Current Status of Spinal Cord Regenerative Therapies: A Review

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Abstract

Spinal cord injury (SCI) is any injury resulting from an insult to the spinal cord that disrupts its major functions, either completely or incompletely, and it can be caused by both traumatic and nontraumatic events. The number of SCI patients has been continuously increasing due to increasing number of motor vehicles and average age of patients is constantly decreasing. After SCI, nerve cells located at the injured site are severely damaged and eventually die, and these dead cells are cleared away by the immune system and in turn a cavity remains. Unfortunately, till date no effective treatment strategy exists ensuring functional recovery after SCI. There is an imperative need for the development of therapies to reduce the enormous physical and financial burdens of people afflicted with SCI. The surgical treatment has been known to aid in rehabilitation, but cannot substantially improve neurologic and functional outcome after SCI. The stem cell–based therapy has been proposed as a promising treatment strategy for SCI. Many of the current strategies for treatment of SCI involve replacing the cells lost to injury with cells derived from alternative sources, such as Schwann cells, oligodendrocyte precursor cells, and neural stem cells. This review discusses the present status of various cell-based therapies, which are being used for treating SCI.

Keywords
- stem cell
- spinal cord
- regeneration
- trauma
- injury

Introduction

Spinal cord injury (SCI) is defined as any injury resulting from an insult to the spinal cord that disrupts its major functions, either completely or incompletely.[1] Spinal cord injuries can be caused by both traumatic and nontraumatic events. The leading causes of traumatic SCI include motor vehicle accidents, falls, sport-related injuries, industrial accidents, gunshot wounds, and assault. Nontraumatic causes of SCI include stroke, infections, degenerative joints/bone disease, neoplasms, and other neurologic conditions, such as multiple sclerosis.[2–4]

The number of patients suffering from SCI is continuously increasing, and each year an estimated 180,000 individuals over the world sustain new injury.[5] In the United States, the incidence of SCI has been estimated to be approximately 40 cases per million people per year or around 12,000 cases per year with around 250,000 individuals living with SCIs.[6,7] The average age at the time of injury has gradually increased from a reported 29 years in the mid-1970s to a current average of around 40. Men are more at risk for SCI than women.[8,9]

The spinal cord contains millions of nerve cells, which are organized into multiple tracts to convey electrical signals between the brain and the rest of the body. Once the spinal cord is injured, these nerve cells located at the injured site are severely damaged and eventually die. The dead cells are cleared away by the immune system and in turn a cavity

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remains.[10] The glial scar tissue that forms around the cavitation prevents axonal regeneration and, as a consequence of this, no more electrical signals can be transmitted, resulting in loss of motor, sensory, and autonomic functions.[11] Many of these injuries result in partial or complete paralysis, and only less than 1% of people are able to recover complete neurologic function.

Unfortunately, till date there has been no effective treatment strategy ensuring functional recovery after SCI. There is an imperative need for the development of therapies to reduce the enormous physical and financial burdens of people afflicted with SCI.

There have been various drug treatment strategies for SCI, but none of them, other than methylprednisolone, have achieved a substantial clinical usage. The surgical treatment has been known to aid in rehabilitation, but cannot substantially improve neurologic and functional outcome after SCI. There have been several experimental studies in animal models, involving strategies such as local spine cooling and oscillating field stimulation aimed at reducing the paralyzing effects of injury and promoting regrowth of functional nerve fibers.[12] Several these experimental treatments have now reached the stage of controlled human trials.[13,14] The stem cell–based therapy has been proposed as a promising treatment strategy for SCI. Many of the current strategies for treatment of SCI involve replacing the cells lost to injury with cells derived from alternative sources, such as Schwann cells (SCs), oligodendrocyte precursor cells (OPCs), and neural stem cells (NSCs).[15] However, none of these developments have reached even limited use in the clinical care of human SCI.[16] Currently, interventions to promote recovery from SCI using the cellular regeneration therapies involve stem cell transplantation with an aim to supply new cells to replace lost ones and to optimize the spinal cord for natural recovery. ►**Fig. 1** shows schematic flow of how stem cell could potentially promote recovery after SCI. Furthermore, evidence continues to show that these implanted cells are able to induce neuronal plasticity and modify their microenvironment by promoting axonal elongation, collateral sprouting, remyelination, and synapse formation.[17]

This review aims to discuss the present status of various cell-based therapies, which are being used for treating SCI.

Various cells used in the regeneration in spinal cord injuries are discussed as follows.

Schwann Cells

Schwann cells (SCs) produce the myelin sheath in neurons. The transplanted SCs play a key role in regeneration of axons after injury, by secreting a variety of growth factors, including neurotrophin-3 (NT-3), nerve growth factor (NGF), brain-derived nerve factor (BDNF), ciliary neurotrophic factor (CNTF), and fibroblast growth factor (FGF) that contribute to neuronal survival, generation of cell adhesion molecules, and extracellular matrix proteins that support axonal growth. The SC also migrates to the site of injury after SCI, despite being part of the peripheral nervous system, and they can be easily expanded in vitro, allowing for autologous cell transplantation to avoid immune response.[18]

Xu et al used nerve guidance conduits (NGCs) containing SC in a complete transaction injury rat model and concluded that the group with NGCs containing SC showed increased remyelination as well as propriospinal and sensory axonal regeneration as compared with controls.[19]

A few other studies have looked at genetically modifying Schwann cells to secrete increased amounts of growth factors, specifically NGF and BDNF.[20–22] Although the genetically modified cells promoted more axonal regeneration than unmodified Schwann cells, no functional recovery was observed with either cell line. Additionally, several studies have used Schwann cells in combination with other strategies for treatment of SCI. Chondroitinase ABC enhances Schwann cell–induced axonal regeneration, but it

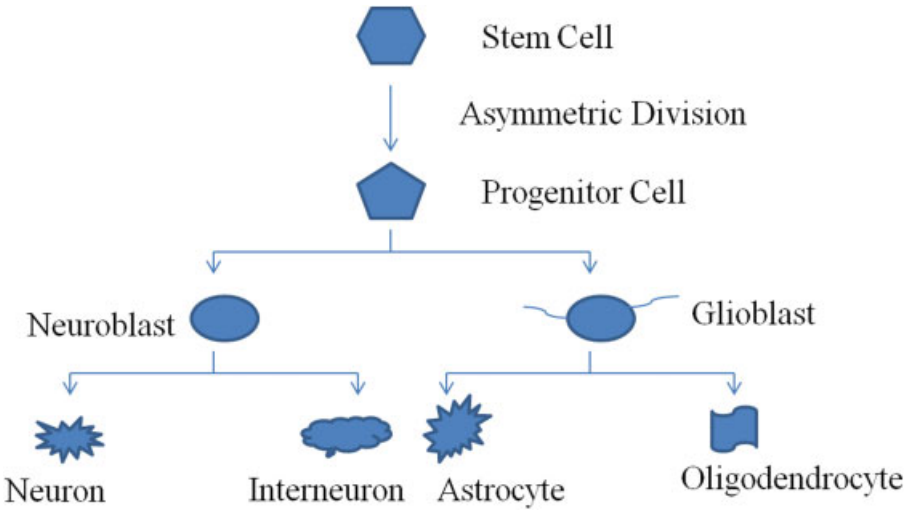

Fig. 1 Schematic illustration showing the stem cell differentiation during recovery after SCI.

does not lead to functional recovery, as studied in T9 dorsal hemisection injury model.[23] Combining cyclic adenosine monophosphate (cAMP) with Schwann cells as therapy for treatment of a T8 mild contusion injury resulted in functional recovery as assessed by the Basso-Beattie-Bresnahan (BBB) score.[24] This study used an inhibitor against the phosphodiesterase that hydrolyzes cAMP to extend its signaling, which allows the neurons to extend into otherwise inhibitory substrates.

Thus, SC works best when used in combination with other therapeutic approaches that minimize the inhibitory nature of the lesion site.

Embryonic Stem Cells

Embryonic stem cells (ESCs) are pluripotent cells found in the blastocyst. They have a capability of differentiating into all three primary germ cell layers and generating all cell types, including oligodendrocytes, astrocytes, or neurons, making ESCs a perfect candidate for cell therapy.[25] However, there have been ethical considerations, surrounding the use of human ESCs, as obtaining them require the destruction of several human embryos or fertilized oocytes.

Mouse ESCs have been investigated as a starting point for developing potential therapies for SCI. These cells are attractive for preclinical studies because they are easy to culture and readily differentiate into cells found in CNS. One of the first studies that looked at using mouse ESCs for treatment of SCI involved a multistep process that promoted the formation of oligodendrocytes and astrocytes for transplantation.[26] Once transplanted into the spinal cord, these cells demonstrated the ability to restore myelination in myelin-deficient *shiverer* rats, indicating the potential of mouse ESCs to treat SCI.

Other studies have demonstrated that mouse ESCs can survive in the spinal cord for more than 50 days, suggesting their potential as a long-term treatment for SCI.[27] A more recent study showed that mouse ESCs pretreated with a lecithinized BDNF implanted 9 days after T9/10 contusion injury showed better differentiation and promoted increased functional recovery afterward, as assessed by BBB compared with cells treated with normal BDNF, demonstrating the influence of growth factor treatments on ESC differentiation.[28] Recent work has looked at different types of genetically modified mouse ESCs and their impact on SCI. Mouse ESCs were modified to overexpress the bcl-2 protein, which blocks apoptosis, and implanted 9 days after contusion injury.[29] However, these cells did not produce an increase in functional recovery and resulted in tumor-like growths. This study illustrates the importance of selecting the appropriate proteins when genetically modifying cells for transplantation.

Another study examined the effect of transfecting mouse ESCs with the L1 adhesion molecule, which promotes neuronal outgrowth, to determine the effect on cell behavior.[30] These cells were injected both rostral and caudal to the injury site 7 days after a T7–9 compression injury. After 1 month, the cells transfected with L1 showed increased survival and migration compared with untransfected ESCs. Overall, mouse ESCs serve a model cell culture system for determining effective strategies for treatment of SCI due to the ease of culture and ability to be genetically modified. These strategies provide a starting place for translating such methods to human ESCs.

Development of methods and strategies for using human ESCs for treatment of SCI allows for translation to clinical studies. One of the challenges of developing human ESC therapies is determining the necessary cues to promote differentiation in the desired cell types, such as oligodendrocytes and motor neurons. Although the work done with mouse ESCs provides a good starting point, the end goal is to determine human ESC differentiation protocols. Similar to the mouse ESCs, testing the ability of human ESCs to promote recovery in rat injury models requires immunosuppression. Many of the human ESC lines that currently exist have been cultured in the presence of mouse feeder cell layers, making them undesirable for use in clinical trials. Other major challenges include developing person-specific ESC lines to allow transplantation without immunosuppression in a clinical setting.

Keirstead laboratory developed protocols that produce large numbers of oligodendrocytes from human ESCs.[31–34] Their time-intensive differentiation protocol involves multiple selection steps with serum-free media to produce OPCs that can differentiate into mature oligodendrocytes in vitro and in vivo.[31] They implanted these cells into myelin deficient shiverer mice 1 week after T10 contusion injury. The transplanted cells integrated into the spinal cord, differentiated into oligodendrocytes and restored myelination.[32]

The clinical applications of human embryonic stem cell (hESC) critically depend on their ability to differentiate toward defined and purified neural cell types in vitro. Recently, considerable progress has been achieved in improving the methods for differentiation of hESC into neural or neuronal precursors prior to cell transplantation in animal models of SCI.[35–38] Despite promising results obtained in preclinical studies, the challenges in using ESCs include determining the correct cues to direct differentiation in the specific desired cell types in vitro and in vivo.[39]

Mesenchymal Stem Cells/Bone Marrow Stem cells

The bone marrow (BM) is a source of stem cells and it is the only organ where two types of stem cells can be found: coexisting and cooperating. The hematopoietic stem cells (HSCs) produce blood cells, whereas mesenchymal stem cells/mesenchymal stromal cells (MSCs) can be found in the stromal layer adjacent to hematopoietic cells and support hematopoietic cells, regulating the microenvironment and facilitating the maturation of blood cells. The MSCs can also be isolated from other regions such as adipose tissue, neonatal tissue (such as umbilical cord and placenta), and fetal tissues such as the lungs, liver, and blood.[40] From a clinical perspective, MSCs are attractive for transplantation

because they are easily obtained from BM and can be transplanted back into the original donor, eliminating the risk of rejection.

In 2006, Moviglia et al reported a combined treatment with MSCs and autoimmune T (AT) cells in two SCI patients. The main goal behind this approach is to control inflammatory activity to create the ideal microenvironment for cell transplantation, as it has been shown that AT cells are essential for tissue repair. Both patients reported motor improvement and no adverse effects.[41]

Pal et al transplanted autologous MSCs via lumbar puncture in 30 patients of SCI. There were no significant differences in the MRI scans taken at baseline and at the 1-year follow-up. Similarly, somatosensory evoked potentials (SSEPs), motor evoked potentials (MEPs), and nerve conduction velocity (NCV) measurements revealed no significant changes. Despite these negative electrophysiologic findings, patients reported significant recovery: initially, the return of bladder sensation, followed by bladder regulation and bowel function, then improvement in sensory perception, and finally in motor function.[42]

Another study compared the transplantation of MSCs to the conventional treatment in SCI, and confirmed the safety and feasibility of using this technique via lumbar puncture; however, even though recovery was reported, it was not found to be statistically significant.[43]

Mazzini et al studied 19 patients of SCI with a goal to study and evaluate the safety and feasibility of MSC transplantation. All patients were monitored for adverse effects of therapy and magnetic resonance imaging (MRI), and tractography suggested no mass at the injection site or within the neuraxis, no syringomyelia or pseudomeningocele formation, and suggested the therapy to be safe. However, the patients did not report any improvement in the quality of life after either form of therapy.[44]

Hofstetter et al transplanted BMSCs into T7 contusion injury model of SCI both immediately following injury and 7 days postinjury.[45] The cells transplanted 7 days postinjury showed better rates of survival and formed bundles that bridged the lesion. Implantation of BMSCs also led to an increase in BBB scores compared with controls.

Various other studies have looked at the feasibility of different methods of intravenous injection for BMSC delivery,[46–48] but a study by Vaquero et al showed that direct injection of BMSCs into the site of a T6–8 contusion injury promoted superior functional recovery when compared with intravenous injections of BMSCs.[49]

Additional work has been done to clarify the mechanisms by which BMSCs promote functional recovery. One study demonstrated that BMSCs help guide regenerating axons across the injury site when implanted 2 days after a T8 contusion injury and can help promote recovery by restoring the stepping control circuitry.[50] A more recent study showed that BMSCs express the gamma aminobutyric acid (GABA) receptor.[51] BMSCs also stimulate phosphoinositide-3-kinase and mitogen-activated protein kinase signaling in neurons, which promotes their survival.[52] All of these mechanisms contribute to the success of BMSC transplantation as a treatment for SCI.

Olfactory Ensheathing Cells

The neurogenesis in olfactory system continues to take place throughout a person's life. The stem cells proliferate to generate new sensory neurons in the basal layer of olfactory epithelium. The stem cells proliferate in the subventricular zone (SVZ) of the forebrain, generating neural progenitors, which migrate to the olfactory bulb to create new interneurons, within the central nervous system (CNS). In case of injury, these neurons are immediately replaced through an increase in neurogenesis.

Because of their ability to guide the connections between the peripheral nervous system (PNS) and the CNS, and to their ability to differentiate into nonolfactory cell types, these multipotent cells are excellent candidates for cell transplantation.[53] OECs can be harvested and then transplanted back into the original human donor, eliminating rejection. Currently, phase I clinical trials using autologous OEC transplantation are underway where these cells have been demonstrated to be safe for up to 1 year after treatment.[54]

There have been several studies in literature supporting the use of OECs reporting improved outcomes in both experimental models[55–59] and clinical settings.[60,61]

The use of genetically modified OECs with the aim of obtaining any additional benefit of recovery in SCI is being considered. The OECs are modified to secrete various growth factors such as NT-3, BDNF, and GDNF.[62,63] The growth factor–secreting OECs have been found to promote the tissue sparing and improve functional recovery after SCI.[63]

Olfactory ensheathing cells can therefore be considered as a promising source to promote regeneration in cases of SCI. Another advantage is that they can be used as autografts. Nevertheless, several preclinical and clinical trials with high number of patients using olfactory ensheathing cells need to be performed to consider the possibility of using OECs as an effective treatment for SCI.

Neural Stem Cells

First described by Altman in 1960, these multipotent cells have the potential to transform into any cell type in the CNS. Neural stem cells (NSC) are remnants from neuroectoderm of early embryos, and are present in embryonic, fetal, and adult nervous systems. During development, these cells divide and differentiate to form the main components of the CNS, that is, the brain and the spinal cord. During adulthood, stem cells reduce and are confined to certain specific regions, such as the spinal cord, and, to a greater extent, SVZ and subgranular zone (SGZ) of the hippocampal dentate gyrus.[64]

Several studies have investigated the potential of NSCs derived from humans to promote recovery in animal models of SCI. One of the first studies looked at the ability of human NSCs derived from fetal brain cultured as neurospheres to promote recovery after T9 contusion injury in both severe combined immunodeficiency (SCID) and myelin-deficient

shiverer mice.[65] These cells differentiated into functional neurons and oligodendrocytes while promoting an increase in functional recovery as assessed by BBB scores.

Other studies have reported good outcomes by using NSCs in combination with other strategies to treat SCI. Teng et al seeded mouse NSCs within polymer scaffolds[66] and implanted them into the lesion site resulting from a T9/10 hemisection. The authors reported good functional recovery as indicated by an increase in BBB scores as compared with animals receiving only cells, even though the transplanted cells did not stain positive for mature cell markers, indicating that they remained undifferentiated.

Some studies suggested that NSCs, genetically modified to produce neurotrophin-3 (NT-3), enhanced the axonal sprouting in animals.[67] Others have used NSC in conjunction with anticiliary neurotrophic factor (CNTF) antibodies, myelin-specific T cells, fibroblasts, combination of growth factors, heparin, laminin, and FGF, and found that the combination of cells was able to produce functional recovery as evidenced by an increase in the BBB.[68–73]

Fibroblasts

Fibroblasts secrete extracellular matrix molecules, such as collagen and glycoproteins, and are easy to obtain and are expandable in culture, thereby making them attractive for use as a cellular therapy. Fibroblasts are also easy to genetically modify, allowing for additional functionality. However, the method of transfection should be carefully considered. Some transfection agents only produce transient expression of the target protein whereas other methods, such as lentiviral transfection, produce stable protein expression, but may cause unwanted mutations upon integrating into the chromosomal DNA of the fibroblasts.

Many studies have been performed to determine the effect of transplanting fibroblasts that overexpress neurotrophins, including NT-3, BDNF, and NGF.[74,75] The growth factor–secreting fibroblasts have been found to promote axonal growth into the injury site as compared with unmodified fibroblasts.[76,77]

Many other groups have also investigated the use of fibroblasts as a potential treatment for SCI. McTigue et al investigated the effects of several growth factors, including NT-3, BDNF, CNTF, NGF, and basic FGF, secreted by genetically modified fibroblasts on regeneration after SCI.[78] The cells modified to secrete NT-3 and BDNF promoted proliferation of endogenous oligodendrocytes and remyelination of damaged axons, advocating the efficacy of using fibroblasts in SCI.

Human Umbilical Cord Blood Stem Cells

Human umbilical cord blood stem cells (HUCBCs) have been shown to have the following properties: They have the potential to differentiate in vitro into cells that are morphologically similar to oligodendrocytes and express oligodendrocyte markers; they secrete factors that prevent further injury; have tropism for the injured area in the spinal cord; can be effective even through remote infusion either by intravascular or intrathecal administration; and improve neurologic function in animal studies.[79] Therefore, the clinical application of HUCBCs to treat SCI is very appealing. In cases of SCI, UCBC could, thus, control apoptosis, demyelination, and scar formation.[80]

Transplantation of CD34 (+) HUCBCs in traumatic SCI rats significantly increased BBB score and the infarct size and blood vessel density at the injured site. However, the transplanted cells survived at least 3 weeks at the injured site, but did not differentiate into neural cells.[81] In a study conducted by Yao et al, 25 patients with traumatic SCI (injury time $>$ 6 months) were treated with HUCBCs via intravenous and intrathecal injection. After 12 months of treatment, they found that autonomic nerve functions were restored and the latent period of somatosensory evoked potentials was reduced.[79] Clinical studies with hUCB are still rare because of the concerns about safety and efficiency.

Conclusion

SCI is a life-changing event with a predilection for young patients who face a long life of physical and emotional struggles. The treatment of acute SCI is an ever-evolving landscape and one that is likely to see tremendous changes over coming years. SCI continues to be at the forefront of scientific research into stem cell and biotechnology innovation, with stem cell implantation, fast emerging as a promising therapeutic option, with the proven advantages of ease of availability, especially the hESCs, their easy administration, and fewer adverse effects. Various studies are underway, identifying the potential areas of damage in SCI, the histopathologic changes and the potential target areas for therapy in SCI models. The combination of basic science and clinical advances brings hope to the current and future sufferers of SCI. For better SCI recovery, more SCI research, systematic preclinical testing of promising therapies, diverse and abundant source of transplantable stem cells, genetically modified stem cells optimized for specific conditions, or the combination therapies are the areas that need to be further explored.

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