Review Article

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Application and current challenges of neural stem cells transplantation in clinical trials of spinal cord injury

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Abstract

Spinal cord injury (SCI) can lead to sensory, motor, and autonomic dysfunction, and even accompanied by persistent pathological pain, seriously affecting the psychosomatic health of patients. At present, there are no effective treatment methods. In recent years, cell transplantation replacement therapy has entered people's field of vision. Functional bioactive cells are transplanted into the host to exert the characteristics of biotherapy so as to repair the injured nerve and repair the function. Neural stem cells (NSCs) are a kind of pluripotent stem cells, which have been well applied in nerve injury repair and tissue engineering regeneration. In preclinical and animal models, NSCs transplantation can well repair SCI and repair part of the function. The therapeutic functions of NSCs include differentiation into neurons, promoting axonal regeneration and myelination, neuroprotection, immunoregulation, and improving the local inflammatory microenvironment of SCI, replacing injured neurons. it is beneficial to the repair and reconstruction of neural network after SCI. NSCs have also achieved some encouraging results in clinical trials of SCI. Transplantation of NSCs into patients with SCI is a safe and feasible treatment, which can partially restore the sensory and motor function of the patients, without obvious side effects. At present, NSCs are in the primary exploration stage in clinical trials, and there are many problems and challenges in their extensive development and application in clinical trials. Therefore, we comprehensively discussed the application of NSCs in clinical trials of SCI and the existing problems and challenges, so as to provide useful information for the application of NSCs in SCI in the future.

Keywords: applications and challenges, clinical trials, neural stem cells (NSCs), spinal cord injury (SCI), treatment

Introduction

Spinal cord injury (SCI) is a devastating neurological condition that can lead to physical injury, psychological stress, and financial burden. In the past 30 years, the global prevalence rate has increased from 236 to 1298 per million people. It is estimated that 250 000–500 000 people worldwide suffer from SCI every year[1,2]. The treatment and late nursing of SCI have brought huge economic burden to the patients' families and society, and it is also a major challenge to the treatment of SCI at present. SCI is a destructive neurological and pathological condition that can lead to major motor, sensory, and autonomic nervous dysfunction. Its pathophysiology includes acute and chronic phases, and accompanied by a series of destructive events, such as oxidative stress, inflammatory events, apoptotic pathways, and motor dysfunction[3,4]. Although inflammation is a protective mechanism after SCI, but an excessive inflammatory response can lead to widespread damage and exacerbate disease progression. In these processes, more important is the activation/transformation of immune cells around the nerve injury (such as M1-type microglia/A1 astrocytes), thereby releasing various inflammatory cytokines (such as IL-1a, TNF-a, and C1q), forming an inflammatory microenvironment that is not conducive to the repair of damaged nerves, resulting in neuronal damage[5–7]. These secondary pathological changes have brought great obstacles to the repair of SCI. Irreversible functional damage after SCI is caused by conductive interference, demyelination, axon degeneration, and synapse loss of neurons at the injured site.

HIGHLIGHTS

- Comprehensively integrated the biological characteristics of neural stem cells (NSCs) and their functions in nerve injury repair.
- The source and application of NSCs transplantation for spinal cord injury (SCI) repair were comprehensively introduced.
- The application and treatment effect of NSCs in clinical trials of SCI were comprehensively discussed.
- The problems, challenges, and insights in the current clinical trials of NSCs transplantation in SCI were discussed.

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Therefore, changing these pathological changes, protecting neuron survival, promoting axon regeneration and re-myelination are conducive to SCI repair and functional repair. Currently, treatment methods for SCI include neuroprotective drugs, small molecule drugs with anti-inflammatory properties (such as methylprednisolone, minocycline, and P2X7 receptor blockers), rehabilitation treatment, and surgical intervention[8–10]. These methods can improve the symptoms of patients with SCI and restore some functions, but they still cannot repair SCI and rebuild neural network function. For patients with severe chronic SCI, drugs rarely provide effective treatment for patients with SCI. Moreover, clinical drug use is difficult to reverse complex pathological changes after SCI, nor is it possible to repair damaged nerves, rebuild neural function, and provide a good basic environment for neurons to survive. In addition, drugs use have their own inherent flaws, including dependence on long-term use, side effects, and truncation. Therefore, it is of great significance to explore a promising treatment strategy.

Recently, cell transplantation technology has been introduced to provide support for SCI repair. Cell transplantation strategies that promote axon regeneration, and neuronal plasticity have shown significant improvements in animal models of SCI[11,12]. NSCs are pluripotent stem cells that can differentiate into three main cell types in the nervous system and play a key role in the development and functional maintenance of the nervous system[13,14]. Compared with the above methods, NSCs have the key advantages of repairing injured nerves and exerting neuroprotective effects, differentiating into neurons to replace injured/apoptotic neurons, secreting various neurotrophic factors to provide a good environment for nerve regeneration, promoting axonal regeneration and myelination, and reconstructing neural network functions. Moreover, for patients with severe chronic SCI, NSCs transplantation can also achieve certain therapeutic effects and restore some functions[15–17]. Furthermore, another advantage of NSCs in nerve injury repair is their immune and anti-inflammatory properties. Transplantation of NSCs into the host can inhibit microglia/macrophage activation and the release of inflammatory cytokines, improve the secondary inflammatory microenvironment, and facilitate axon regeneration and re-myelination[18]. Transplantation of NSCs with a halving dose of the SOX9 gene showed good integration characteristics, mainly differentiated into motor neurons, reduced the accumulation of glial scar matrix, promoted long-distance axon growth and connection between neurons and host, and improved the motor and somatosensory functions of recipient animals[19]. These all reveal that NSCs have unique biological characteristics in nerve injury repair. They can not only differentiate into damaged/apoptotic neurons, but also provide a good nutritional basic environment for nerve regeneration and improve catastrophic inflammatory microenvironment.

These studies have revealed the huge potential of NSCs in the treatment of SCI. Given the encouraging results achieved by NSCs in animal models, researchers are gradually applying and transitioning to clinical trials, which also shows that transplantation of NSCs into SCI patients is a safe and feasible. A few clinical trials have shown that NSCs transplantation can treat SCI and restore some functions in patients. Although the current basic research field has demonstrated that NSCs transplantation has achieved encouraging therapeutic effects in the application of SCI, they can promote nerve regeneration after SCI and improve sensory and motor function. The transition of NSCs to clinical trials demonstrates the safety and feasibility. However, there are still differences on the exact therapeutic effect of NSCs in clinical trials, there is a lack of sufficient and reliable evidence, and there are also many problems and challenges that hinder their extensive clinical development. Although NSCs are in the early stages of clinical trials, but they are a promising treatment for SCI.

Functional characteristics of NSCs in nerve injury repair

The journey of neural development begins with neuroepithelial cells, a type of pluripotent NSCs, pseudo-layered cells lined up in the nerve lumen. Neuroepithelial cells begin to divide symmetrically and retain stem cell characteristics[20]. After the formation of neural tubes, neuroepithelial cells transform into radial glial cells, which produce progeny and intermediate neural precursor cells through asymmetric division, differentiate into neurons, and induce neuron migration[17,21]. As the embryo develops, radial glia can differentiate into oligodendrocytes and astrocytes (near birth, radial glial cells change their characteristics to produce NSCs, a reservoir of neurogenesis and embryogenesis in adult life)[22,23]. NSCs are pluripotent stem cells with the potential for self-renewal and differentiation. NSCs are widely distributed in the cerebral cortex, hippocampus, striatum, olfactory bulb, ependymal area/subeventricular area of the lateral ventricle, spinal cord of the embryonic, and fetal and adult central nervous system. NSCs can differentiate into neurons, astrocytes, and oligodendrocytes, and demonstrate that they can be renewed to the extent that they provide a large number of brain cells[17,24]. NSCs promote nerve repair through inherent neuroprotective and immunoregulatory properties, release of neurotrophic factors, activation of endogenous NSCs, and intercellular signal transduction[25]. At present, there is also some recognition of the functional role of NSCs in nerve injury repair. The biological functions of NSCs in nerve injury repair include neuroprotection, promotion of axon regeneration, secretion of neurotrophic factors, and immunomechanism[17,26,27] (Fig. 1).

Neurotrophic factor secretion and axonal growth promotion by NSCs

NSCs secrete a variety of neurotrophic factors, including brain-derived neurotrophic factors (BDNF), fibroblast growth factors (FGF), nerve growth factors (NGF), insulin-like growth factors, neurotrophin-3 (NT-3), and hepatocyte growth factors. The secretion of these nutritional factors provides a favorable foundation environment for nerve regeneration[24,28]. Neurotrophic factors secreted by NSCs increase survival and regeneration of endogenous neurons. These neurotrophic factors are important regulators of central nervous system development and function, reduce cell death in host endogenous neurons, promote axon/dendritic connections, and enhance the survival of transplanted NSCs[29,30]. C17.2 NSCs can naturally secrete large amounts of multiple neurotrophic factors before transplantation, including NGF, BDNF, and glial cell-derived neurotrophic factor[31]. NSCs were transplanted into cervical spinal cord lesions in adult rats, it was found that the transplanted NSCs expressed neurotrophic factor genes *in vivo* and promoted the extensive growth of host axons[31]. Moreover, NSCs have been genetically modified to produce NT-3, expand the impact of NSCs on host axons[31]. NSCs can produce neurotrophic growth factors and differentiate into mature neurons to rebuild the injury site[32].

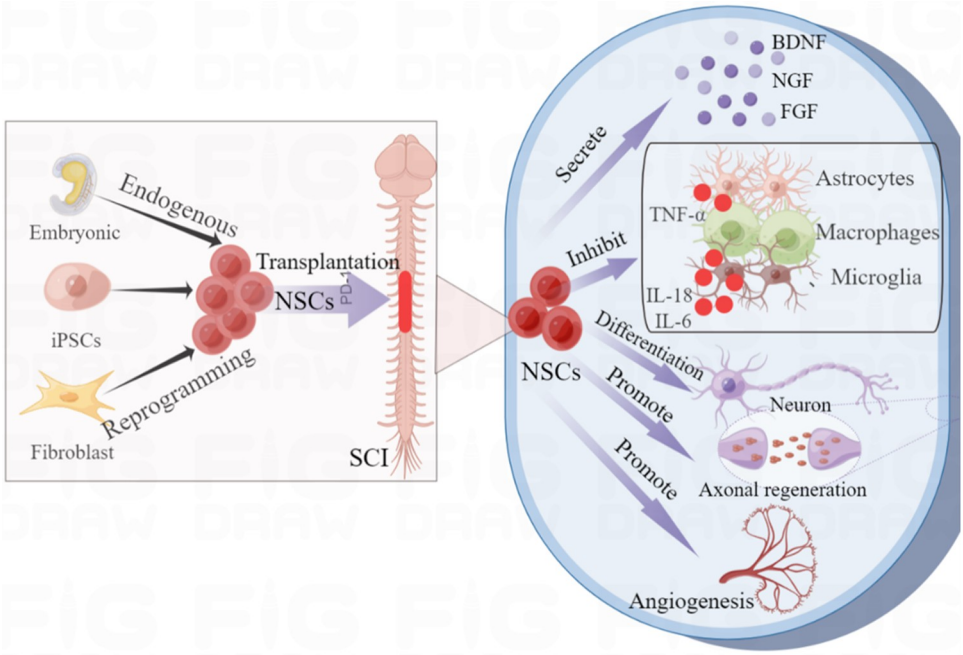

Figure 1. Functional role of NSCs in SCI NSCs derived from endogenous embryonic tissue, iPSCs, and somatic cell reprogramming are transplanted to the site of SCI can secrete various neurotrophic factors (such as BDNF and NGF), inhibit the activation of immune cells (macrophages, microglia, and astrocytes) and the release of inflammatory cytokines to exert anti-inflammatory and immunoregulatory effects, differentiate into functional neurons, promote axonal regeneration, promote vascular formation, rebuild capillary networks, and facilitate the repair of SCI.

These studies have shown that neurotrophic factors secreted by NSCs are functionally related to neuron activation and axon growth processes. However, additional research is needed to elucidate the complex molecular signals regulated by the NSCs secretion group, a necessary step for clinical translation.

Immune regulation and anti-inflammatory effects of NSCs

Neuroinflammation mediated by immune cell activation/penetration and cytokine release plays an important role in SCI[33,34]. NSCs transplantation can safely and effectively reduce neuroinflammation, promote nerve regeneration, reduce the degree of spinal cord fibrosis, reduce the area of damaged tissue and cavities, and promote axon regeneration and myelination[35]. Human NSCs transplantation can reduce the inflammatory response after infarction and promote nerve regeneration by inhibiting local glial cell activation and mediating the infiltration of peripheral immune cells[36]. This also demonstrates that NSCs have biological functions of inhibiting glial cell activation and anti-inflammation. Therefore, restoring nerve damage by enhancing immune intervention with NSCs represent a potential treatment. Before transplanting NSCs, using immune cells in vitro to promote the differentiation of NSCs and adoptive transfer of immune cells to the damaged site may lead to a more favorable prognosis after SCI. In addition, we need to study the functional role of NSCs in the acute and chronic stages after activation of specific immune cells, which will help accelerate the application of NSCs and immune cells as therapeutic strategies.

NSCs promote myelination and axon regeneration

Without additional intervention, the regenerated axons remain mostly unmyelinated (<10%) and limit functional repair[37]. NSCs transplantation promotes axon regeneration and new myelination after SCI[38]. NSCs have the ability to self-renew and differentiate into glia, including oligodendrocytes, cells responsible for the formation and maintenance of myelination and the regeneration of demyelinated axons. Because of these properties, NSCs are considered a promising source of cells for reconstructing damaged neural circuits and promoting myelin regeneration. Transplantation of human NSCs significantly increased the total number of regenerated and myelinated axons in a multichannel poly (lactide-co-glycolide) bridge, and could be integrated into the host spinal cord as myelin oligodendrocytes and synapse-integrating neurons, and promoted regeneration[37]. Implantation of NSCs with IGF-1 gel can promote the growth of neurites in lesions after SCI, enhance neuron regeneration and myelin regeneration[27]. Human NSCs transplanted into Shiverer immunodeficient mice preferentially differentiated into oligodendrocytes. These oligodendrocytes produced dense myelin sheath with normal nodular tissue, ultrastructure, and axon conduction speed, and myelin sheath still forms in the central nervous system 5–7 weeks after transplantation[39]. The study reveals that NSCs can produce functional myelin sheath even in animals with severe symptomatic myelin deficiency.

NSCs promote angiogenesis

NSCs promote angiogenesis and are conducive to nerve damage repair[40,41]. In the hippocampus of adult mammals, NSCs are in close contact with a dense network of capillaries[42]. Adult hippocampal NSCs express vascular endothelial growth factor, and the loss of NSC-specific vascular endothelial growth factor results in the isolation of NSCs and their intermediate progenitor daughter cells from the local vascular system[42]. NSCs

maintain their proximity to the vascular system through self-stimulated vascular endothelial growth factor signals, supporting their movement to and/or adhesion to local blood vessels[42]. Studies have shown that transduced NSCs express vascular endothelial growth factor. Transplantation of NSCs expressing vascular endothelial growth factor improves spatial learning and memory ability in rats, inhibits oxidative stress damage, inflammatory response, and histopathological damage[43].

All of these reflect the important functional role of NSCs, which can provide a good nutritional foundation for the regeneration of damaged nerves through the expression and paracrine of various neurotrophic factors. Moreover, NSCs transplantation can inhibit the activation of immune cells, reduce the release of inflammatory cytokines, improve the inflammatory microenvironment of nerve damage, and facilitate the survival and extension of new axons. Furthermore, NSCs can also promote axon regeneration, myelin formation, and integrate with host neurons to rebuild neural network functions. In short, NSCs have shown great application prospects in nerve injuries, including SCI, but the detailed mechanism of their work in the body still requires better research in the future to explore and understand.

Differentiation of NSCs into neurons promotes SCI repair

Primary or secondary injury to the spinal cord can lead to axonal degeneration/demyelination and neuronal apoptosis. Neuronal degeneration/apoptosis is a key factor hindering SCI repair and functional recovery. Therefore, it is crucial to protect neurons, promote axon regeneration, and lead regenerated axons to form new neural connections with axons distal to host injury. With the continuous exploration of neural injury regenerative treatment strategies, people have found that active cells from different sources can be genetically modified, cell fusion and epigenetic modification to induce/differentiate into functional neurons, and can be transplanted into the host to replace lost/apoptotic neurons play a role in repairing damaged nerves[44–46]. These differentiated neurons have high transformation rates, no ethical issues, short induction cycles, and no risk of tumor formation[17,47,48]. Based on these advantages, induced differentiation of cells into neurons has broad prospects in cell replacement, disease model establishment, drug screening, and personalized medicine in the treatment of SCI.

NSCs transplantation is recognized as a promising therapeutic strategy to replace neurons lost after SCI. NSCs have the potential to differentiate into neurons. When transplanted into a host, they can differentiate into functional neurons that simulate the spinal cord conduction microenvironment, rebuild interrupted neural electrical signal transmission after SCI[49,50]. Transplantation of NSCs can form connections with host cells, differentiate into mature neurons, and promote the repair of SCI[51,52]. A double-crosslinked conductive hydrogel (BP@Hydrogel) containing black phosphorus nanoplates can induce behavioral and electrophysiological recovery in mice with complete spinal cord transected by reducing inflammation and promoting endogenous NSCs to form functional neurons and synapses under rotating magnetic fields[53]. A pure water-driven nickel-zinc micromotor based on self-built electrochemical fields is proposed. The micromotor can be magnetically guided and accurately approach the target NSCs. Through electrochemical fields, biological electrical signals can be exchanged and communicated with endogenous NSCs, allow regulated proliferation and direct neuronal differentiation *in vivo*[54]. Fut9 gene-modified NSCs effectively differentiate into neurons to promote functional and histological recovery after SCI[32]. *In vitro* experimental data show that porous PHPMA hydrogel (NeuroGel) can promote the differentiation of NSCs into neurons and inhibit the differentiation of NSCs into glial cells. *In vivo*, it was shown that when the implant was placed in the rat, a dense network of neurons formed could be seen, which promoted the recovery of spinal cord tissue[55]. These studies have shown that NSCs have the characteristics of differentiated neurons in vivo and in vitro, can replace/repair damaged neurons and promote the repair of SCI. This also reveals that the migration of NSCs and neuronal differentiation *in vivo* can be further enhanced by the use of bioactive materials, providing new possible treatment strategies and approaches for the repair of SCI. However, combined use may produce more beneficial therapeutic effects with a full understanding of the interaction precursors between NSCs and biomaterials, such as the compatibility and degradation between the two.

Source of NSCs for SCI repair

NSCs can be derived from endogenous NSCs, human embryonic stem cells and human fetal brain NSCs/progenitor cells, induced pluripotent stem cells (iPSCs) and direct reprogramming. This lays a solid foundation for the source of NSCs and their development in the field of tissue repair and regeneration. During embryonic development, there are two key areas of proliferation: the ventricular area (VZ) and the subventricular area (SVZ). NSCs are located in the VZ area of the neural tube and produce various types of cells needed to build the central nervous system[56]. NSCs in the VZ can preserve the stem cell bank in a symmetrical or asymmetrical division and migrate to the SVZ, create the ability to proliferate or differentiate[57,58]. The SVZ of the adult brain is a site where neural progenitor cells (NPCs) proliferate early in development and contains resting NSCs. However, these resting NSCs are activated after damage to the central nervous system and participate in the repair process. NSCs can be obtained from the adult SV or SVZ or from primitive embryonic stem cells[17,58]. NSCs are permanently present in the SVZ of the adult brain to ensure lifelong neurogenesis during periods of plasticity or adverse damage to the neural network. NSCs can be isolated from the SVZ or SV and obtained by culture *in vitro*. These cultured NSCs can be identified by their markers such as Nestin, Sox5, Sox2, or NeuroD[59,60]. NSCs isolated from the SVZ of monkeys and rats maintained their ability to proliferate for at least nine generations. In addition to expressing Nestin, Sox2, and GFAP, these NSCs also have the ability to self-renew and can differentiate into neurons, astrocytes, and oligodendrocytes[17]. Studies have shown that NSCs/neural precursor cells can be obtained through primary spinal cord culture. Cells were isolated from canine spinal cord fragments and cultured in serum-free culture medium supplemented with EGF and FGF-2 growth factors[61]. Neurospheres formed bands on their periphery that can migrate and communicate with other neurospheres. Identification of phenotypic markers of the characteristic genes Sox2, Nestin, GFAP, and β-tubulin III confirmed these neurospheres extended by NSCs[61].

iPSCs have made invaluable contributions to regenerative medicine, bypassing ethical issues in the use of human embryos.

Efficient and effective methods to transform human iPSCs into differentiated derivatives. iPSCs have the plasticity to generate, repair, and change nervous system functions. iPSCs are useful for powerful large-scale development and disease modeling research and for regenerative medicine providing a source of cells is crucial[62–64]. NSCs differentiated from iPSCs can be confirmed by a variety of methods, such as RNA-Seq, flow cytometry, immunocytochemistry, and RT-qPCR[65,66]. Orphan nuclear receptor NR2E1 (TLX) genes with different structures are stably introduced into iPSCs and cultured in differentiation medium. The stable expression allows iPSCs to rapidly and autonomously differentiate into NSCs, which can be expanded to 45 generations[67]. Long-term cultured NSCs maintain highly homogeneous expression of NSCs-specific biomarkers and the potential for neuronal differentiation[67]. Studies have shown that differentiation and induction of iPSCs to differentiate into NSCs are stable and effective. The transplanted NSCs derived from iPSCs can survive and migrate to ischemic brain areas to differentiate into mature neural cells, with the potential to restore neural function in rats damaged by stroke[68]. Transplantation of iPSCs-derived NSCs into SCI rats showed that these transplanted cells could differentiate into neural cells and astrocytes, but did not differentiate into oligodendrocytes. Some transplanted cells remained undifferentiated and proliferated[69]. NSCs derived from purified iPSCs were transplanted into a rat model of cervical contusion. These cells were able to survive and differentiate into neurons and astrocytes, improved the motor function of the forelimbs[70]. However, both internal (genetic and epigenetic) and external (culture conditions and duration) factors can affect the stability of NSCs, which may also lead to possible risks for iPSCs-derived NSCs transplantation. Moreover, iPSCs transplantation has some shortcomings, such as low survival rates, potential for tumor formation at the transplant site, and high cost-effectiveness in developing therapies. Therefore, it is crucial to develop optimized solutions, including standard protocols for collecting cells, ideal times for cell delivery, guiding cell-directed differentiation, and safe and effective routes for drug delivery.

Reprogramming, including microRNAs-activated cell reprogramming, chemical induction, and traditional transcription factors that transform adult cells into different cell types, represents a promising approach in the field of regenerative medicine, potentially creating a cell source for cell replacement therapy[71–73]. Some studies have provided another guarantee for the source of NSCs by reprogramming somatic cells such as fibroblasts and astrocytes into NSCs through transcription factors or chemical inducements. Mouse fibroblasts were transformed into NSCs through a cocktail of compounds. These induced NSCs have a gene expression profile similar to true NSCs, can proliferate and differentiate into glial cells and functional neurons[74]. Overexpression of the transcription factor Ptf1a carried by lentiviruses reprogrammed human or mouse fibroblasts into NSCs. The obtained NSCs strain has strong self-renewal ability and can differentiate into various types of neurons, astrocytes, and oligodendrocytes *in vivo* and *in vitro*[75]. Mature astrocytes can be directly transformed into NSCs by a single transcription factor Oct4, and NSCs display typical neurosphere morphology, true NSCs gene expression, self-renewal ability, and pluripotency. Interestingly, continuous sonic stimulation enhances Oct4-driven reprogramming of astrocytes to NSCs, improves conversion efficiency[76]. This efficiently reprograms astrocytes to generate NSCs, provides an alternative treatment method for autologous cells to treat SCI. The current method of inducing NSCs production from somatic cells is still slow and inefficient. Recently, NSCs have been quickly and effectively produced from human or mouse fibroblasts using a single microRNA (Mir-302a)[77]. These NSCs exhibit morphological, molecular, and functional properties similar to adult and mouse NSCs, respectively, and can be expanded for more than 20 generations *in vitro*. This may provide a rapid and effective strategy for generating NSCs in terms of cell origin for basic research and clinical applications. Compared with iPSCs-induced NSCs, directly transformed cells do not need to undergo iPSCs status, avoiding the risk of forming tumors after transplantation into SCI. This is because genes that can be used for exogenous transcription factors can be integrated into the host cell genome to induce genomic mutations in directly reprogrammed cells. Although a source of cells can be provided by reprogramming somatic cells into NSCs, these NSCs are unlikely to be used in clinical studies due to safety issues associated with the use of foreign genes and viral transduction.

Application of NSCs in clinical trials of SCI

The clinical treatment of SCI has always been a difficult and key issue that urgently needs to be solved. At present, a variety of methods have been used clinically to jointly treat/improve the functional symptoms of patients with SCI. These methods include physical therapy, drug therapy, surgical treatment, and related treatments such as spinal cord electrical stimulation. Although these treatment methods can improve a certain degree of sensory and motor function, but they still cannot promote SCI repair and axon regeneration, inhibit/improve catastrophic complications, protect/inhibit neuron loss/apoptosis, and improve function. Therefore, exploring a promising treatment method is particularly important. Given the functional role of NSCs in nerve injury repair, different studies have achieved exciting results in pre-clinical trials/animal models. Transplantation of NSCs from different sources into animal models can promote SCI repair and improve neural function, which has been affirmed by different studies[78,79]. Given these impressive results, researchers have gradually transitioned to explore its therapeutic effects in clinical trials, and there has been some progress.

NSCs have been transplanted into patients to explore their functional role in treating SCI. Use of NSCs in clinical trials shows feasibility and safety[80,81]. In a clinical trial, four patients with T2-T12 SCI were treated with spinal internal fixation removal, laminectomy, and epidural incision, followed by six directional injections of NSI-566 NSCs. All patients were well tolerated. No serious adverse reactions occurred 18–27 months after transplantation[82]. The International Standards for Neurological Classification of Spinal Cord Injury neurological assessment of one patient showed a two-level improvement in bilateral sensory and motor sensations (from T8 to T10) at 6, 12, and 18 months, and a one-level improvement in sensory and motor sensations at 27 months compared to baseline before treatment. The Brain Motor Control Assessment showed an increased response of lower limb muscles to strengthening movements[82]. Another patient experienced new spontaneous activity in the right rectus abdominis muscle and left paraspinal

muscles from T6 to T8 and improved sensation between bilateral T6 and T9. MRI imaging of all patients showed varying degrees of focal myelopathy, and one patient had bone fragments in the spinal canal at the T11 level. No new spinal cord or soft tissue swelling, enhancement, or fluid accumulation was noted after surgery or subsequent imaging[82]. These data support the safety of NSI-566 transplantation to the site of SCI, but this safety trial lacks statistical power and the control group needed to evaluate the functional changes caused by cell transplantation. Recently, human spinal cord-derived NSCs (NSI-566) were transplanted into 4 subjects with SCI from T2 to T12 to investigate the feasibility and safety of their treatment. All four subjects tolerated cell transplantation well, and two patients had improvements in nerve injury levels, motor scores, and sensory scores. Subjects 'neurological improvement remained stable at a level over both two and 5 years. Two of the four patients had an overall decrease in postoperative pain scores, including discomfort and allodynia in the transition area, pain at the surgical site, and neuropathic pain and nociceptive pain in other areas. Two subjects had durable neuromuscular improvements and increased neuromotor and sensory scores. MRI in all patients showed varying degrees of focal myelomalacia[83]. There was no radiographic evidence of immediate or delayed complications after NSCs injection, including no new areas of spinal cord and soft tissue swelling or enhancement of fluid accumulation[83].

A clinical trial was conducted to study the safety and efficacy of intramedullary transplantation of pedestrian NSCs in 29 patients with chronic traumatic SCI. In the first year after injection, serious adverse events occurred in 4 of 12 chest subjects and 9 of 17 patients with cervical spondylosis. But they did not believe safety issues related to cellular bone marrow injections[84]. Cervical MRI showed a slight increase in T2 signal intensity in 8 of the 17 transplant subjects, with no decrease in motor activity or neuropathic pain. All T2 signal changes disappeared within 6–12 months after transplantation[84]. The safety and efficacy of transplanting human NSCs into cervical spinal cord in patients with chronic C5-7 tetraplegia within 4–24 months after injury were verified in II phase. The dose of cell transplantation increased from 15 million cells to 30 million cells. The patient's upper limit of motor function score (UEMS) did not decrease after cell transplantation. The higher dose of cells (40 million) did not achieve greater improvement on UEMS than the lower doses (15 and 30 million). GRASP (a clinical impairment measure) strength/manual muscle testing and squeezing ability for all participants improved. GRASP intensity and contractility increased by 4.17 and 1.08 points, while contractility performance decreased by 3.5 points, and the spasticity of the upper limbs was generally stable and the spasticity of the upper limbs was generally reduced[85]. Three of the six participants reported no pain on day 28. Two patients had no pain, but reported musculoskeletal pain in the head/neck/shoulder area on day 28. One subject reported musculoskeletal shoulder pain, but did not experience pain on day 28, while musculoskeletal neck pain and neuropathic pain occurred in the thighs, calves, and feet. One-year MRI follow-up data showed the presence of vertebral resection surgery and no significant spinal cord restraint, spinal cord softening, cyst formation, spinal kyphosis, and instability[85]. This also suggests the safety, tolerance, and feasibility of increasing the number of transplanted cells *in vivo*. In another phase I/IIa trial, 20 million human NSCs were transplanted into the thoracic spinal cord of 12 subjects (T2–T11 motor integrity, sensory insufficiency). After 6 years of follow-up, five subjects showed improvement in neurologic function in more than 1 neurologic function pattern, including segmental recovery of light touch, acupuncture, deep anal pressure, and maintained this improvement through at least the first year of follow-up. Two of seven patients developed some sensation below the lesion, and AISA-A increased to B[86]. All patients experienced reliable improvement in sensation, while lower limb motion scores remained unchanged. Cervical spinal imaging showed no tumor formation or malformation in the injured area, and no obvious secondary changes on the spinal cord surface[86] (Table 1). These studies have shown that NSCs transplantation is safe and feasible in treating SCI patients, but there are still differences in treatment effectiveness.

Challenges and existing problems of NSCs in clinical trials

Although the application of NSCs in animal models has been unanimously recognized and made great progress, the therapeutic value of NSCs in clinical trials is still different. As mentioned earlier, NSCs transplantation is safe and feasible in clinical trials, but their therapeutic effect on the functional improvement of patients with SCI are not very ideal, and even some side effects when discharged from hospital. At present, these clinical trials are not enough to support the extensive development and application of NSCs in patients, which may be closely related to many important problems that need to be solved.

The source of NSCs. Although NSCs can be obtained from many sources, such as embryonic tissue, reprogramming, and iPSCs. However, NSCs are obtained from embryonic tissue, but their limited sources, technical problems in isolation, and low purity hinder the widespread development of NSCs therapy in clinical trials. In addition, there are ethical and moral problems with obtaining cells from embryonic tissue. At present, emerging technologies continue to improve and can be used to obtain a large number of NSCs and through identification, promote the development and application of NSCs. iPSCs can differentiate into a large number of high-purity NSCs, but this process takes a long time, and accompanied by safety problems, the transformation into clinical application becomes complicated, and there is genetic instability/uncertainty in the process of transformation/differentiation, which may lead to the risk of tumorigenesis or malformation. Reprogramming somatic cells into NSCs, such as chemical, growth factor, or microRNA-induced cell transdifferentiation, overcomes these shortcomings of the iPSCs-induced NSCs process and provides a very attractive strategy for clinical large-scale production and clinical trials of NSCs. Future direct reprogramming can use free plasmids to replace viral vectors to express lineage-specific transcription factors without the need for transgene integration. Moreover, the use of RNA, miRNA, protein, or small molecule compounds is another safe way to produce clinically applicable somatic cells that transform into NSCs. Another problem faced by direct reprogramming is low conversion efficiency and poor survival rate after direct reprogramming cells are transplanted. As the molecular mechanisms and signaling pathways in the direct reprogramming process are understood, more effective direct reprogramming methods will be developed to generate large-scale induced NSCs for clinical and research use. Therefore, it

Table 1

Clinical trial study of NSCs transplantation in SCI

Cell source	Types of SCI	Cell transplant dose	Transplantation method	Follow-up time	Treatment outcome	Side effects	Refs
Human spinal cord-derived neural stem cells (NSI-566)	T2-T12 SCI	450 000 cells/45 μL of the vehicle	Midline bilateral stereotactic injection	18–27 months	In two subjects, one to two levels of neurological improvement were detected using the ISNCSCI motor and sensory scores. Transplantation of NSCs into patients is feasible and safe.	no serious adverse events	[84]
Human spinal cord-derived NSCs (NSI-566)	SCI from T2 to T12	2×10^5 cells/mL	Bilateral injection into remaining tissue on the side of the injury site	5 years	All subjects tolerated the cell transplantation well, and both patients had improvements in levels of nerve damage, motor scores, and sensory scores. The subject's neurological improvement remained stable during the follow-up period.	No obvious side effects	[85]
Human-derived NSCs	Patients with chronic traumatic SCI with complete and incomplete sensation in the chest and neck	20 M cells and 40 M cells	Intra-bone marrow injection of neural stem cells	6–12 months	8 of the 17 transplant subjects had mild increases in T2 signal changes and no hypokinesia or neuropathic pain occurred. All T2 signal changes subsided.	Four of the 12 thoracic subjects experienced four serious adverse events and nine of the 17 cervical subjects experienced 15 serious adverse events, but they did not consider safety issues related to cell or manual bone marrow injections.	[86]
Human NSCs	Chronic C5-7 quadriplegic patients	Dose increased from 15 000 000 cells–40 000 000 cells	Injection of stem cells into the bone marrow	1 year	There was no evidence of additional SCI, new lesions, or syringeal formation after transplantation. The use of incremental cell doses in patients is safe, tolerable, and feasible. Patients with higher exercise progress (UEMS) and Redefinition Assessment of Strength, Sensitivity, and Prevention (GRASPP) showed a trend towards progress in exercise.	No obvious side effects	[87]
Human central NSCs	Thoracic spinal cord patient (traumatic, complete T2-T11 movement, incomplete sensation)	20 million cells	Open neurosurgery injection above and below the spinal cord injury site	6 years	Short-term and long-term surgery and medical treatment for NSCs transplantation are safe and tolerable. Sensation improved reliably in 5/12, and there was no change in lower limb motion scores.	There was no tumor formation or malformation in the lesion area after transplantation, and there were no structural changes in SCI secondary lesions.	[88]

ISNCSCI, International Standards for Neurological Classification of Spinal Cord Injury.

is necessary to further improve transformation efficiency and ensure induction of NSCs to maximize use and optimize clinical results. To deal with this problem, combined with existing research, we believe that selecting the appropriate vector to carry specific drugs or target genes is the best-optimized solution. Exosomes can deliver siRNAs, microRNAs, and chemotherapeutic drugs and are considered to be major candidates for delivery vehicles for nerve injury treatment[87,88]. It is speculated that small molecule compounds delivered through exosomes can target tissues/cells, practice precise reprogramming, and greatly improve the efficiency of cell reprogramming. In addition, efficient cell sorting techniques will continue to be developed to purify directly transformed cells. In the future, skin fibroblasts will be directly reprogrammed into NSCs closer to their natural state, which will provide an adequate source of cells for clinical transplantation for the treatment of SCI.

The therapeutic effect of NSCs transplantation in animal disease model has been successful and exciting results have been obtained, but the success of animal model should not be directly applied to clinical trials. This is related to the huge physiological differences and biological characteristics between humans and animals. We should fully and detailedly grasp the functional mechanism and functional characteristics of NSCs in basic research, gradually establish clinical standardized treatment plan and implementation plan, and better transition to the stage of clinical trial exploration. In preclinical studies, neurotrophic factors (such as BDNF, NGF, and VEGF) can be overexpressed in NSCs through genetic modification or transfection to enhance their paracrine function, provide a nutritional microenvironment during SCI and promote neural network reconstruction. By transfecting these neurotrophic factors into NSCs, the secretory function of NSCs can be improved and provide a good environmental basis for SCI.

As mentioned above, NSCs/NPCs are multipotent stem cells with the ability to self-renew. They can differentiate into neurons, astrocytes, and oligodendrocytes, and can pass through the blood–brain barrier, thereby replacing damaged cells at the injury site. Moreover, they can secrete a variety of chemokines and neurotrophic factors to benefit nerve regeneration[89]. NSCs transplantation can reduce the inflammatory response, prevent cell apoptosis, inhibit the formation of glial scars, and shrink the injury cavity, thereby promoting the recovery of electrophysiological activities and sensorimotor functions after injury[90,91]. However, transplanting NSCs directly into damaged areas has limited therapeutic effect, which may be due to the difficulty of stem cells surviving and uncontrolled differentiation. Therefore, it can be used for clinical treatment to enhance NSCs activity and direct differentiation of neurons through a combined approach (using bioactive materials such as hydrogels or scaffolds). Three-dimensional (3D)-printed constructs can provide a conducive microenvironment for NSCs survival, proliferation, and differentiation in the injured areas. Koffler *et al*[92], printed a precision-tailored 3D scaffold via a microscale continuous projection printing technology (μCPP) ladened with NPCs. In rat SCI model, the scaffold promoted axons extension into the 3D scaffold and regeneration to caudal side of the injured spinal cord, and significantly improved the functional outcomes[92]. NSC-laden scaffolds by 3D bioprinting are beneficial to the survival and differentiation of NSCs cells into neurons because it simulates the natural environment of the spinal cord, and promote nerve regeneration and motor function recovery[93]. Besides, Yang *et al*[94], created NSCs-laden scaffolds via extrusion-based 3D bioprinting. NSCs were wrapped in an ECM-like hydrogel to form a 3D nerve fiber-like structure. The NSCs in the scaffold survived and improved the microenvironment in the injured site, and subsequently promoted nerve regeneration, the formation of nerve relay, and effective motor function recovery in rats[94]. Although the use of bioactive materials in basic research has significantly enhanced the functional effect of NSCs in treating SCI, but the combination application does not seem feasible in short-term clinical trials due to the interaction between the combination grafts and the effectiveness and safety of the combination therapy. There is still insufficient evidence to confirm the effectiveness and safety of the combination therapy. Therefore, based on a full understanding of NSCs transplantation therapy, we should control the performance, degradation, safety, and metabolism of bioactive materials in the body, so as to evaluate the safety and effectiveness of joint strategies for treatment. With the continuous exploration in the field of nerve regeneration, the use of combined biomaterials has great application prospects in SCI.

The standardized culture system of NSCs *in vitro* to obtain high purity and stable cells, the best time for transplantation, and cell dose. All these can lead to unexpected side effects or even treatment failure in clinical trials. Cell culture *in vitro* can be affected by internal and external environment, such as cell contamination may lead to cell function instability and abnormal cells. It is recommended to conduct in-depth sequencing of each batch of NSCs products and check for side effects, including tumorigenesis. Currently, there is no unified standard for the best time of NSCs transplantation, which should be evaluated for each type of acute, chronic, and subacute SCI. Moreover, the methods and strategies of NSCs transplantation. At present, the routinely used techniques include vein transplantation, intracranial transplantation, intrathecal spinal cord transplantation, or intrathecal transplantation, which can be well completed by surgeons, and clinical trials have shown that patients can be well tolerated. However, there are great differences in the therapeutic effects of different transplantation methods, such as, NSCs are transplanted into the host through vein, but these cells can flow through the veins throughout the body, which may cause other side effects and few cells may play a therapeutic role in SCI. Therefore, it is necessary to further explore the best transplant method to solve these problems.

The survival rate and location tracking of NSCs transplantation *in vivo* are also the key to poor therapeutic effect. Therefore, to improve and promote the survival rate and survival time of NSCs *in vivo*, advanced imaging techniques are needed to monitor the physiological status of NSCs transplanted *in vivo* and give full play to the function of NSCs in the treatment of SCI. Studies on the transplantation of NSCs into damaged nerves, including SCI, have not reported on cell survival and colonization in the injured part, nor have they quantified and tracked the survival of transplanted cells. This may be related to the fact that tracking the transplanted cells may be very difficult over time after the cells are transplanted into the host. Therefore, it is necessary to improve tracking and quantification methods, and use new methods to label transplanted cells, such as nuclear probes and paramagnetic nanoparticles to label cells, to further understand the situation of transplanted cells *in vivo*.

Although SCI functional recovery can be achieved in a variety of ways, including genetic regulation, cell transplantation, and biomaterials, these combinations have produced greater effects in SCI and can lead to better functional recovery[95,96]. However, these methods only achieve axon regeneration and do not spontaneously lead to meaningful functional recovery. Therefore, the formation and remodeling of functional neural circuits is crucial for recovery from SCI. In recent years, functional electrical stimulation has developed into an important therapeutic intervention that clinicians can use to help patients with SCI regain the ability to stand, walk, reach, and grab[97]. Brain-computer interfaces (BCI), as the foundation of a new generation of neural prosthesis devices, can drive robotic systems or stimulate muscles to restore the movement of paralyzed individuals by recording electrical signals from the brain, implanting electrodes to record the expected movement of paralyzed parts of the body, and decoding electronic signals from the motor cortex[98]. BCI is an emerging SCI intervention strategy that can be used to restore vitality to paralyzed limbs. BCI based on local field potential can control spinal cord stimulation and improve forearm function in cervical SCI rats[99]. BCI, combined with functional electrical stimulation, transmits electrical pulses directly to surrounding nerves and muscle tissue through electrical stimulation, activate paralyzed muscles, cause contractions, and assist in specific body movements[100]. A clinical trial reported that BCI intervention promotes meaningful cortical sensorimotor plasticity and ultimately maximize the restoration of arm function in traumatic subacute SCI in the neck[101]. BCI's invasive technology has shown hope in the treatment of SCI, but currently existing research focuses on the application of BCI to improve the rehabilitation and quality of life of SCI patients. There is no technology that can restore complete functional autonomy of SCI patients. The current techniques and results of BCI vary widely, as BCI is still in the early stages of development and further clinical research is needed to optimize the prognosis of SCI patients. In addition, the feasibility and stability of the BCI system in long-term application in patients are still controversial. Future studies should obtain a larger number of electrodes and reliable data through more data to confirm this stability. However, through searching the literature, no relevant studies have reported the application of NSCs combined with BCI in the treatment of SCI.

Conclusion

SCI can lead to disastrous consequences, including sensory, motor, and autonomic dysfunction, which seriously affect the quality of life of patients. There are no effective treatment methods. The emergence of cell transplantation provides a foreground treatment for SCI. NSCs are a kind of pluripotent stem cells that maintain the growth and development of the nervous system. NSCs transplantation has been widely used in the field of nerve injury repair and nerve regeneration. Different studies have confirmed the therapeutic function of NSCs in SCI. The mechanisms of promoting the repair and functional recovery of SCI include differentiation into neurons, secretion of neurotrophic factors, immune regulation and anti-inflammation, promote axonal regeneration and myelination. NSCs transplantation has achieved some results in clinical trials of patients with SCI. Although the application of NSCs in clinical trials is feasible and safe, the therapeutic effect of NSCs is still not ideal, and there is a lack of sufficient evidence to prove it. At present, the development of clinical trials of NSCs transplantation is still very limited and is currently in the preliminary exploration stage. There are many problems and challenges, which hinder the extensive clinical development of NSCs transplantation. Despite these problems and challenges, it will not prevent researchers from further exploring and solving them, and these problems will eventually be solved with the passage of time. In short, NSCs are a kind of therapeutic cells with foreground in the treatment of SCI.

Ethical approval

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Consent

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Author contributions

Completed the writing and design of articles, data collection and revision, and obtained funds: W.-j.Z; completed the revision, design, supervision and agreed to the final version of the article: J.Z.

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The authors declare no conflicts of interest.

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All data generated or analyzed during this study are included in this article. And we have not used other data that has already been published. All the data presented in this article are original results derived from this study.

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