SCI in the chronic phase, which improves the level of sensation, increases MEP reactive area and enhances trunk stability [188]. In the clinical trial NCT02510365, Xiao et al. observed a gradual recovery of electrical conduction and walking ability after transplantation in the acute phase [175].

#### Immunotherapy benefits MSC transplantation

Immunotherapy on SCI modulates innate and adaptive immune responses, which improves the intense inflammation after SCI [231]. Methylprednisolone (MP) is considered as a significant innate immune suppressant with anti-inflammatory and neuroprotective effects [232]. It exerts neuroprotective function by mitigating oligodendrocyte apoptosis induced neuron death through the inhibition of lipid peroxidation. It is also demonstrated of the enhancement of motor scores after administration of MP in clinical trials [233]. Human immunoglobulin G (hIgG) serves as a regulator of the adaptive immune response, which promotes the integrity of the BSCB by downregulating inflammatory enzymes and upregulating

the expression of tight junction proteins, thereby reducing the infiltration of immune cells. It induces neutrophils to migrate into the spleen by increasing chemoattractant there, leading to a reduction in the inflammatory cascade in the spinal cord [234, 235].

There is little research on the combination of MSCs and immunotherapy. The inflammatory response following SCI poses a significant threat to the survival of MSCs, particularly during the early stages. Employing immunotherapy to suppress the inflammatory response at the lesion site can create a conducive environment for MSCs. Nevertheless, prior to implementation, it is imperative to assess the influence of MP on MSCs and the safety of immunotherapy to avert potential systemic complications.

### Technologies for tracking transplanted MSC

In preclinical experiments, MSCs labeled with human nuclear antigen or green fluorescent protein (GFP) can be tracked through immunofluorescence imaging at cellular level [124, 236],while transfection with luciferase followed by in vivo imaging system (IVIS) allows imaging MSCs at the macroscopic level [237]. However, they are not suitable for clinical transformation for inaccuracy, lack of real-time capability as well as safety concern. Some research has already been conducted to identify suitable imaging tools to facilitate the clinical translation of MSC transplantation. Magnetic resonance imaging (MRI) is a non-invasive imaging modality with precise surveillance of MSCs labeled by exogenous contrast agents such as iron-oxide nanoparticles and gadolinium diethylenetriamine pentaacetic acid (Gd-DTPA), which shows safe and effective outcomes [238–240]. Besides, a combined ultrasound (US) and photoacoustic (PA) imaging technology is developed with capability for accurate guidance and quantitative imaging, which enables real-time tracking of MSCs and provides precise navigation for the safety of clinical MSC transplantation [241, 242].

A schematic diagram of MSC applications is provided to summarize the current common approaches (Fig. 3). In addition to the aforementioned methods, functional electrical stimulation (FES) holds promise as a rehabilitation training technique due to its effectiveness. The intricate pathological process of microenvironment imbalance following SCI involves multiple phases, targets and pathways. Merely focusing on a singular aspect is inadequate for comprehensive clinical rehabilitation. Consequently, a multiple-target and multiple-disciplinary treatment (MDT), such as combining MSC transplantation with other approaches is suggested.

## Limitation and future direction

Although MSC transplantation therapy has a relatively robust experimental foundation and some clinical evidence, there exist several limitations. The majority of current animal models employed in studies are rodents, which may not precisely reflect the pathological progression following SCI in humans. Consequently, the direct application of these findings to humans lacks accuracy, necessitating the inclusion of large animal models, especially non-human primate for further investigation, like in stroke research [243]. In terms of anatomical structure, their spinal cord closely resembles that of humans. Regarding effectiveness of SCI modeling, the post-injury periods and microenvironment changes they undergo are more analogous to those observed in humans. For evaluation metrics, evaluation criteria and identical imaging techniques in clinical trials can be employed. Additionally, inadequate translational studies and clinical trials impede the advancement and widespread implementation of this therapy. Furthermore, the ethical boundaries and risk assessment pertaining to this matter remain unresolved, especially for antigenicity and tumorigenicity.

In future research, it is imperative to conduct further investigation into the paracrine function and accurate underlying mechanism of MSCs. The specific and precise regulation will be the most efficient and economical approach. In order to facilitate the clinical application of MSCs, it is necessary to link preclinical experiment with clinical trials. More clinical trials are also warranted for risk assessment and to determine the optimal transplantation strategy. Additionally, the standardization and commercialization of MSCs are crucial for achieving large-scale production and widespread availability.

## Conclusions

SCI results in significant neural impairment, imposing a substantial burden on both the patient and society. The initial mechanical trauma directly causes tissue destruction and triggers subsequent inflammatory cascades. The disrupted balance between NF and pro-NF, along with damaged vasculature contributes to an unfavorable nutrient microenvironment. The inhibitory glial scar formation and insufficient intrinsic mechanisms hinder axonal regeneration. As a promising therapeutic approach, MSCs modulate the imbalanced microenvironment through its paracrine abilities and direct cellular communication. The administration of MSCs has been shown to mitigate inflammation, restore the nutrient balance and improve the inhibitory microenvironment, ultimately resulting in the regeneration of axons and the recovery of neurological function. Clinical trials and the optimal transplantation strategy are discussed. Various forms of

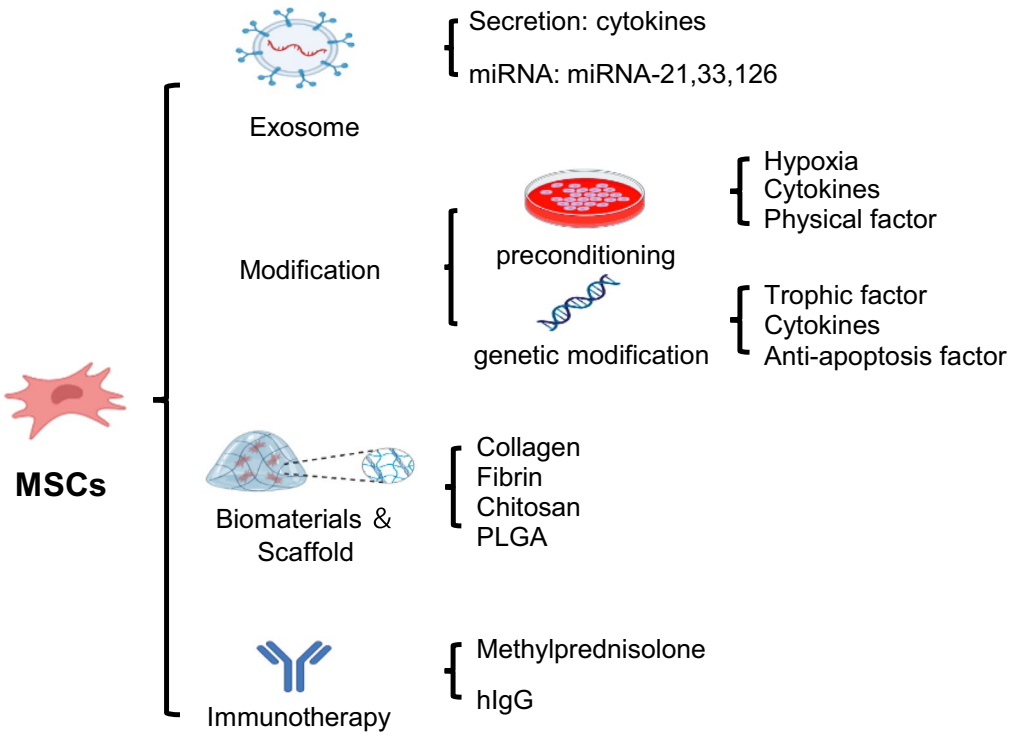


**Fig. 3** Different applications for MSCs. Exosomes show promising effect, which do not raise ethical concerns and possess the ability to evade capture by the liver and lungs as well as easily cross through the BSCB. The modification of MSCs, through methods such as preconditioning and genetic modification, can enhance the regulatory and adaptive capabilities of MSCs in different microenvironments. Co-transplantation with biomaterials and scaffolds can improve the survival and targeting rate of MSCs. Immunotherapy can be employed as a preliminary treatment to create a more conducive environment for MSCs

MSC application, such as exosomes, modification and combination with biomaterials, have been explored. At present, it is crucial to acquire a comprehensive and thorough comprehension of MSC regulation on the imbalanced microenvironment following SCI. Further research that is more specific is warranted to propel us towards an era of enhanced precision and targeted regulation.

**Abbreviations**

| | |
|---|---|
| ADMSCs | Adipose derived mesenchymal stem cells |
| AFMSCs | Amniotic fluid derived mesenchymal stem cells |
| ASC | Apoptosis-associated speck-like protein containing a CARD |
| ASIA | American Spinal Injury Association |
| AT | Adipose tissue |
| BBB | Basso, Beattie, and Bresnahan Scale |
| BDMs | Blood-derived macrophages |
| BDNF | Brain-derived neurotrophic factor |
| BMS | Basso Mouse Scale |
| BSCB | Blood spinal cord barrier |
| BM | Bone marrow |
| BMSCs | Bone marrow derived mesenchymal stem cells |
| CBMSCs | Cord blood mesenchymal stem cells |
| CCL2 | C-C motif ligand 2 |
| ChABC | Chondroitin ABC |
| CMSCs | Canine mesenchymal stem cells |
| CNS | Central nervous system |
| COX-2 | Cyclooxygenase-2 |
| CRMP-2 | Collapsin response mediator protein-2 |
| CSF | Cerebrospinal fluid |
| CSPG | Chondroitin sulfate proteoglycan |
| CST | Cortical spinal tract |
| DAMP | Damage-associated molecular pattern |
| DLK | Dual leucine-zipper kinase |
| DRG | Dorsal root ganglion |
| EFMSCs | Epidural fat derived mesenchymal stem cells |
| EV | Extracellular vesicle |
| FES | Functional electrical stimulation |
| $GABA_AR$ | G-aminobutyric acid type A receptor |
| GAG | Glycosaminoglycan |
| Gd-DTPA | Gadolinium diethylenetriamine pentaacetic acid |
| GDNF | Glial cell-derived neurotrophic factor |
| GFP | Green fluorescent protein |
| Glu | Glutamate |
| GM-CSF | Granulocyte–macrophage colony-stimulating factor |
| HGF | Hepatocyte growth factor |
| Hif-1a | Hypoxia-inducible factor-1α |
| hIgG | Human immunoglobulin G |
| HUCMSCs | Human umbilical cord-derived mesenchymal stem cells |
| IDO | Indoleamine 2,3-dioxygenase |
| IL | Interleukin |
| IVIS | In Vivo Imaging System |
| KCC2 | $K^+/Cl^-$ cotransporter 2 |
| LAR | Leukocyte antigen-related |
| LZK | Leucine zipper kinase |
| MAG | Myelin-associated glycoprotein |
| MAI | Myelin-associated inhibitors |
| MDT | Multiple-disciplinary treatment |
| MHC-I | Major histocompatibility complex I |

| | |
|---|---|
| MHC-II | Major histocompatibility complex II |
| MIF | Macrophage migration inhibitory factor |
| MMP-2 | Matrix metalloproteinases-2 |
| MP | Methylprednisolone |
| MRI | Magnetic resonance imaging |
| MSCs | Mesenchymal stem cells |
| MT | Microtubules |
| NF | Neurotrophic factor |
| NF200 | Neurofilaments 200 |
| NGF | Nerve growth factor |
| NgR1 | Nogo receptor 1 |
| NgR2 | Nogo receptor 2 |
| NLR | Nod-like receptor |
| NLRP3 | Nucleotide-binding oligomerization domain-like receptor protein 3 |
| NMDA | N-methyl-D-aspartate |
| NO | Nitric oxide |
| NT-3 | Neurotrophin-3 |
| NT-4 | Neurotrophin-4 |
| NT-5 | Neurotrophin-5 |
| OMgp | Oligodendrocyte myelin glycoprotein |
| OPC | Oligodendrocyte precursor cells |
| PA | Photoacoustic |
| PAMP | Pathogen-associated molecular pattern |
| PB-2 | Plexin-B2 |
| PDM | Peripheral-derived macrophages |
| PEMF | Pulsed electromagnetic fields |
| PGE2 | Prostaglandin E2 |
| PirB | Paired immunoglobulin-like receptor B |
| PKC | Protein kinase C |
| PLGA | Poly lactic-co-glycolic acid |
| PNN | Perineuronal nets |
| Pro-NF | Neurotrophic factor precursor |
| PRR | Pattern recognition receptor |
| PTPσ | Protein tyrosine phosphatase σ |
| RNS | Reactive nitrogen species |
| ROCK | RhoA-associated protein kinase |
| ROS | Reactive oxygen species |
| RPTPs | Protein tyrosine phosphatases receptors |
| RNS | Reactive nitrogen species |
| ROCK | RhoA-associated protein kinase |
| ROS | Reactive oxygen species |
| RPTPs | Protein tyrosine phosphatases receptors |
| SCI | Spinal cord injury |
| SDF-1 | Stromal cell-derived factor 1 |
| TGF-β | Transforming growth factor β |
| TLR | Toll-like receptor |
| TNF-α | Tumor necrosis factor-α |
| Trk | Tropomyosin-related kinase |
| TSG-6 | Tumor necrosis factor-stimulated gene-6 |
| UC | Umbilical cord |
| UCBMSCs | Umbilical cord blood mesenchymal stem cells |
| UCMSCs | Umbilical cord derived mesenchymal stem cells |
| US | Ultrasound |
| VEGF | Vascular endothelial growth factor |
| WISCI | Walk Index for SCI |
| YAP | Yes-associated protein |

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**Author contributions**
YFL, CXZ and SQF select the topic and determine the review structure. YFL and LQL searched the literature. YFL wrote the manuscript and drafted the figures and tables. CXZ, RZ, YLP and SQF critically revised and edited the manuscript. All authors read and approved the final manuscript.

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