Stem cell-based therapies to treat spinal cord injury: a review

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Abstract: Spinal cord injury (SCI) is a devastating condition and major burden on society and individuals. Currently, neurorestorative strategies, including stem cell therapy products or mature/functionally differentiated cell-derived cell therapy products, can restore patients with chronic complete SCI to some degree of neurological functions. The stem cells for neurorestoration include neural stem cells, mesenchymal stem cells, embryonic stem cells, induced pluripotent stem cells, etc. A better understanding of the merits, demerits and precise function of different stem cells in the treatment of SCI may aid in the development of neurorestorative strategies. However, the efficacy, safety and ethical concerns of stem cell-based therapy continue to be challenged. Nonetheless, stem cell-based therapies hold promise of widespread applications, particularly in areas of SCI, and have the potential to be novel therapeutics, which contributes to the repair of SCI. This review mainly focused on recent advances regarding the stem cell-based therapies in the treatment of SCI and discussed future perspectives in this field.

Keywords: spinal cord injury, neural stem cells, bone marrow-derived mesenchymal stem cells, adipose-derived stem cells, embryonic stem cells, induced pluripotent stem cells

Introduction

Spinal cord injury (SCI) is a devastating condition resulting from traumatic accidents with a high morbidity and mortality, and there are no effective treatments available by conventional treatments.1-3 Currently, neurorestorative strategies, including cell therapy (stem cell products or mature/functionally differentiated cell-derived cell therapy products according to classification of US Food and Drug Administration,85 neurostimulation/neuromodulation, neuroprosthesis, neurotization or nerve bridging, neurorehabilitation and combined therapies, can restore patients with chronic complete SCI to some degree of neurological functions.4 From the inception of the Orphan Drug Act in 1983 through March 31, 2010, the Office of Orphan Products Development received 27 applications for orphan designation of stem cell-based products (SCBPs); the sources of orphan-designated SCBPs include human embryonic stem cells (hESCs), fetal porcine cells, peripheral blood, umbilical cord blood, mesenchymal tissue, olfactory tissue and bone marrow.5 The weight-bearing nature and flexible nature of vertebrae make it particularly susceptible to general SCI.6 SCI is characteristically composed of primary injury and secondary injury.7 The primary phase is a direct consequence of physical trauma to the spinal cord, and it can cause the discontinuation of axonal projections to release neurotoxic compounds and inflammatory mediators that contribute to neuronal and oligodendroglial cell
The secondary pathological injury is a complex damage in the cellular level and occurs over the hours, days and even weeks following the initial injury, including loss of myelin, degeneration of axons and formation of a glial scar that inhibits spontaneous regeneration, and these processes can cause a significant degeneration and consequent functional loss. Furthermore, some factors can contribute to the events after primary SCI, including unbalance of ion, glutamate release and lipid peroxidation. Until now, a considerable amount of research has been carried out to find a more effective way to restore SCI functions. However, there is still no neuroprotective and regenerative therapies for the injured spinal cord.

Stem cell transplantation was first applied to the patients with cancers of the blood and bone marrow in the 1950s. Stem cells are defined as cells with the ability to renew themselves continuously and possess pluripotent ability to differentiate into many cell types under particular physiological conditions. Stem cells are classified into embryonic stem cells (ESCs), fetal stem cells (FSCs) or adult stem cells (ASCs) based on their origin. Stem cell-based therapies hold promise of widespread applications particularly in SCI areas. The main advantage of stem cells for treating SCI is their self-renewal capacity. Cell transplantation is considered to be a feasible means to compensate for injury-induced cell and tissue loss following SCI.

The goal of this brief review was to review the recent studies about stem cell-based therapies and advances in the SCI treatment. Further understanding of the potential of stem cell-based therapies may lead to additional therapeutic alternatives in SCI.

**Neural stem cells (NSCs)**

NSCs are multipotent prototype or stromal cells having multilineage potential that can differentiate into adipocytes, myocytes, osteocytes and chondrocytes, and the MSCs can be isolated from bone marrow, adipose tissue, placenta, amniotic fluid and umbilical cord. In the current review, we present our recent understandings on the applications of bone marrow-derived mesenchymal stem cells (BMSCs) and adipose-derived stem cells (ADSCs) in the treatment of SCI.

MSCs are multipotent prototype or stromal cells having multilineage potential that can differentiate into adipocytes, myocytes, osteocytes and chondrocytes, and the MSCs can be isolated from bone marrow, adipose tissue, placenta, amniotic fluid and umbilical cord. BMSCs are one of the most studied cell types for SCI. BMSCs can be isolated from bone marrow because of their property of tendency to adhere to tissue culture plastic. BMSCs have the following advantages: easy to isolate and culture, low immunogenicity, pluripotency and capability to form different phenotypes. Although previous studies have shown that the transplantation of BMSCs can increase sensory function after SCI, the effects on SCI-induced chronic neuropathic pain are unclear. BMSCs have been reported to overcome germ layer commitment and differentiate into neuron-like cells expressing neuronal markers. The transplantation of BMSCs can promote the secretion of various immunoregulatory macromolecules that contribute to
create regenerative microenvironments in the injured spinal cord.41 The immunoregulatory phenomenon was considered to be a multifactorial process that requires both direct intercellular contacts and contact-independent paracrine signaling governed through various molecules, including prostaglandin E2 (PGE2), interleukin 6 (IL-6), interleukin 10 (IL-10), inducible nitric oxide synthase (iNOS), indoleamine 2,3-dioxygenase (IDO), tumor necrosis factor-inducible gene 6 protein (TSG-6) and so on.42 These molecules participate in the processes of proliferation, differentiation, migration or apoptosis in different immune cells.43 Furthermore, a previous study suggested that infusion of BMSCs could lead to a more reducing cysteine (Cys) and glutathione (GSH) redox state and had antioxidant effects in vivo.44 A previous study suggested that treatment with BMSCs had a positive effect on behavioral outcome and histopathological assessment after SCI.45 In other studies, the authors injected BMSCs intravenously, and this could facilitate remyelination after SCI and locomotor recovery.46,47 Compared with intraspinally injection, intravenous injection has the advantage of minimally invasive and minimal hemorrhage incidence.48 A recent study showed that using magnets could increase the targeting efficiency and enhance the efficiency of stem cell delivery in SCI.48,49 However, the underlying mechanisms of BMSCs’ protective effects in SCI are still unclear and need to be further investigated.

ADSCs are isolated from the stromal vascular fraction (SVF) of adipose tissue, and they have a strong resemblance to BMSCs due to their common cell surface markers, similar gene expression profiles and similar differentiation potentials.50–52 ADSCs have recently been identified as alternative stem cells, and previous studies have shown that the cells could survive and integrate into the spinal cord.53–55 ADSCs have been an ideal choice for cell replacement therapy, and ADSCs have the following advantages: easy to isolate and culture, reliable biosafety and free of immunogenicity.55,56 A previous study that used rat models of spinal cord contusion injury showed that injection of ADSC-transdifferentiated motor neurons into the impact site and transplantation of glial cell line-derived neurotrophic factor–gelfoam complex into the myelin sheath after 7 days exhibited beneficial effects on recovery of motor function.57 Transplanted ADSCs can significantly decrease astrocytic network and stimulate axonal sprouting.58 Moreover, transplantation of three-dimensional (3D) cell mass of adipose-derived stem cells (3DCM-ADSCs) significantly improved functional recovery compared with transplantation of ASCs, and this finding may be effective for the treatment of SCI and neural ischemia.59 Neurogenin-2 (Ngn2) is a gene that promotes neuronal differentiation, and a previous study showed that transplantation of Ngn2-overexpressed ADSCs can improve the local microenvironment and promote the functional recovery after SCI.60 Therefore, this study suggested that ADSCs might provide an ideal source for further stem cell research with potential therapeutic application for SCI.

Embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs)

ESCs are pluripotent cells derived from the inner cell mass of a 1-week blastocyst.61 The chief difficulty with the use of ESCs is the immunogenicity, and then, ESCs can stimulate the immune response.62 Transplantation of ESCs may be a practical approach to treat SCI because they can be repeatedly passaged in culture and differentiated into neuronal or glial cells for transplantation.63 ESCs can directly differentiate to oligodendrocytes and secrete trophic factors, such as hepatocyte growth factor and brain-derived neurotrophic factor (BDNF), which may be beneficial for promoting axonal regeneration and neurite outgrowth after SCI.64 In another study, the implanted ESCs could not only restore lost myelin in the injured spinal cord but also differentiate into mature oligodendrocytes that were capable of myelinating axons.65 Thus, at present, transplantation of hESC-derived neural cells, including neuron and oligodendrocytes, is a promising therapy for SCI.66

iPSCs are a type of pluripotent stem cells that can be created from adult somatic cells by “reprogramming” via the transduction of pluripotency genes.67 iPSCs are envisaged as advanced source for cell replacement therapy in regenerative medicine.68 The key advantage of iPSCs for human cell therapy is avoiding immune rejection, ethical constraints and tissue donation.69 However, iPSCs have the potential for uncontrolled proliferation and even tumor formation after transplantation in SCI models.69 Therefore, a careful screening of oncogenic capacity is essential prior to transplantation. Some previous studies have developed useful and attractive methods to solve the problem of tumorigenicity. Itakura et al70 found that immunoregulation could ablate tumors that are formed after transplantation, and this probably has to do with the infiltration of inflammatory cells, such as lymphocytes and microglia. Another study showed that inhibition of Notch signaling could induce the NPCs to more mature cells with limited proliferation.71 Furthermore, a recent study showed that neuroepithelial-like stem cells from human iPSCs (hiPSCIt-NES cells) could differentiate into neural lineages in the
mouse model of SCI and promote functional recovery of hind limb motor function after transplantation. Romanyuk et al used a rat model of thoracic contusion in SCI, and the result showed that transplantation of neural precursors derived from human iPSCs (iPSC-NPs) 1 week after SCI promoted tissue sparing and improvement in motor function. The transplantation field of iPSCs is rapidly progressing, and transplantation of iPSCs' products can produce functional recovery by replacing lost cells and/or modulating the microenvironment; yet iPSCs should be transplanted with caution because of the safety of the cell lines and the risk of tumor formation, which can be harmful to patients.

**Problems to overcome in the application of stem cells for SCI**

In this review, we focus on the stem cell therapies that have the potential to repair the injured spinal cord, and the comparison between different types of stem cells is summarized in Table 1. Although recently stem cell therapies have made great progress and are widely accepted in the treatment of SCI for promoting morphological recovery and functional recovery via various mechanisms (Figure 1), but they still face great challenges. First, the insufficient sources and ethical constraints concerning stem cells greatly hindered their clinical application. Although iPSCs are envisaged as an advanced source for cell replacement therapy without these problems, yet studies with animals are urgently required to demonstrate the efficacy and safety of replacement therapy of iPSCs. Moreover, the issue of stem cell tumorigenicity needs to be considered. Second, the transplanted stem cells often maintain in the state of undifferentiation, and the neuronal induction efficiencies are lower than desirable. The inhibitory microenvironment of injured spinal cord is mainly due to local expression of inhibitory factors and glial scar, and the microenvironment can limit the regenerative capacity of endogenous or transplanted cells. Therefore, how to improve the microenvironment after SCI is related to the survival and differentiation of transplanted stem cells.

Furthermore, another problem is that the transplanted stem cells must be able to make right connections with the host neural network. During recent decades, with the development of tissue engineering, engineered biomaterials have been explored for their ability to support axonal regrowth and neuronal differentiation, and more and more biomaterials are applied to stem cell-based replacement

**Table 1** Comparison between different types of stem cells

<table>
<thead>
<tr>
<th>Types of stem cells</th>
<th>Therapeutic mechanisms and advantages</th>
<th>Disadvantages</th>
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<tbody>
<tr>
<td>NSCs</td>
<td>Neuronal replacement therapy</td>
<td>Undifferentiation or differentiation along the glial lineage after transplantation</td>
</tr>
<tr>
<td></td>
<td>Remyelinate the demyelinated axons</td>
<td>Ethical constraints</td>
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<td></td>
<td>Secrete neurotrophic factors</td>
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<tr>
<td></td>
<td>Ameliorate T-cell receptor-mediated T-cell activation and inhibit signaling of inflammatory cytokines in immune cells</td>
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<tr>
<td>MSCs</td>
<td>Immunomodulation</td>
<td>Tumorigenicity</td>
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<td></td>
<td>Anti-apoptotic effects</td>
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<td></td>
<td>Secrete neurotrophic factors and cytokines</td>
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<td></td>
<td>Permissive cellular substrate for promotion of host axonal growth</td>
<td></td>
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<tr>
<td></td>
<td>Easily extracted and cultivated in large numbers</td>
<td></td>
</tr>
<tr>
<td></td>
<td>No ethical constraints</td>
<td></td>
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<tr>
<td>ESCs</td>
<td>Can be repeatedly passaged in culture</td>
<td>Immunogenicity</td>
</tr>
<tr>
<td></td>
<td>Differentiate into neuronal or glial cells</td>
<td>Ethical constraints</td>
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<tr>
<td></td>
<td>Secrete trophic factors</td>
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<tr>
<td>iPSCs</td>
<td>Avoid immune rejection</td>
<td>Tumorigenicity</td>
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<tr>
<td></td>
<td>No ethical constraints and tissue donation</td>
<td>Genetic and epigenetic abnormalities</td>
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**Abbreviations:** NSCs, neural stem cells; MSCs, mesenchymal stem cells; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells.

**Figure 1** Major stem cell sources and the therapeutic focus of transplantation of stem cells in SCI.

- SCI, spinal cord injury; BMSCs, bone marrow-derived mesenchymal stem cells; ADSCs, adipose-derived stem cells; ESCs, embryonic stem cells; iPSCs, induced pluripotent stem cells.
therapy of SCI. The biocompatible scaffold can provide
3D space; appropriate chemical, physical and mechanical
properties for cell proliferation and differentiation and tissue
formation and has the potential to transform the inhibitory
microenvironment into a permissive microenvironment,
which further promotes axonal regrowth.76,77 Several
types of biomaterials have been suggested for achieving
improved functional recovery in the patients with SCI. In a
recent study, implanting cetuximab-modified linear-ordered
collagen scaffolds (LOCS) into SCI lesion sites in dogs
could decrease chondroitin sulfate proteoglycan (CSPG)
at the lesion site and results in neuronal regeneration and sig-
nificant locomotion recovery.78 Furthermore, new 3D culture
systems have emerged recently that have demonstrated not
only the feasibility of creating complex, organized spinal
tissue but also how bioengineered 3D scaffolds can be used
to control the transplant microenvironment.79 A previous
study demonstrated that a collagen/heparin sulfate scaffold
fabricated by a 3D bioprinter could enhance the mechanical
properties of collagen and provide continuous guidance
channels for axons, which would improve the neurological
function in the patients with SCI.80 Moreover, nanoparticles
can deliver the therapeutic molecules to the target tissue of
interest and reduce the side effects of untargeted therapies in
unwanted areas; hence, nanobiomedical systems are believed
to be potential to provide new therapeutic availability for the
treatment of SCI.81 Thus, combining neural tissue-engineered
scaffolds with stem cell-based therapies can improve trans-
plant efficacy and foster host tissue regeneration, and this
combination is the future direction of the stem cell-based
therapies following SCI.

Moreover, administration of drug or trophic factors is also
a treatment strategy for promoting regeneration of injured spi-
nal cord, and the combination therapy is expected to provide
superior clinical effectiveness.82 Karimi-Abdolrezae et al83
added growth factors (epidermal growth factor, basic fibroblast
growth factor, platelet-derived growth factor) to chondroitinase
ABC (C-ABC) and administrated them in the rats after SCI,
and this treatment could enhance proliferation of endogenous
NPCs, increase new vascular formation and suppress inflam-
matory reaction. In another study, semaphorin-3A inhibitor
was administered to transect spinal cord of rats, and it could
promote the elongation of neuronal axons and enhance the
Schwann cell-mediated myelinization and angiogenesis.84

Taken together, although stem cell-based therapies had
some problems at present, but they still have good developing
prospects, and further investigations will be needed in order
to improve the transition to the clinic.

Conclusion
The main goals of stem cell-based therapies for SCI are the
neuron replacement and neurological, structural and func-
tional restoration after SCI. Stem cell-based therapies hold
great promise to become an effective therapeutic approach
for SCI. Although there are a few different types of stem
cells that can serve as a renewable cell source in cell-based
therapy for patients suffering from SCI, yet which type of
stem cells is most suitable for cell replacement therapy in
patients with SCI still needs to be clarified. Furthermore,
the efficacy, safety and ethical concerns of stem cell-based
replacement therapy continue to be challenged.

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Disclosure
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