Stem cell therapy in spinal trauma: Does it have scientific validity?

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Abstract

Stem cell-based interventions aim to use special regenerative cells (stem cells) to facilitate neuronal function beyond the site of the injury. Many studies involving animal models of spinal cord injury (SCI) suggest that certain stem cell-based therapies may restore function after SCI. Currently, in case of spinal cord injuries, new discoveries with clinical implications have been continuously made in basic stem cell research, and stem cell-based approaches are advancing rapidly toward application in patients. There is a huge base of preclinical evidence *in vitro* and in animal models which suggests the safety and clinical efficacy of cellular therapies after SCI. Despite this, data from clinical studies is not very encouraging and at times confounding. Here, we have attempted to cover preclinical and clinical evidence base dealing with safety, feasibility and efficacy of cell based interventions after SCI. The limitations of preclinical data and the reasons underlying its failure to translate in a clinical setting are also discussed. Based on the evidence base, it is suggested that a multifactorial approach is required to address this situation. Need for standardized, stringently designed multi-centric clinical trials for obtaining validated proof of evidence is also highlighted.

Key words: Spinal cord injury, stem cells, neurologic recovery, clinical trials
MeSH terms: Spinal cord injury, stem cell research, clinical trial

Introduction

Spinal cord injury (SCI) is the most devastating ailment that can afflict mankind. A complete injury causes permanent loss of sensation and movement in the affected limbs and trunk, loss of bowel, bladder and sexual functions, thus causing extreme psychological stress.[1] It not only causes disability, but also has a profound impact on the social and economic prospects of the individual and the whole family.[2] The high aggregated costs of treatment and prolonged hospitalizations are an economic burden not only to the individual's family, but also to the society.

Accidents are the main cause for SCI and youth in their prime of life are the most commonly affected.[3] Till date, there is no established therapeutic intervention capable of restoring significant neurological function after SCI. With recent advances in stem cell research there has been a tremendous hope for the development of new treatments for many serious diseases amongst researchers, clinicians and the individuals suffering from such diseases. Rigorous scientific and medical evidence is a must to harness the potential of these cells to create a standard mode of therapy which may then be offered as a clinical alternative to other existing standard therapies. In the following passages, we attempt to cover the evidence base for regeneration and repair after SCI. Both preclinical and clinical studies have been critically analyzed for graft survival, axonal regeneration, safety and sensory and motor functional recovery. Finally, we discuss the necessity for multi-disciplinary approaches using combinatorial strategies to achieve robust cellular regeneration associated with neurological and functional improvement.

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Pathophysiology of the spinal cord

Spinal cord injury is caused by direct mechanical damage to the spinal cord that usually results in complete or incomplete loss of neural functions such as mobility and sensory function.[4] The nature and extent of the injury varies widely, depending on the site of injury and its severity. A primary injury refers to a mechanical trauma to the spinal cord leading to a disruption of the spinal cord tissue. SCI can result from contusion, compression, penetration or maceration of the spinal cord.[5] The most common injury mechanism is contusion of the spinal cord at the moment of injury, and the prolonged

compression caused by vertebral bony structures and soft tissues that have become dislodged.[6] During the injury process, the spinal cord might be hyper-bent, over-stretched, rotated, and lacerated,[7] but the white matter is usually spared.[8] The primary injury to the spine triggers a number of pathophysiological processes, which may lead to a prolonged secondary injury phase.[9] These pathophysiological processes are the key determinants of the final extent of neurological deficits.[4,10] The secondary phase can be divided into the immediate (≤2 h), acute (≥2-48 h), sub-acute (≤14 days) intermediate (≤6 months) and chronic stages (≥6 months) of SCI. The pathophysiological processes, which are most affected relate to three major bodily systems - the nervous system, the immune system and the vascular system.[11]

The pathophysiological changes that occur within these different phases are distinct. (1) Acute phase: Edema, ischemia, hemorrhage, reactive oxygen species production and lipid peroxidation, glutamate-mediated excitotoxicity, ionic dysregulation, blood-spinal-cord barrier permeability, inflammation, demyelination, neuronal cell death, and neurogenic shock. (2) Sub-acute phase: Macrophage infiltration, microglial activity, astrocyte activity and scar formation, and initiation of neovascularization. (3) Chronic phase: Wallerian degeneration, glial scar maturation, cyst and syrinx formation, cavity formation, and schwannosis. The end of spontaneous post-SCI changes is identified as a pathophysiological phenomenon with solid glial scar formation, syrinx formation, and neuronal apoptosis. There is retraction and demyelination of spared axons, which may induce permanent loss of sensorimotor functions that is unresponsive to treatment.[12] To select the best time-point for therapeutic cell transplantation, an understanding of the timeline of secondary damage cascades is important.[13]

The persistence of secondary injury mechanisms leads to further neuronal cell death and the interruption of the descending and ascending axonal tracts culminating in glial scarring. The scar forms a hostile environment for axon regeneration due to secretion of molecular inhibitors of axon growth as well as physical impenetrability.[14] The intrinsic capacity for regrowth of CNS axons over long distances if provided permissive environment suggests that the failure of CNS neurons to regenerate is due to the defects lying in the environment rather than within the CNS neurons.[15] A multitude of regenerative (cell growth and survival) as well as nonregenerative (physical and biochemical) events need to function in tandem to restore functionality of the neuron.[16]

Targets for repair

Based on the pathophysiology of SCI, several targets for intervention have been proposed to minimize damage and promote repair and regeneration.[14,16] The same are summarized in Figure 1. Based on these targets several physicochemical and cellular strategies have been employed at preclinical and/or clinical level to evaluate their safety and efficacy [Figure 2].

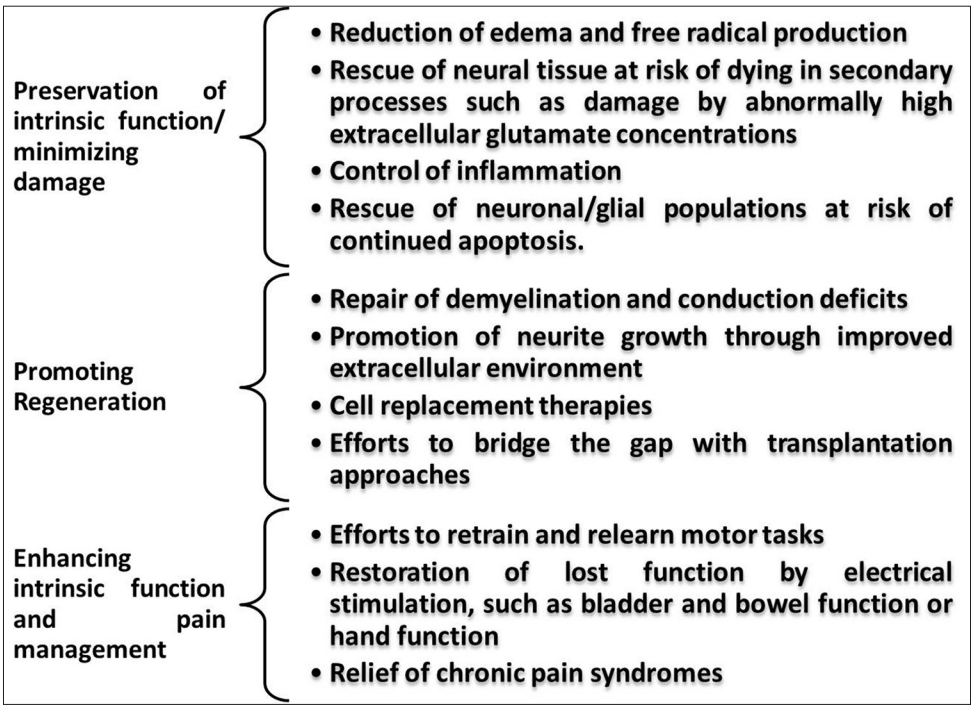

Figure 1: Schematic diagram showing targets for Intervention after spinal cord injury (SCI): Potential targets for repair and regeneration after SCI are listed in the left pane and the proposed approaches to achieve these targets are listed in their right

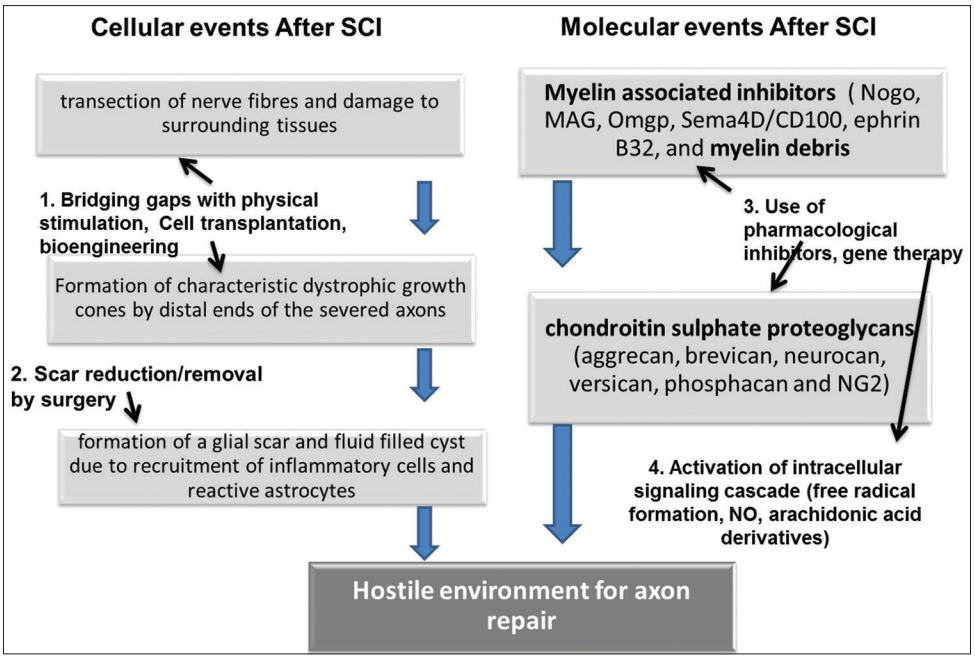

Figure 2: Strategies to promote regeneration after spinal cord injury (SCI). The cellular and molecular events which result in the creation of a hostile environment for axon repair following SCI are delineated. The strategies (1–4) employed to promote neuronal repair and regeneration by providing a permissive environment along with the level at which they act

In order to promote functional recovery, stem cell transplantation must suppress the inflammatory response, inhibit neuronal apoptosis and necrosis, enhance neuronal regeneration, and promote axon regeneration and remyelination.[17]

Materials and Methods

We searched MEDLINE for the search term "(stem cell OR stem OR hematopoietic OR mesenchymal) AND (SCI OR hemisection OR contusion injury OR dorsal column injury OR complete transection OR corticospinal tract injury) from 1st January 2000 to 28th February 2014. Our initial search retrieved 2076 articles, of these 1494 were animal studies, and 981 were human studies. If required, recovered papers were reviewed for further relevant references. Further cross-referencing was undertaken with EMBASE, Cochrane Library, ongoing trials databases and Google and Google Scholar to corroborate findings and resolve discrepancies, if any.

Preclinical evidence

Cell transplantation after SCI may promote neuronal regeneration and function by (1) Secreting neurotrophic molecules at the lesion site; (2) acting as a scaffold for axonal regeneration; (3) replacing the lost/damaged cells.[18]

A number of cell populations have been tested for their safety and efficacy after SCI. These include

- Embryonic cells
- Umbilical cord cells
- Mesenchymal cells
- Hematopoietic cells
- Olfactory ensheathing glial cells
- Progenitor cells
- Schwann cells (SCs)
- Induced pluripotent stem cells (iPSCs).

A large volume of preclinical evidence exists indicating the efficacy of cell transplantation in case of animal models of SCI and is discussed below.

Embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent stem cells derived from the inner cell mass of the early embryo.[19] They can replicate indefinitely and differentiate into all three germ layers and generate all cell types of the body. ESCs were the first population of cells tested for its regenerative potential. The cells could differentiate into neuronal cell types both *in vitro* and *in vivo* in animal models. However, due to their capability to differentiate into all cell types they were found to be tumorigenic.[20] In recent times, instead of direct transplantation, derivatives of these cells have been used to analyze their potential for neuronal regeneration. Several groups have derived neural progenitor/stem cells, motor neurons, oligodendrocyte progenitor cells, and olfactory ensheathing cells (OECs) from ESCs *in vitro*, and then transplanted these cells into various animal models to study restoration of neural function. The transplanted population in these cases have a restricted potential to

differentiate and are generally progenitor cell populations derived from the pluripotent ESC. The use of such restricted population thus reduces the risk of tumorigenesis. Such stem cell-derived neuronal progenitor cells (NPCs) are hailed to be a promising population for neuronal repair. Such NPCs may possess variable characteristics depending on how they were derived.

Kumagai *et al*.[21] generated two primary and secondary neurospheres from the ESCs and demonstrated that only the secondary neurospheres were effective in promoting functional recovery in the rodent sub-acute SCI model. This functional recovery was attributed to paracrine secretion from the transplanted gliogenic neurospheres rather than direct cell replacement. Other populations derived from ESCs include motor neurons,[22] neural stem cells (NSC),[23] NPCs, [24] GABAergic neurons,[25] oligodendrocyte progenitor cell (OPC) populations.[26] These populations have demonstrated various degrees of functional restoration in animal models. Table 1 describes in brief the cell populations derived and their effect upon transplantation in animal models.

Due to the efficacy of neuronal regeneration and neuronal function promotion by the ESC derived NSCs, a variety of engineered ESC-NSC populations have been developed for SCI by several groups. These include, ESC-NSC expressing neural cell adhesion molecule L1.[31] Transplantation of L1-overexpressing substrate adherent ESC-derived neural aggregates into a mouse SCI model resulted in an increased number of surviving cells, enhanced neuronal differentiation, reduced glial differentiation, and increased tyrosine hydroxylase expression as compared to wild type cell aggregates.[32]

Similarly, engineered cell population expressing neurogenins, a family of a basic helix-loop-helix transcription factors involved in specifying neuronal differentiation, have been reported by several groups. Perrin *et al*. and Shapiro *et al*. utilized Neurogenin-2 expressing ESC-derived NPCs and reported full restoration of weight support and significant improvement of functional motor recovery in rats after severe spinal cord compression injury. Partial restoration of serotonin 5HT1A receptor expression, which plays a major role in locomotion and is particularly affected after SCI, was also observed.[33,34]

Successful differentiation of human ESC-derived NPCs (hESC-NPCs) with collagen scaffolds into neurons and glia was observed in the hemisection rat model.[24] The transplanted cells also promoted hindlimb locomotor recovery and sensory responses with observed migration of transplanted stem cells toward the lesion site.[24] Niapour *et al*. reported that co-transplantation of hESC-NPCs and SCs resulted in significant motor function recovery as compared to control and single transplantation groups.[35] This study suggested that the co-transplantation might be a feasible strategy to enhance neuronal differentiation and suppress glial differentiation and thus promote functional recovery.

Table 1: Cell population derived from ESCs and their effect upon transplantation in animal models

Cell population derived	Animal model	Effect upon transplantation	References
Primary and secondary neurospheres	Rat subacute SCI model	Axonal growth promotion, remyelination, angiogenesis, and significant locomotor functional recovery by secondary neurospheres only	21
Motor neurons derived from a co-culture of endothelial cells and ESCs treated with retinoic acid and sonic hedgehog	Adult mouse SCI model	Significant recovery of sensory and motor function	22
Longterm self-renewing rosette-type neural stem cells	Mouse SCI model	Enhanced remyelination and axonal regeneration and survival of endogenous neurons	27
ESC-derived motor neuron progenitors	Adult rat SCI model	Enhanced sprouting of endogenous serotonergic (5-HT) projections, enhanced survival of endogenous neurons, enhanced gross tissue sparing, and decreased phosphorylation of stress-associated protein kinase which can result in apoptosis, immune activation, and inflammation	28
ESC - derived GABAergic neurons	Hemisection rat model	Significant reversal of decreased PWTs after intrathecal transplantation	25
ESC-derived oligodendrocyte progenitor cells	Rat contusion SCI model	Improved axon remyelination and motor function	26
ESC-derived oligodendrocyte progenitor cells	Contusion SCI model in rats during the acute phase after injury	Survival, migration and integration into the spinal cord tissue	29
ESC-derived oligodendrocyte progenitor cells	Cervical contusion rat model	Significantly improved forelimb stride length and a reduced demyelination and more oligodendrocyte remyelinated axons than Schwann cell remyelinated ones in transplanted group	30

ESCs=Embryonic stem cells, SCI=Spinal cord injury, 5-HT=5-hydroxytryptamine, PWTs=Paw withdrawal thresholds

Embryonic Stem Cell-derived populations have been utilized for combinatorial strategies also. Combination of motor neuron progenitors along with ESC-OPCs in a complete transection SCI rat model[36] resulted in significantly better BBB scores with significantly higher amplitude of motor evoked potential (MEP) in electrophysiological evaluation as compared to individual populations. Salehi *et al*., in another combinatorial study, transplanted OECs and ESC - derived motoneurons into contused SCI rats.[37] They reported significantly better regeneration and functional restoration as compared to that observed when individual populations were transplanted. Illes *et al*. have reported the presence of intrinsically active neurons (IANs) in neuronal populations derived from mouse ESCs. They proposed that presence of such IAN populations in cell grafts may be a prerequisite to attain functional activity following interventions involving transplantation of neural tissues.[38] Lee TH has reported a substantial improvement of motor function due to transplantation of mouse ESCs in a rat model of clip-compression SCI.[39]

Induced pluripotent stem cells

Although ESCs possess great potential due to their ability to differentiate into all cell types that are useful for therapeutic purposes, such as transplantation, they raise significant ethical concern as most of the ESCs arise from human embryos. Recent discovery that somatic cells can be reprogrammed to a pluripotent state may be used to circumvent these concerns. The reprogrammed cells called iPSCs, exhibit functional similarities to ESCs and present an exciting area of research.

These cells have been used to derive cell populations equivalent to those derived by ESCs and tested in case of SCI.

Like ESCs, long term self-renewing rosette-type NSC population has been derived from iPSC (lt-iPSC-NSCs).[23] This population possesses stable neuronal and glial differentiation ability and capacity of generating functional mature neurons *in vitro*. [23] Upon transplantation into the mouse SCI model, enhanced remyelination and axonal regeneration along with survival of endogenous neurons was observed.[27] iPSC derived GABAgeric neurons have been tested in the hemisection rat model and were found to significantly reverse decreased paw withdrawal thresholds.[25] This indicates that this may be a potential solution for the loss of sensory function after SCI.

In a recent study, Sareen *et al*. have reported survival, migration and integration of human neural progenitor cells generated from iPSCs in nude rats. The authors have proposed that the iPSC derived NPC may be an alternative to human fetal tissue derived NPCs.[40]

Mesenchymal stem cells (MSCs)

Mesenchymal stem cells are stromal cells that have the ability to self-renew and also exhibit multilineage differentiation. This self-renewing, multipotent stem cell population was initially identified from the bone marrow (BM). According to the statement of International Society for Cellular Therapy, the definition of multipotent MSCs must (1) Adhere to plastic when cultured in standard conditions; (2) they must express CD105, CD73, and CD90, and lack the expression of CD45, CD34, CD14, or CD11b, CD79a, or CD19 and HLA-DR surface molecules; and (3) they must be able to differentiate to osteoblasts, adipocytes and chondroblasts *in vitro*. MSCs have been reported to differentiate into osteoblasts, chondrocytes, adipocytes, neural cells and myoblasts, *in vitro*.[41,42] Due to their multipotent nature, source availability and comparative safety, these cells have been advocated as a promising cell source for repair. MSCs have been reported to evince spontaneous neuronal differentiation when implanted into both irradiated mice[43,44] and humans.[45] Furthermore, allogeneic MSCs were reported to be well tolerated after intradermal injection in horses.[46,47] Mezey *et al*.[44] and Brazelton *et al*.[43] infused BM cells intraperitoneally in rats and have reported neuronal differentiation in the brain of host animals.

Transplantation of MSCs in SCI animal models has been reported to promote sensorimotor function recovery and bladder function recovery via neural lineage differentiation, neurotrophic paracrine effects and posttrauma inflammation regulation.[48-50] Abrams *et al*. observed that the injury-induced sensitivity to mechanical stimuli was significantly attenuated upon MSC injection in contusion SCI rats.[51] They also reported improvement of locomotor function in SCI rats after transplantation of MSCs.

The major limitations in the therapeutic *in vivo* application of MSCs for SCI is their low survival rate after graft, the lack of neural differentiation, glial scar formation, cystic cavity formation, the inhibitory cellular environment and the transplantation time-point.[48,52,53]

Furthermore, significant effects on the outcome are observed depending upon the route of transplantation of MSCs. Intravenous (IV) transplantation of MSCs was reported to result in significantly better BBB motor score as compared to intralesional transplantation in SCI rats.[54] Similarly, IV cell administration in severe contusive SCI rats in acute and sub-acute phase resulted in significant locomotor recovery.[55] Intrathecal co-administration of NPCs and MSCs did not lead to any migration to the injury site.[56]

Implantation of MSCs into the spinal cord or lesion site has not been reported to promote neuronal

differentiation.[52] However, Boido *et al*. have reported, significantly reduced lesion volume and improved hindlimb sensorimotor functions after transplantation of mouse MSCs into the lesion cavity of compression SCI mouse model.[53] Similar results have been reported by Gu *et al*. after transplantation of BM MSCs into the epicenter of the injured spinal cord of rats [54] In such cases, it is hypothesized that the engrafted MSCs may generate a favorable environment for functional recovery through modulating the post-SCI inflammatory response and by having neurotrophic paracrine activity.[53] The therapeutic effects of MSC transplantation on the sensorimotor deficits in animal SCI models have been clearly confirmed by a large number of studies.[52-54,57]

In order to overcome the limitations of direct MSC transplantation, several strategies have been employed that include pretransplantation neural differentiation, neurotrophic gene transduction, glial cell co-transplantation, and tissue engineering.[51,53,56,58] Rostral and caudal injection of neural modified human bone-marrow derived MSCs to the T-8 lesion immediately after SCI in the rat model resulted in significantly improved locomotor function.[15,58-65] Transplantation of neurally differentiated rat MSCs (NMSCs) into the epicenter of a contusive lesion resulted in the recovery of motor function as well as significantly shortened initial latency, N1 latency and P1 latency of the SSEPs.[58,66] Co-transplantation of autologous neural differentiated and undifferentiated MSCs in the contusion lesion cavity at T8–T9 level of rats' spinal cord reported a significantly higher BBB score as compared to controls.[59]

Genetically modified MScs have been also been tested in some *in vivo* studies. Populations tested include MSCs over expressing basic fibroblast growth factor (bFGF),[60] and Neurotrophin-3 (NT-3) gene.[61]

Song *et al*. have demonstrated improvement of hind limbs' motor function after allograft of bone MSCs in rats with acute injury to their spinal nerve.[67] In another study, Yin *et al*. have reported the antiapoptotic effect of MSC transplantation in adult rats after spinal cord ischemia-reperfusion injury. This suggests that MSCs may affect cell regeneration and repair through control of apoptosis following SCI.[68]

One of the major barriers to spinal cord regeneration is the glial scar, which hampers the movement of regenerating cells and does not support the survival of implanted cells and their neural differentiation. Biological scaffolds are now gaining importance for providing substratum as well as neurotropins to aid cell survival, differentiation and proliferation. Platelet-rich plasma scaffolds in combination with BDNF have been shown to support survival and neural differentiation of human MSCs.[69] Gelatin sponge (GS) scaffold system, which was constructed by ensheathing GS with a thin film of poly-(lactide-co-glycolide) (PLGA), also has been reported to support rat MSC adherence, survival and proliferation *in vitro*, and in the rat SCI model, the seeded scaffolds were shown to attenuate inflammation, promote angiogenesis, and reduce cavity formation.[15] Other scaffolds and cell combinations tested include PLGA scaffolds system and human MSCs,[62] a combination of Matrigel and neural-induced adipose-derived MSCs (NMSCs).[70] In recent times, the use of fibrin scaffold along with MSC injection has been reported to result in the formation of longitudinally-aligned layers of MSCs growing over the spinal cord lesion site. This was associated with migration of host neurites into the MSC architecture. Such a strategy provides control over cell integration into the tissue after intraspinal injections hence enhancing localization of the cell graft.[71]

Sources of MSCs other than BM have also been identified by researchers, such as, adipose tissue,[73] amniotic fluid,[76] placenta,[72,73] umbilical cord blood (UCB),[74,75] and in several fetal tissues including liver, lung, and spleen.[76] Of these the MSCs from UCB and adipose tissue are sources choice with many advantages such as ease of collection, availability and proliferative capacity.

Neural stem/progenitor cells

Neural stem/progenitor cells (NS/PCs) were first demonstrated in the subventricular zone of the mouse in 1989[77] and were isolated from the mouse striatal tissue and subventricular zone for the first time in 1992.[78] These cells were capable of self-renewal and generating the main phenotypes (neurons, astrocytes, and oligodendrocytes) of CNS cells *in vitro* and *in vivo*.[79] After transplantation into the injured spinal cord, NS/PCs generate mature neural phenotypes and provide neural functional recovery in some SCI models.[80]

Neural stem/progenitor cells have been transplanted *in vivo* for studying their therapeutic potential after SCI. In most cases, *in vivo* transplanted NSCs have shown a preferential capability of differentiating into glial lineages, especially astrocytes.[80] The direct transplantation of NSCs or NPCs has not been always efficient for functional recovery after SCI. Transplantation of fetal NPCs, derived from fetal rats, into the dorsal column lesion site of adult rats, resulted in only a minor sensory function improvement with no restoration of the motor function recovery.[81] Pretreatment of human NSCs with bFGF, heparin, and laminin before transplantation into the contusion lesion of rats led to an optimized survival rate, neuronal and oligodendroglia differentiation, and improved trunk stability.[82] Tarasenko *et al*.[5] reported that the spinal cord microenvironment can probably change the differentiating fate of grafted NSCs. Transplantation

of NS/PCs obtained from myelin-deficient shiverer mutant mice (shi-NS/PC) into the lesion site of rats demonstrated that the remyelination capability of wt-NS/PCs was vital to motor and electrophysiological functional recovery.[83] Transplantation of the Olig2 expressing NSC into the contused spinal cord has been reported to increase the volume of spared white matter and reduce the cavity volume. This was associated with thickened myelin sheath which may be due to differentiation of NSCs into oligodendrocytes.[84]

Injection of a combination of NSCs and OECs into the spinal cord lesion of rats has been reported to lead to hindlimb locomotor functional recovery.[85] Salazar *et al*. have reported a significant improvement in locomotor recovery in early chronic SCI mouse model after NSC transplantation.[86] They have also demonstrated that the transplanted NSCs differentiated into oligodendrocytes and neurons and that astrocytic differentiation was rare. The authors also reported the integration of transplanted human NSCs with host cells. Emgard *et al*. have reported a neuroprotective effect of human spinal cord derived neural precursor cells in two different rat models of SCI.[87] In a recent study, Nemati *et al*. transplanted adult monkey NSCs into a contusion model of SCI in the rhesus monkey and reported homing of MSCs to the lesion and improved hindlimb performance.[88]

Olfactory ensheathing cells

Olfactory ensheathing cells permit growing axons from neurons of the nasal cavity olfactory mucosa to re-enter the olfactory bulb (OB) of the brain and form synapses with second-order neurons.[87] These cells are present in the olfactory epithelium and are considered as a special class of glial cells which exist in both the peripheral nerve system (PNS) and CNS, and share certain features and functions with astrocytes as well as SCs.[89] By virtue of their cell-specific properties, OECs are more likely to rescue neural function in the injured spinal cord as compared with SCs.

Recent studies have shown that rodent OECs can support axonal regrowth when transplanted into experimental models of SCI[90] and are also able to form myelin sheaths around regenerated or demyelinated axons, thereby permitting rapid saltatory conduction to occur.[90,91] It has therefore been proposed that OECs may be suitable cells for transplant-mediated repair of spinal cord trauma or nonrepairing foci of demyelination (such as may occur in chronic multiple sclerosis). These data indicate that transplanted OECs have a repair repertoire that is similar to that of SCs, but may have advantages over these because of their ability to migrate and integrate within areas of astrocytosis that are characteristic of damaged CNS.[92,93]

Following transplantation into a localized electrolytic lesion of the corticospinal tract in adult rats, OECs supported unbranched, regenerative growth of corticospinal axons and restoration of a corticospinal-dependent paw-reaching function.[90] OECs promoted regeneration after complete transection of the spinal cord[94] and restored rapid and secure conduction across the transected dorsal columns of the rat spinal cord[95]with recovery of motor function.[96] Human OECs were also shown to remyelinate the demyelinated spinal cord of the rat.[95] Other groups have shed doubt on the functional improvements induced by OECs grafts, and have suggested that they are caused by a trophic support mechanism and not the birth of new neurons, which means that the therapeutic potential of OECs after SCI may be limited.[93,97] Centenaro *et al*.[99] and Aoki *et al*.[100] in their olfactory tissue transplantation studies suggest that OECs may be of limited use in promoting recovery after SCI.

The disparity in the results reported by different groups may be attributed to the cell population used, donor, injury models, graft preparation, time of transplantation and the transplantation procedure.[101-104]

To address the issue of the time of transplantation or "transplantation window", Muñoz-Quiles *et al*.[104] compared the motor function recovery after OB derived OEC (OB-OEC) transplantation into completed transection injured rats among sub-acute chronic and nontreatment groups. They reported a 10% higher percentage of recovery in sub-acute transplantation group than the chronic group with motor axons growing beyond into the lesion site, indicating a rostral to caudal the lesion site crossing phenomenon.[104] Based on these finding Li and Lepski in their review, proposed that sub-acute or chronic cellular transplantation to bypass the acute phase after spinal trauma combined with scar ablation may be a potentially effective strategy to achieve regeneration and/or repair after SCI.[105]

Another factor that determines the survival and fate of the transplanted tissue is *in vitro* culture conditions. OECs with longer preculture times were found to be less effective as compared to those with shorter preculture times.[106]

Although the application of OECs for regeneration after SCI has been questioned, several studies support the potential of OECs to be protective/regenerative in nature.[107] OECs have been combined with cAMP treatment[108-111] and laser puncture,[112] genetically modified for NT-3 production, and co-transplanted with other cell types[113] in order to boost the efficacy of OEC transplantation. Although most of such combinations have resulted in better efficacy as compared to OECs alone, a few have failed to do so. Co-transplantation of OECs with MSCs did not lead to any significant

synergistic effects on neural function improvement as compared to OEC alone.[36,114,115]

Schwann cells

Schwann cells were discovered by Theodor Schwann in 1839 and were found to provide myelination of peripheral axons. Schwann cell precursors (SCP) were found in developing stem cells within neural crest. When connected to nervous fibers, SCs or precursors lead to myelination of peripheral axons.[114] In the human and large animals, SCI leads to the formation of a cavity and a glial scar. Due to this, the ends of the regenerating axons at the edge of the scar become dysmorphic and cannot progress further leading to termination of axon regrowth.[116] It has been demonstrated that after SCI, if these injured neurons are grafted into a peripheral neural environment, which facilitates growth and remyelination, they can recover their morphology and electrophysiological function.[117] SCs are an important part of the PNS and are vital for the myelination of peripheral axons. Park *et al*. have reported that transplantation of SCs into a demyelinated spinal cord slice, *in vitro*, promotes survival and secretion of neurotrophic factors which may aid intrinsic neuronal regeneration.[118] SC transplantation has also been reported to lead to remyelination of demyelinated axons and axonal sprouting.[119]

In the past studies, SCs used were isolated from peripheral nerves and cultured *in vitro* to provide enough number of cells for the transplantation. In recent times, alternate sources for SCs have been used. The SCs have been derived from different stem cell populations or neural progenitors like, MSCs[120,121] adipose-derived stem cells,[120] and skin-derived precursors (SKPs).[122] Mesenchymal stem cell-derived SCs were tested by Park *et al*. and Xu *et al*. *in vitro* and were found to support axon remyelination and sprouting.[118,121] Biernaskie *et al*. derived SCs from SKPs which were isolated from the rodent or human skin.[122] Upon transplantation of these SKP-SCs in the murine contused model lesion site bridging effect, increased size of spared tissue, and reduced reactive gliosis was observed which was better in the SKP-SC group as compared to control and SKP only.[121] In addition, significant enhancement of locomotor recovery was observed although there was no restoration of sensory function. Therapeutic potential of SCP in an acute SCI model was tested by Agudo *et al*.[123] They reported a successful integration of the graft into the host tissue, and a robust bridging effect which extended rostrocaudally due to immediate cell injection into the lesion site after surgery.[122] However, no significant difference in motor function was observed between the SCP and control group.

Similar to other cell types, SCs have also been genetically modified and tested. SCs overexpressing chondroitin sulfate proteoglycans have been reported to suppress the expression of glial fibrillary acidic protein and chondroitin sulfate proteoglycan in reactive astrocytes, induce robust migration of astrocytes extending in parallel to the regenerated axons and remyelinate the axons.[1,124] Co-transplantation strategies and use of scaffolds and matrices have revealed that Matrigel and biodegradable scaffolds could promote cell survival and/or axon regeneration; however, functional activity was not significantly enhanced.[125] Co-transplantation of SC and MSC was found to be a promising strategy to enhance cell survival, axon regeneration and functional activity.[126] However, co-treatment of SC with cAMP enhancer and cAMP analog did not exhibit enhanced locomotor function as was reported previously. This could be attributed to experimental group arrangements, consistency of injury severity, appropriated statistics, and animal surgery.[18,30,127,128] Kanno *et al*. have reported improved axonal regeneration and motor function following transplantation of SCs which were genetically modified to secrete neurotrophin in combination with chondroitinase.[129]

Clinical evidence

Based on the vast body of preclinical evidence, scientists and clinicians have been eager to explore the therapeutic effects of cell transplantation on spinal cord patients. Various cell types, different administration strategies, and different kinds of SCI patients have been involved in clinical trials or therapeutic settings. A plethora of patient testimonials and case studies have reported the clinical safety and efficacy of cell transplantation after SCI. However, there is a paucity of data from valid clinical trials. The data from such validated trials report several obstacles that are inherent to human studies including ethical issues differences in anatomy, and differences in underlying pathophysiological processes. Until now, no promising cell therapies that are safe and effective for SCI patients have been achieved.

Bone marrow transplantation for SCI has been the focus of attention in the last few years. There have been extensive preclinical studies which have demonstrated their potential role. Transplanted BMCs were found to improve neurological deficit in CNS injuries model by generating neural cells or myelin-producing cells[92,128] BMCs have been transplanted by direct injection into the injured spinal cord,[129,130] IV injection,[131] intrathecal injection[131] or injection into the spinal artery.[132]

The uses of BMCs for stem cell therapy in SCI subjects has more advantages compared with ESC use and therefore are more widely used. BM stem cell-based therapy is not associated with carcinogenesis, which sometimes occurs in ESC therapy,[134] Immunological rejection or graft-versus-host reactions, caused by allograft,[133] and extensive scientific

data on BMCs has been accumulated from wide-ranging experiences in BM transplantation for hematological diseases.

Moreover, several hypotheses have been proposed to explain the role of BM stem cells in SCI models. First, BMCs improve neurologic deficit by generating either neural cells or myelin-producing cells.[128,135] Second, transplanted BMCs do not differentiate into neurons; rather, they work by guiding axonal regeneration by producing extracellular matrix. Third, transplanted BMCs promote compensatory mechanisms to reorganize neural network, and activate endogenous stem cells.[136] The studies have been summarized in Table 2.

Preclinical studies suggest behavioral efficacy due transplantation of human UCB cells and suggest that benefits may come from secretion of factors by transplanted cells,[53] however, only a few small "open label" human studies have been conducted with varying claims of benefit. Currently, a planned SCI trial by China SCI network is being conducted (ClinicalTrials.gov Identifier: NCT01046786).

The potential use of ESCs and iPSCs in clinical applications has vastly interested both researchers and clinicians. This has also has gained the attention of media. However, several issues remain to be addressed regarding their safety and efficacy.[153-156] One of the most widely publicized trials has been the hESC OPCs, GRNOPC1, within patients who were suffering from complete thoracic level paraplegia with the loss of motor and sensory function.[157,158] To date, there are no serious adverse events in the long term followup reported, however, in November 2011, Geron announced that it had ended its SCI stem cell research program largely due to financial reasons. Though the concept of using ESC or iPSC derived cells for regeneration and repair is very tempting due to reasons of ease of availability and low immunological risks, still a vast body of preclinical evidence is needed before the therapeutic potential of these cells may be tested in a clinical setting.

Human trials published so far have had various flaws in design and documentation. Initiatives of various associations in educating clinicians as well as individuals regarding cellular therapies and clinical trial design in case of SCI go a long way in promoting moral, ethical and scientifically validated use of cellular interventions.[159-163]

A stem cell clinical trial in SCI needs to address several open ended questions with respect to cell population (ESC derived progenitors, MSCs, OECs, etc.), cell dosage (number of cells and number of interventions), subject selection (acute vs. chronic, level of injury) and outcome measures. In our experience, we have not been able to duplicate the efficacy to stem cell interventions in case of chronic[149] as well as acute SCI (unpublished results). The current proof of evidence points in the direction of undertaking trials which include other repair strategies such as predifferentiation, scaffolds, growth factors, etc., along with cell transplantation to achieve repair and regeneration post-SCI. Transplanting partially differentiated "progenitor cell" populations may be more effective than the pluripotent or multipotent populations. Disparity in preclinical evidence data versus clinical evidence.

Despite huge base of preclinical evidence in support of restoration of neuronal function through cellular interventions, the clinical evidence has not been that encouraging. There still remains a huge gap between the "bench" and the "bedside" which remains to be bridged. The factors which contribute to this are:

- Difference in the injuries between the animal models and human SCI
- Choice of the animal model
- Cell population used
- Patient selection criteria
- Spontaneous recovery confounding interpretation of results
- Poor trial design
- Lack of standardized outcome measures in a clinical trial.

Conclusion

The list of experimental therapies that have been developed in animal models to improve functional outcomes after SCI is extensive. There is a vast body of preclinical evidence which supports the therapeutic potential of cell transplant in facilitating spinal cord regeneration and/or repair after SCI. However, preclinical studies have their inherent limitations dependent upon the mechanism of injury and the animal model used. Recent publications on the mechanisms involved in repair and regeneration post-SCI provide valuable insights regarding the potential barriers to regeneration after SCI. These need to be addressed by scientists and clinicians to define new strategies for achieving repair. Basic scientific research should be directed toward providing a rational basis for tailoring specific combinations of clinical therapies to different types of SCI. Functional regeneration should be the primary goal of any approach being tested, and it is important that this is tested by scientifically validated and universally accepted outcome measures and tools. Due to the involvement of multiple cell type and the complexity of SCI, it is becoming increasingly clear that a single approach may not be successful in achieving SCI repair.

Table 2: Summary of published clinical trials on cellular therapy for SCI

Study	Study type	Study population	Cell population	Route of administration	Result	Limitation
Ichim *et al.*[139]	Case report	A 29-year-old and ASIA scale type A classified patient	MSC+CD34 cells	Multiple intrathecal and IV injections	Significant improvements in sensory function and lower limbs muscle strength recovery	Insufficient evidence in the study to support that the recovery was due to cell graft and not spontaneous
Kishk *et al.*[140]	Case controlled	Chronic complete SCI patients	MSCs	Monthly intrathecal administration	No significant improvements over the controls	Not a randomized controlled trial
Bhanot *et al.*[141]	Case report	Chronic SCI patients	Autologous MSCs	At the site of injury after a laminectomy	Equivocal results	Insufficient evidence and poor trial design
Park *et al.*[142]		Six complete acute SCI subjects	Autologous bone marrow cell transplantation in conjunction with administration of granulocyte macrophage-colony stimulating factor		5 subjects showed improvement in AIS grades with no serious complications. Authors conclude procedure to be safe	
Karamouzian *et al.*[143]	Nonrandomized controlled clinical trial	Eleven SCI patients with complete thoracic injuries	Autologous MSCs	Lumbar puncture	No significant improvements over the controls	
Syková *et al.*[144]		20 subjects with complete SCI	Unmanipulated autologous bone marrow	Intraarterial application	Suggested that transplantation within a therapeutic window of 3-4 weeks following injury would play an important role in any type of stem cell SCI treatment	
Yoon *et al.*[145]	Phase I/II open-label and nonrandomized study on 35 complete SCI subjects		Autologous bone marrow cell transplantation in conjunction with the administration of granulocyte macrophage-colony stimulating factor		Acute and sub -acute groups respond better than chronic SCI groups	
Jarocha *et al.*[146]	Preliminary study	Children with chronic SCI			Safety and feasibility of transplantation of autologous bone marrow mononuclear cells	Though the authors claim to a certain degree of neurological improvement due to the cell transplant, this evidence base provided is inconclusive
Huang *et al.*[147]	Prospective clinical study	16 subjects with chronic SCI	Fetal olfactory ensheathing cells		Feasible and safe	No efficacy studied
Lima *et al.*[148]	Pilot clinical study	AIS A subjects 6 months to 6.5-year post injury	Olfactory mucosa autografts	Transplanted into lesions after a myelotomy and scar removal	Feasible, relatively safe and potentially beneficial	Efficacy of the reported procedure could not be replicated in human chronic thoracic SCI
Chhabra *et al.*[149]	Single-blind, controlled pilot study Phase I/IIa	6 AIS A subjects	Olfactory mucosa autografts	Transplanted into lesions after a myelotomy and scar removal	Feasible and is safe up to 1-year post-implantation. No efficacy reported	Small number of trial patients

contd...

Table 2: *Contd...*

Study	Study type	Study population	Cell population	Route of administration	Result	Limitation
Mackay-Sim *et al.*[150]	Single-blind, controlled, Phase I clinical trial	3 AIS A subjects	Olfactory mucosa autografts	Cultured and purified olfactory ensheathing cells from nasal biopsies injected into the spinal cord lesion	Feasible and is safe up to 1-year postimplantation	Small number of trial patients
Lammertse *et al.*[151]	Randomized controlled trial with single-blinded primary outcome assessment	43 participants (26 treatment, 17 control)	Activated macrophages	Injection into the caudal boundary of the SCI	No significant improvement over the control group	
Zhou *et al.*[152]	Case report	6 subjects	Autologous activated Schwann cells	Laminectomy and cell transplantation	Reported recovery	More replicable pre-clinical data, preferably in large animals, is needed before undertaking further clinical studies using the SC population
Saberi *et al.*[153]	A 2-year followup for the safety assessment	4 subjects with chronic SCI	Schwann cells		Suggest the safety of clinical trials for SC therapy	Poor subject selection criteria and post assessments. Transient paresthesia or increased muscle spasm has been reported following transplantation of purified SCs in patients with chronic SCI (28-80 months posttrauma)
Amr *et al.*[154]	Case series of 14 patients	Chronic SCI	Co-transplantation of bone marrow derived MSCs with chitosan-laminin scaffold and peripheral nerve grafts after chronic SCI	Stem cells injected into the whole construct of cord defect and contained using a chitosan-laminin paste	Sensory and neurological improvements	Randomized controlled studies needed to validate data

SCI=Spinal cord injury, MSCs=Mesenchymal stem cells

In the current scenario, the need for multi-disciplinary involvement is essential as a single approach to achieve functionally effective axonal regrowth and sprouting may be ineffective due to the complex nature of SCI and the number of cell populations involved. A multi-factorial approach involving cell populations, scaffolding matrix, growth factor supplementation and scar removal is required to address this situation. Along with this, multi-centric studies involving standardized and validated approach, a stringent trial design with appropriate outcome measures and rehabilitation protocol are a must to understand and achieve the potential of cellular therapy in case of SCI. A clinical trial program with appropriate clinical trial design and ethical conduct is key for achieving this goal.

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