Chapter 9

Stem Cell Therapies for Cervical Spinal Cord Injury

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Additional information is available at the end of the chapter

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Abstract

Cervical-level injuries account for the majority of presented spinal cord injuries (SCIs), yet there are few therapies that successfully improve the overall quality of life for patients. Regenerative therapies aimed at ameliorating deficits in respiratory and motor function are urgently needed. Cellular transplantation strategies are a promising therapeutic avenue. These strategies seek to overcome the inhibitory environment of the injury site, increase native regenerative capacities, provide scaffolding to bridge the lesion, or replace injury-lost neurons and glia.

Numerous considerations must be taken into account, however, when designing effective cellular transplantation therapies, most notably of which is cell source. Each cell source offers its own unique attributes—both positive and negative—that directly correspond with functional outcomes and clinical translation. Here we discuss three different cell types currently used in cellular transplantation strategies to treat cervical SCIs: mesenchymal stem cells (MSCs), embryonic stem cells (ESCs), and induced pluripotent stem cells (iPSCs). By illustrating the characteristics of each cell type and outlining the studies and clinical trials in which they have been featured, we hope to provide the reader with a detailed understanding of both their capabilities and also their potential drawbacks in experimental and clinical settings.

Keywords: cervical spinal cord injury, stem cell therapies, cellular transplantation, functional outcomes, regenerative strategies

1. Introduction

1.1. The impact of cervical SCI

Spinal cord injuries (SCIs) create a formidable encumbrance on the US healthcare service with over half of all injuries occurring at the cervical level. While most causes of SCI can be attributed

to accidents or violence, the incidence of cervical-specific injuries continues to rise from particularly distinctive causes [1,2]. This is in part due to the ever-increasing spectrum of injury types, such as those sustained in direct military environments or as a result of changes in tactical armor design [3–6]. Others include improvements in emergency medicine leading to better survival rates [7], the growth of the aging population as a result of advances in preventative care [8–10], and lifestyle choices leading to structural degradation of the cervical spine [11,12].

Survivors of cervical SCI are faced with dramatic life changes owing to lengthy and repeated hospitalizations and the need for full- or part-time caretakers, overall resulting in a loss of personal independence. Combined with a frequent inability to maintain employment or contribute to the workforce, patients incur substantial financial expenses—over the course of their lifetime, a 25-year-old SCI patient can expect to accrue up to $4.5 million in direct costs alone. Overall, SCI costs the nation upward of $40.5 billion annually, as per a 2009 report by the Christopher and Dana Reeve Foundation [1,2]. Although recent advances have resulted in increased survival rates, quality of life still remains poor; patients encounter a gradation of sensory deficits, respiratory deficits, motor dysfunction, and paralysis based on their specific injury location. Therapies designed to ameliorate some of these complications, even partially, are drastically needed and would make a radical impact in easing the financial, emotional, and physical burden experienced by cervical SCI patients.

1.2. The pathophysiology of cervical SCI

The cervical spinal cord contains the long tracts connecting the rostral and caudal portions of the central nervous system (CNS), as well as sensory and motor neurons. Cervical SCI in mammals initiates large zones of necrosis at the site of injury, creating gaps in the circuitry and preventing communication within the CNS. Axons within the spinal cord fail to regenerate after injury and retract toward the soma from the lesion border. Overall this culminates in crucial changes to normal upper limb function in mammals and disrupts motor function in humans resulting in paralysis and diaphragm-mediated respiration [13].

SCI is characterized by two distinct phases: primary and secondary injuries. During primary injury, the delicate spinal cord tissue is mechanically compromised due to shearing and compression forces, either by direct contact or inadvertently through manipulation of the vertebrae. This leads to mechanical injury, disruptions in vasculature and respiration, neurogenic shock, inflammation, membrane compromise, and alterations in ion and neurotransmitter levels [14–16]. While the primary injury phase leads to an immediate and often serious impairment of neurological function, it is the secondary injury phase that typically dictates the full magnitude of injury. There are approximately 25 established mechanisms to date by which this occurs, but still much ambiguity as to how these pathways converge upon each other to determine the full manifestation of injury [17,18]. Overall, this biochemical cascade activates the ischemic pathway, inflammation and immune responses, swelling, and neuronal apoptosis and leads to neurotransmitter imbalances that underlie excitotoxicity [19–25].

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1.3. Regeneration and plasticity of the CNS

Prior evidence suggested that the adult mammalian CNS did not regenerate, predominantly due to the unlikely event of axonal regeneration through the inhibitory milieu of the spinal lesion [26]. However, some degree of functional recovery is often seen, possibly as a result of reorganization of spared circuitry from innate axonal sprouting of spared and intact fibers [26–28]. Experimental evidence has shown that this process can be influenced and axonal regeneration encouraged via the use of other synergistic therapies. These include the addition of neurotrophic growth factors [29–32], the deletion of inhibitory factors typically associated with the lesion [33–35], and rehabilitation regimens and physical activity [36–38]. Despite this, the innate regenerative capacities of the CNS are often overwhelmed by the extent of injury and functional recovery is limited at best.

Given SCI's multifactorial pathophysiology and the inherent complexity of the CNS, any potentially successful treatment must be effective in positively addressing multiple deficits. Cellular transplantation therapies offer an attractive means of accomplishing this by repopulating SCI-lost neurons and glia, increasing native regenerative capacities through trophic and immunomodulatory factors secreted by transplanted cells, and providing scaffolding to bridge the inhibitory milieu of the lesion site [20,29,30,32,39–43]. Furthermore, the potency of stem cells makes them an ideal candidate by circumventing the impediments of harvesting and transplanting adult neurons. By promoting neurite regeneration and replenishing appropriate cell populations, it may be possible to reconnect rostral and caudal neural circuitry and restore function.

In recent decades, the therapeutic promise of cellular intraspinal transplantation has gained significant interest and has eventuated in preliminary clinical trials. Numerous preclinical experiments have been developed to target SCI using peripheral nerve bridges, Schwann cells,

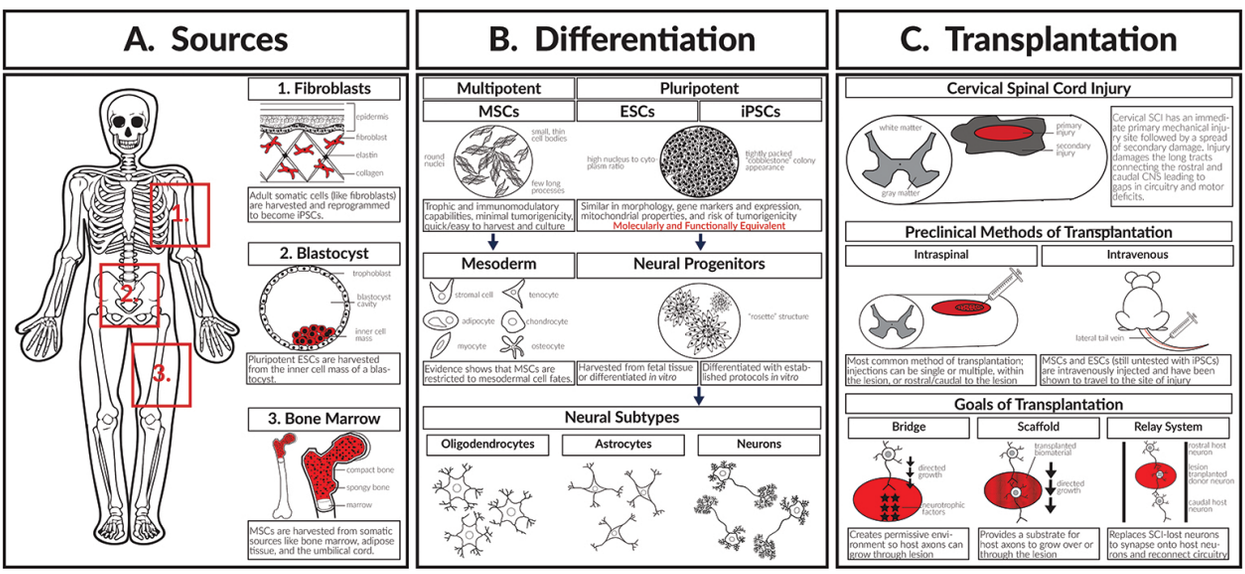

Figure 1. A summary of the different stem cell types. Panel (A) shows the origin and isolation of the cells, (B) shows their differentiation potential, and (C) describes transplantation methods used and their intended goals for treatment of cervical SCI.

olfactory glia, mesenchymal stem cells (MSCs), embryonic-derived stem cells, and induced pluripotent stem cells (iPSCs). However, the vast majority of these do not address cervical-level injury [44–52].

Here, we highlight the current literature on embryonic, mesenchymal, and induced pluripotent stem cell-based cellular transplantation with an emphasis on cervical-level SCI. We discuss the benefits and disadvantages of each cellular source, and consider possible future therapeutic avenues. **Figure 1** provides a summary of these cellular types and their origins, including transplantation methods used and their intended goals for treatment of cervical SCI.

2. Mesenchymal stem cells (MSCs)

2.1. Isolation and purification of MSCs

Transplantation of mesenchymal stem cells (MSCs), also known as bone marrow stromal cells (BMSCs) or mesenchymal progenitor cells, is a strategy currently being investigated to ameliorate the array of deleterious effects following SCI. The terms mesenchymal stromal cells and mesenchymal stem cells have been interchangeably used in the published literature; however there are demonstrable differences between the cells. Mesenchymal stem cells are a subset of stromal cells that maintain the same fibroblast morphology and specific marker expression; however they also have the potential for self-renewal and to differentiate into adipocytes, chondrocytes, and osteoblasts *in vitro* [53–55]. For cultured cells to be defined as MSCs, they should demonstrate the following: (1) adherence to plastic under culture conditions, (2) expression of CD105, CD73, and CD90, (3) lack of expression of CD45, CD34, CD14/CD11b, CD79/CD19, and HLA-DR surface markers, and (4) possession of the transdifferentiation potential to mesodermal lineages *in vitro* [56].

MSCs reside in a wide variety of tissues but are typically extracted from bone marrow and adipose tissue and to a lesser extent the umbilical cord. Their wide distribution and perivascular origin [57] account for their capability to sense and respond to injury by secreting trophic and anti-inflammatory factors [58,59]. The first report of MSCs isolation from bone marrow was by Friedenstein and colleagues. [60]. The spindle-shaped cells isolated were defined as colony-forming units (CFU) with the potential for *in vitro* culture for further transplantation *in vivo* [61]. It was only in 1999 that Pittenger and colleagues established the multi-lineage differentiation potential of MSCs into distinct mesodermal lineages [62]. Originally, Friedenstein and colleagues cultured MSCs by plastic adherence. Since then, many groups have modified this technique by expanding MSCs as a suspension culture [63–65].

Since its origin, bone marrow-derived MSC culture techniques have been continuously validated and improved. While isolation via plastic adherence is effective, the isolation of such cells does not yield a purified population of MSCs leading to varied growth kinetics and differentiation capabilities [66]. The complications of a heterogeneous population can be overcome by the purification of MSCs by using single specific surface markers such as Stro-1, CD271, Stro-3, CD73, and CD2000 [65,67]. Transplantation of MSCs, selected as above,

following SCI in a rat model translated to marked improvement in functional recovery and increased tissue sparing [68].

Compared to iPSCs or embryonic stem cells (ESCs), MSCs overcome the ethical concerns of isolation, as MSCs can be extracted from one's own bone marrow or adipose tissue [69]. Recent data has indicated the principal therapeutic advantages of MSCs are their neuroprotective [70,71] and immunomodulatory [71,72] properties.

2.2. Regenerative potential of MSCs

The ubiquitous presence of MSCs around blood vessels makes them more amenable to respond to cues from tissue damage. Recent reports attribute the immunomodulatory function of MSCs as suitable for regenerative therapies and thereby tissue repair. Experimental evidence has shown that the immunomodulatory effect of MSCs is possibly due to their ability to suppress T-cell proliferation by secreting soluble factors such as TGF-beta and hepatocyte growth factor and not via apoptosis [73]. MSCs can also exert their immunomodulatory effect by shifting the balance in favor of regulatory T-cells, known suppressors of the immune system that are triggered by anti-inflammatory cytokines such as IL10. Aggarwal and Pittenger [74] co-cultured populations of immune cells with human MSCS and demonstrated that MSCs altered the secretory cytokine profile, restored balance between helper T-cells and macrophages, reduced pro-inflammatory cytokines, and increased anti-inflammatory molecules, thus favoring the induction of a tolerant anti-inflammatory phenotype [74,75]. Shifting and quenching the inflammatory response then redirects the body's resources toward tissue repair and growth [76,77]. Thus far, preclinical studies have demonstrated the potential for MSCs and their secreted factors to repair damaged tissue through their immunomodulatory and neuroprotective properties.

2.3. Treating cervical SCI with MSCs: toward clinical application

Clinically, the majority of interventions in treating SCI are pharmaceutically based and designed primarily to manage pain and control inflammation. With recent advances in stem cell therapy, there has been an increased interest in clinical studies evaluating the safety and efficacy of stem cells. MSCs are considered a favorable option for transplantation due to a number of factors: ease of isolation, rapid clinical expansion of cultures [78], ability to be cryopreserved and regenerated without loss of potency [79,80], minimal risk of tumorigenicity [81,82], multipotent capabilities, and the clinical possibility for autologous transplantation [83,84]. Furthermore, MSC transplantation has been tested widely in clinical trials and considered safe in a variety of neurological, cardiovascular, and immunological diseases [85]. As such, there is great potential for MSCs as a treatment for SCI, which has been well documented within the literature [53,68,86,87]. There are a number of clinical trials (both ongoing and completed) to test the potential of using MSCs to treat SCI (for the most current information, please refer to www.clinicaltrials.gov). Despite promising results of MSC therapies in animal SCI models and potential for clinical translation, there is yet to be an FDA approved treatment available for SCI patients.

Further investigation is needed to fully understand the basic delivery of MSCs and the mechanistic role in cervical SCI. It has not been established that engraftment and differentiation of MSCs are even needed for a therapeutic effect, and less than 1% of MSCs survive for longer than a week when systemically administered [88–90]. This survival rate would be similar in intraspinal injection. A transplantation study by Paul and colleagues compared the efficacy of hMSC engraftment when delivered either via lumbar puncture (LP), intravenous transfer (IV), or direct injection into the injury site in a rat C5 subtotal hemisection model [91]. Based on their results assessing engraftment volume (direct injection > LP > IV), glial scarring (no difference seen after 21 days of MSC transfer), and host immune response (direct injection had the highest host immune response), it can be concluded that MSC delivery via LP is a viable alternative. LP can overcome some of the difficulties of delivering MSCs in patients at the clinical setting. As a validation, a clinical trial performed by a Japanese group evaluated the effect of MSCs treatment in a single patient with a C5 fracture dislocation [92]. On day 13, he received an autologous MSC transplant via lumbar puncture and showed gradual improvement over the 6-month period in both motor and sensory scores graded according to the American Spinal Injury Association (ASIA) Scoring for International Standards for Neurological Classification of Spinal Cord Injury (ISNCSCI). Though this is a single case reported, it appears to be promising for the future of MSCs as an intervention in cervical SCI.

2.4. Sources of MSCs

2.4.1. From bone marrow stromal cells (BMSCs)

Bone marrow stem/stromal cells (BMSCs) are the most commonly used stem cell source for transplantation in experimental SCI models. These multipotent cells are derived from the heterogeneous stroma of bone marrow, which is also comprised of hematopoietic stem cells (HSC). MSCs are separated from other cells (like HSCs) by the expression of distinctive cell surface markers [53,67]. Though there has been much speculation about the transdifferentiation capacity of MSCs into neuronal and glial lineages [71,93], Hofstetter and colleagues reported that transplanted MSCs could not be induced to differentiate toward a neuronal fate, either *in vitro* or *in vivo*, in spite of the fact the MSCs displayed weak expression of NeuN (a neuronal marker) [94].

As one of the first identified sources of MSCs, BMSCs have been well studied for their anti-inflammatory, neurotrophic, and neuroprotective functions in SCI [95,96]. BMSCs transplanted into the rat spinal cord after a contusion injury demonstrated sensorimotor enhancements, partly due to their anti-inflammatory effects by attenuating activation of microglia and astrocytes [97]. To further support their therapeutic role in SCI, BMSC transplantation has been shown to reduce cavity formation, enhance axonal growth, and also prevent neuronal apoptosis [86]. While the majority of the studies have explored MSCs potential in a thoracic injury model, very few studies demonstrate their effect in a cervical SCI model; here we summarize studies relevant to cervical SCI.

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One of the earliest studies to investigate transplantation within the cervical region investigated the use of rat-derived BMSCs in a combinatorial approach with cyclic adenosine monophosphate (cAMP; a neuronal stimulator) and neurotrophin-3 (NT-3; neurotrophic factor) [98]. Using a dorsal column injury as a model system, cAMP and NT-3 were injected 5 days prior to a C4 transection at L4 to precondition the DRG soma. BMSCs were then transplanted 7 days post injury. This combinatorial approach showed successful axon regeneration throughout and beyond the injury site after 12 weeks that was augmented by preconditioning with cAMP and NT-3. Despite this, functional recovery was not supported. The authors attribute the failure to the regenerating axons not reaching their target in the gracile nucleus, a major region that intercepts sensory information.

MSCs can secrete anti-inflammatory molecules and neurotrophic factors, which can lead to immunomodulation and tissue regeneration. In addition, they can also be engineered to secrete factors specific to CNS regeneration. In 2005, Lu and colleagues examined the ability of BMSCs to promote repair in the injured cord by secreting growth factors that overcome the inhibitory environment of the lesion [99]. Native or neurally induced rat-derived BMSCs were modified to either express human brain-derived neurotrophic factor (BDNF) in an acute injury or NT-3 in a chronic injury model [100]. By 1 month post transplantation within a C3 dorsal column lesion, BMSC grafts (both native and neurally induced) supported the growth of host and sensory motor axons, a finding that was augmented by either BDNF or NT-3 transduction. Transduction with neurotrophic factors substantially increased the number of coerulospinal, raphespinal, sensory, and motor axons penetrating the lesion site. Supporting these results, Novikova and colleagues transplanted BMSCs that were pretreated with Schwann-cell differentiating factors into a rat C4 hemisection model and demonstrated that BMSCs prestimulated to secrete neurotrophic factors can also contribute to inhibition of astrogliosis and the post-injury inflammatory response [101].

Another group investigated the combination of BMSCs with rat-derived neural progenitor cells (NPCs) co-transplanted in a rat C3 dorsal hemisection transection model [102]. As demyelination is a significant obstacle following SCI, the strategy was employed with the hypothesis that BMSCs would drive NPCs toward a mature oligodendrocyte lineage. In contrast with their *in vitro* data where BMSCs sufficiently redirected NPC differentiation toward an oligodendrocyte lineage, the group's *in vivo* data failed to demonstrate the same 6 weeks post transplant.

Human-derived BMSCs are a clinically attractive transplantation strategy because they can be reintroduced into patients as autografts or allografts. One preclinical study examined the transplantation of human-derived BMSCs from the iliac crest of four different healthy donors into a rat C3–C4 hemisection model [66]. Regardless of donor source, BMSCs survived and filled the lesion site with minimal migration and substantial axonal growth by 2 weeks post transplantation. By 11 weeks post transplant, no BMSCs were present within the lesion site, having been replaced by host oligodendroglial cells. Axonal infiltration into the lesion site and functional recovery varied by donor; however there was no direct correlation between the amount of axonal growth and forelimb function. One possible explanation for this is that

individual donor-based distinctions in the secretory profile of BMSCs contributed to the varied outcome.

As challenging as the pitfalls within previous results may appear, there are currently two active clinical trials (Phase I NCT02574572 and Phase II/III NCT0167644) approved by the FDA to study the safety and efficacy of BMSCs transplanted in patients with chronic SCI. In conjunction with human studies, a clinical study tested the dose-dependent efficacy of autologous human-derived BMSCs in 13 patients with a mix of cervical (five patients) and thoracic (eight patients) chronic SCI. They found no deleterious effect on the patients; however only one of the 13 patients showed improvement [103]. It is possible that the absence of statistical significance can be attributed to the difference in observation of positive outcomes. For example, while one patient showed an improvement in motor power, two other patients had a patchy improvement in sensory outcome below the level of injury. At the chronic stage, the formation of glial scar tissue around the injury lesion is possibly too dense for growing axons to penetrate. While the transplantation of allogenic BMSCs was deemed safe in patients with chronic SCI, there is a need for preclinical studies to establish the mechanism of MSC's contributory role in chronic SCI.

While spinal cord injury models are in the majority represented by a contusion or hemisection injury, damage via herniated discs is also quite a common cause of disability. A herniated disc under traumatic events can lead to spinal cord injury. A discectomy is the surgical removal of the herniated nucleus pulposus of a vertebral disc to reduce pressure on the spinal cord or radiating nerves. Clinically, a discectomy is treated by fusing artificial prostheses to replace the intervertebral disc. In a preliminary proof-of-concept study utilizing an ovine model, one group sought to replicate the intervertebral disc by formulating allogeneic BMSCs with a chondrogenic agent, pentosan polysulfate, to form a cartilaginous matrix when implanted into the animal at the C3–C4 and C4–C5 levels [104]. The implant was devoid of any adverse events, and histological evidence showed predominantly cartilaginous tissue within the interbody cages. This was further confirmed by CT scans at 3 months post transplantation that showed significant bone formation in the cohort receiving BMSCs with pentosan polysulfate when compared to the cohort that received BMSCs alone. Although this particular study had its limitations, it further illustrates the potential for MSCs in preserving spinal function and advancing regenerative medicine.

2.4.2. From adipose tissue (AMSCs)

Adipose tissue is equally attractive as a cell therapy source, due to its minimally invasive harvesting procedure. From a clinical standpoint, adipose-derived stem cells (AMSCs) can easily be obtained from the large quantities of fat tissue that are removed by routine and safe procedures such as liposuction and abdominoplasties. Adipose tissue also contains supportive stroma that can be isolated and differentiated toward mesodermal lineages [105].

While a vast majority of the literature indicates that AMSCs support axonal growth, a study utilizing transplanted human-derived AMSCs in a rat C3–C4 hemisection was found to

significantly reduce sprouting of the descending serotonergic fibers at the injured site [106]. The authors attributed this to several cumulative factors including enhanced survival of neurons and axons, attenuating the need for excessive sprouting, and reduced astroglial and microglial reactivity favoring the growth of the fibers into and across the transection site. Consistent with other MSC transplantation studies, there was no improvement in functional recovery despite promising microanatomical changes.

Use of AMSCs in humans has been validated for safety and toxicity in both a preclinical testing and a Phase I clinical trial [82]. It is important that every batch of stem cells prepared for transplantation into humans is processed under strict GMP conditions and the cultured cells are verified for absence of toxins and tumorigenic potential in preclinical testing. The purpose of this study was to evaluate any tumorigenic potential for hAMSCs. Twelve weeks post transplantation, the safety of transplanted AMSCs showed no significant difference in adverse events, ECG monitoring, and physical examinations. Though there was no statistical significance in ASIA score, individual patient scores showed improvement at different levels for motor and/or sensory assessment.

2.4.3. From human umbilical cord blood (UMSCs)

Human umbilical cord blood-derived MSCs (UMSCs) offer various therapeutic advantages in SCI treatment with reversal of SCI pathophysiology (downregulation of apoptotic genes and secretion of neurotrophic factors) in as little as 5 days post injury [107]. In an example using a thoracic SCI, transplantation of UMSCs was reported to have been transdifferentiated toward neuronal and oligodendroglial phenotypes. This was viewed as being a successful strategy as evidenced by improved functional motor outcome [86]. These transdifferentiated oligodendrocytes supported the injured spinal cord in remyelination by secreting neurotrophic factors [108]. UMSCs demonstrate a potential application in the treatment of SCI by their reported ability to transdifferentiate into neuronal lineages. For further information on UMSCs in thoracic injury, readers are referred to the review by Park and colleagues [109].

The first clinically based translational study to use UMSCs was in a rat model of radiation myelopathy, in which significant improvement of the microenvironment previously affected by radiation therapy was observed [110]. Radiation myelopathy is a rare, yet serious complication of cancer radiotherapy. Even though radiation myelopathy is not classified as a traditional SCI, there are many similarities in pathophysiology such as vascular damage and demyelination. This particular case study was relevant, as the group studied the efficacy of UMSCs in a radiation myelopathy model restricted to the cervical spine. Administration of the UMSCs clearly improved both microvessel and endothelial cell density, along with functional improvement in blood flow. Concurrent with other studies utilizing MSCs, UMSCs were able to reverse injury induced inflammation by reducing pro-inflammatory and increasing anti-inflammatory cytokines within the spinal cord. **Table 1** shows eight preclinical and clinical trials involving the transplantation of MSCs, describing study design, injury model, observed outcomes, and noted adverse effects.

Study and reference (PMID)	Study designs	Preclinical or clinical trials	Functional Observations	Histological/ imaging datas	Adverse events
Long-term results of spinal cord injury therapy using mesenchymal stem cells derived from bone marrow in humans (22127044)	Cells: autologous MSCs harvested from iliac bone Dosing: MSCs (8×10^6) directly injected into spinal cord and (4×10^7) were injected into intradural space Sample size: *N*=10 patients with ASIA class A or B injury caused by traumatic cervical SCI After 4 and 8 weeks of first injection, 5×10^7 injected via lumbar tapping Measurements: grading of motor power, MRI, electrophysiological recordings	Clinical	At 6-month follow-up: - Six out of 10 patients showed improvement in motor skills - Three out of 10 showed gradual improvement in daily activities	MRI showed a decrease in cavity size and the appearance of fiber-like low-signal intensity streaks	None observed
Intrathecal transplantation of autologous adipose-derived mesenchymal stem cells for treating spinal cord injury: a human trial (26208177)	Cells: autologous ADMSCs isolated from lipoaspirates of patient's subcutaneous fat tissue Dosing: 9×10^7 cells per patient intrathecally through lumbar tapping at day 1, 1 month, and 2 months Sample size: *N*=14 patients with 12 for ASIA A, 1 for B, 1 for D Six patients were injured at cervical, 1 at cervicothoracic, 6 at thoracic, and 1 at lumbar levels Measurements: MRI, hematological	Clinical	At 8-month follow-up: - ASIA motor score was improved in five patients - ASIA sensory score was improved in 10 patients - Voluntary and contraction improvement was seen in two patients - One patient showed median nerve improvement in somatosensory	MRI: no significant interval change for presence of tumorous growth EEG: no significant change after transplantation	Urinary tract infection, headache, nausea, and vomiting were observed in three patients

Study and reference (PMID)	Study designs	Preclinical or clinical trials	Functional Observations	Histological/ imaging datas	Adverse events
	parameters, electrophysiology ASIA motor/sensory scores were assessed before and after transplantation		evoked potential test		
Chronic spinal cord injury treated with transplanted autologous bone marrow-derived mesenchymal stem cells tracked by magnetic resonance imaging: a case report (25885347)	Cells: BM-derived MSCs retrieved from iliac crest were labeled with superparamagnetic iron oxide nanoparticles Dosing: intrathecal transplantation into lumbar spine with 30×10^6 cells (50:50 ratio of both labeled and unlabeled cells) After transplantation, the patient was placed in the Trendelenburg position for 24 hours Sample size: *N*=1 patient with an incomplete SCI from the atlantoaxial subluxation	Clinical	- ASIA B score did not change over 12 months - At 2 days, 6 months, and 12 months post operative, both upper and lower limbs motor score had not changes from preoperative levels - Light touch and pin prick test also did not change	MRI: showed positive signal from labeled cells in the cervical region after 48 hours No change at the structural level of injured spinal cord at any follow-up	After transplantation, patient experienced fever, headache, and myalgia with increased neurologic pain after 12 months
Transplantation of autologous bone marrow mesenchymal stem cells in the treatment of complete and chronic cervical spinal cord injury (23948102)	Cells: prepared from BM collected from iliac spine Dosing: 8×10^5 cells/μl in 25 μl slowly injected to a depth of 3 mm at multiple sites in the central dorsal area across the junction of injured and uninjured spinal cord Sample size: *N*=40 patients with complete and chronic cervical SCI divided into control and treatment groups	Clinical	- Ten patients from treatment group showed significant clinical improvement in motor, sensory, and residual urine volume - Nine patients showed changes in AIS scores - Eight out of 20 patients in the treatment group	N/A	One or two patients in the treatment group developed fever and reported headaches

Study and reference (PMID)	Study designs	Preclinical or clinical trials	Functional Observations	Histological/ imaging datas	Adverse events
			showed significant improvement in postoperative EMG		
Localized delivery of brain-derived neurotrophic factor-expressing mesenchymal stem cells enhances functional recovery following cervical spinal cord injury (25093762)	Cells: WT-MSCs or BDNF-MSCs MSCs derived from BM of adult transgenic rats expressing GFP and transduced with murine leukemia virus encoding BDNF MSCs characterized by cell surface expression of CD105 and lack of CD45 Dosing: 2×10^5 cells injected intraspinally at C2 at time of injury Measurements: immunohistochemistry, electromyogram (EMG)	Preclinical: unilateral spinal cord hemisection at C2 in Sprague-Dawley rats; male	N/A	Retrograde labeling with CTB showed localization of MSCs near injection site and primarily in the white matter At day 14 after transplantation, all rats treated with BDNF-MSCs showed functional recovery of diaphragm	N/A
Bone morphogenetic proteins prevent bone marrow stromal cell-mediated oligodendroglial differentiation of transplanted adult neural progenitor cells in the injured spinal cord (23770801)	Cells: NPC isolated from sub-ventricular zone and BMSC isolated from bone marrow Dosing: immediately following the transection, cell suspensions (2 μl) containing either only BMSCs (0.6×10^5 BMSCs/μl; *N*=5), only NPCs (1.8 × 105 NPCs/μl; *N*=5), or a mixture of NPCs and BMSCs (1.2×10^5 NPCs/μl mixed with 0.3×10^5 BMSCs/μl; *N* =6) were administered by a single injection into	Preclinical: C3 complete transection in adult female Fischer 344 rats	None	In vitro assays demonstrate blocking of BMP signaling enables BMSC-induced differentiation of NPCs to oligodendrocytes	

Study and reference (PMID)	Study designs	Preclinical or clinical trials	Functional Observations	Histological/ imaging datas	Adverse events
	the center of the lesion site				
Neuroprotective and growth-promoting effects of bone marrow stromal cells after cervical spinal cord injury in adult rats (21521004)	Cells: harvested from femur and tibia of rats and differentiated to a Schwann-cell phenotype Dosing: 10–12 × 10^6 cells were injected into lateral funiculus at approx. 1 mm from the rostral and caudal site to the lesion Measurements: immunohistochemistry	Preclinical: C4 hemisection in Sprague-Dawley rats; female	N/A	At 6–8 weeks: NF-positive fibers, serotonin-positive raphespinal axons and CGRP sensory axons were seen in the injured cord	N/A
Grafting of human bone marrow stromal cells into spinal cord injury: a comparison of delivery methods (19182705)	Cells: frozen hMSC thawed and transplanted Dosing: animal groups received either 1 × 10^6 cells transplanted via LP or IV or 1.5 × 10^5 cells directly at injury site Measurements: histology, immunohistochemistry	Preclinical: right subtotal hemisection at C4–C5 Sprague-Dawley rats; female	N/A	Cells delivered via LP showed: - Early tissue sparing in immunostaining for GFAP - Reduced host immune response staining for ED1 (macrophage) and CD5 (pan T-cell marker)	N/A

Table 1. Tabulated summary of preclinical and clinical trials involving the transplantation of MSCs; for each trial the study design, injury model, observed outcomes and adverse effects are described.

3. Embryonic-derived stem cells (ESCs)

3.1. Derivation of embryonic stem cells

Embryonic stem cells (ESCs) represent an intriguing avenue to pursue in the race to understand and treat neurodegenerative pathophysiology. Their pluripotency makes them an extremely versatile option when compared to the limitations of MSCs, and the basic cell culture techniques governing them have been established for decades. ESCs are pluripotent cells derived from the inner cell mass harvested at the embryonic blastocyst stage. Unlike multi-

potent stem cells, such as MSCs, that are limited to mesoderm lineages, ESCs have the ability to give rise to all three germ layer lineages: the ectoderm, endoderm, and mesoderm. The capacity to generate neuronal lineages under directed conditioning makes them an ideal target for cell-based therapies of the nervous system [111–113]. Furthermore, ESCs possess the ability to self-renew, a key characteristic of stem cells that allows for a potentially indefinite source of cells.

ESCs were first harvested and cultured in the early 1980s from murine sources and eventually followed by human sources in the late 1990s [113–116]. Key differences in the *in vitro* culturing of these cells were responsible for the almost 20-year gap between the successful harvesting of murine versus human-derived ESCs. Murine ESCs are capable of surviving without the support of fibroblast feeder layers but require the addition of leukemia inhibitory factor (LIF). This led to earlier xeno-free expansion and characterization than human sources, which were later found to rely on the addition of fibroblast growth factor (FGF) to retain their pluripotency and stem cell characteristics [117].

Despite the positive attributes of ESCs, there are certain shortcomings that cannot be overlooked. Current research utilizing human-derived ESCs is still limited by the lack of chemically defined, *in vitro* culture conditions and is often dependent on the extracellular matrix and growth factor support of Matrigel substrates [118–120]. Additionally, the harvesting and culture of human-derived ESCs raise ethical concerns and extended culturing has been debated to lead to karyotypic stability. *In vivo* use of ESCs often leads to teratoma formation and the need for immunosuppressant drugs, all of which pose a problem for usage in clinical studies [111,121–123]. Current research strategies have centered around developing high-efficiency, high-purity differentiation protocols in order to generate committed or progenitor cell populations that limit their teratogenicity and enhance their therapeutic potential.

3.2. The regenerative potential of ESCs

In treating SCI, groups have looked to generate neural and glial-specific lineages such as oligodendrocytes, astrocytes, and neurons from ESCs. The majority of differentiation protocols lead to high astrocyte and oligodendrocyte populations and relatively few neurons, likely due to the proliferative capacity of the supporting glial cell types compared to neurons [40,111,124–126]. Differentiation methods developed by the McDonald and Keirstead groups have extensively researched the high-efficiency generation of oligodendrocyte progenitor cells (OPCs), which have proven useful in improving myelination and functional outcomes of increased weight support and partial hindlimb gait coordination after thoracic SCI [40,127–131].

Elegantly designed directed differentiation protocols from the Jessell research group using retinoic acid and sonic hedgehog have led to generation of spinal motor neurons from ESCs, which expressed progenitor motor neuron (pMN) marker Olig2 followed by classic motor neuron markers, Isl1, Hb9, and choline acetyltransferase (ChAT) [132]. When grafted into embryonic day 27 chick spinal cords, these cells have shown integration into the ventral spinal cord and acquire appropriate native motor pool identities [132,133], indicating a potential cell source for lost motor neuron pools after SCI. The Keirstead group has also investigated the

spontaneous differentiation of human ESC-derived neural progenitor cells (NPCs) into various neuronal phenotypes such as cholinergic, serotonergic, dopaminergic (DA) and/or noradrenergic, and medium spiny striatal neurons [130,134]. Unfortunately, when ES-derived NPCs are transplanted as a heterogenic population, over-proliferation of undifferentiated stem cells can occur and potentially lead to tumor formation [135]. Further research has investigated the ways to mitigate this over-proliferation through antibiotic selection in order to generate high-purity progenitor motor neurons and committed motor neuron populations [136–138].

The first study to demonstrate that stem cells could be induced toward a cortical projection lineage utilized murine ES cells [139]. Appropriate culture conditions and *in vitro* patterning led to the generation of neural precursor cells that initially express forebrain progenitor-specific genes and later features of cortical pyramidal neurons. Most interestingly, transplantation of these cultures into various locations in the mouse brain led to area-specific axonal and dendritic growth, connectivity and integration within the host CNS, and axon extension to developmentally appropriate targets. These results suggest that it is possible to drive stem cells toward committed neural subtypes that can integrate into anatomically relevant circuits *in vivo*.

With all of these various cell populations, numerous cell-based treatment strategies for SCI have been investigated; however the overwhelming majority of these studies have been completed in thoracic injury models. Despite cervical injuries comprising more than 50% of the SCI population, very little preclinical research has been completed to date in this representative model [111].

3.3. Treating cervical SCI with ESCs

The first study to use embryonic-derived stem cells for therapy in a cervical SCI model was from Sharp and colleagues, looking at the use of human ESC-derived OPCs in a severe midline contusion cervical SCI rat model [140]. The hESC-derived OPCs used in this study were derived using the same protocol [130,131,141,142] as those used in earlier thoracic injury models from the Keirstead group [39,142] and in the Geron™-sponsored, clinical trial (first to use human ESCs as a therapeutic) that was halted in 2011 due to financial and safety concerns [143,144]. This study found that transplanting hESC-derived OPCs 7 days post injury led to a decrease in lesion area accompanied by robust white and gray matter sparing 8 weeks post transplant. Transplanted cells remained localized within the lesion epicenter with minimal rostral or caudal migration. Notably, the preservation of endogenous motor neurons was correlated with increased forelimb function. Finally, the use of transplanted hESC-derived OPCs led to differential changes in spinal cord gene expression for neurons, growth factors, apoptosis, and inflammation [140]. As such, injured animals with transplanted cells saw gene expression levels more closely matching their uninjured counterparts when compared to transplant-naïve animals. Since the discontinuation of the Geron™-sponsored thoracic injury clinical trial, Asterias Biotherapeutics, Inc., has undertaken the use of hESC AST-OPC1 cells for clinical use in a new Phase I/IIa efficacy and safety trial for cervical-level injuries (NCT02302157).

While most of the literature utilizes acute injury models to study the intervention of stem cells, one group investigated the use of hESC-derived OPCs in a rat cervical chronic injury model. In the 2013 study by Sun and colleagues, Olig2+ GFP+ OPCs derived from mouse ESCs were transplanted 4 months post rat cervical irradiation injury [145]. The irradiation injury model is a chronic injury commonly caused by radiotherapy for cancer treatment that results in the death of oligodendrocytes, which leads to severe demyelination and increased axon death. By 8 weeks post transplant, there was significantly less demyelination in addition to improved forelimb locomotor function using a clinical degree of weakness scale. Transplanted hESC-OPCs differentiated primarily into mature oligodendrocytes that expressed myelin basic protein. These cells were produced using a retinoic acid/sonic hedgehog differentiation protocol similar to the ones developed by the Jessell and Zhang groups [132,146].

The Keirstead group has also exploited the potential for ESCs to differentiate into neural lineages for use in cervical SCI treatments. They demonstrated successful use of hESC-derived progenitor motor neurons (pMNs) in a cervical SCI contusion model. The pMNs were generated using a retinoic acid differentiation protocol over 28 days and were shown to be Olig1/2+, as well as Tuj1 and Hb9 positive. Electrophysiology of these cells indicated glutamate receptors after 8 weeks in culture and could innervate both human and rodent muscle tissues. *In vivo*, reduced SCI pathology, or lesion size, and greater endogenous neuron survival and growth were observed in hESC-pMN transplanted spinal cords. Furthermore, these outcomes correlated with increased functional recovery on the balance beam task [147]. The authors did note, however, that differentiation of transplanted cells was dependent on location. Cells that were found in the distal ventral horn led to increased differentiation, whereas cells confined to the site of injury reverted to a progenitor state.

Other embryonic or fetal-derived tissues have been used in spinal cord injury therapies, including whole fetal spinal cord transplants (pioneered by Reier and Anderson) and fetal neural progenitor/stem cells taken from brain and spinal tissue [51,111,123,148–157]. In the study by Diener and Bregman, transplantation of fetal spinal cord tissues into cervical hemisections led to supraspinal growth, axon projections, improved skilled forelimb function indicating transplanted cell survival, and potential local circuit formation. Further investigation of fetal spinal cord lineage-restricted NPCs by various groups has indicated differentiation of transplanted cells into all three neural lineages, in both cervical and thoracic level injuries, with long distance cell migration and axon growth leading to functional improvement [123,148–151,158]. Two clinical trials have begun since 2013 investigating the use of fetal-derived tissues for spinal cord injury [144]. StemCells, Inc.® have begun a safety and efficacy study using human-derived CNS-stem cells (proprietary source) in cervical SCI after completing a thoracic injury trial [159]. Neuralstem, Inc., is also investigating the use of fetal stem cells in SCI [160,161] after promising results using such cells in ALS treatment. This trial, however, is determining the efficacy of these cells in a thoracic injury model. While these cells have proven useful in improving functional outcomes after SCI in preclinical trials, they are subject to the same ethical concerns as those for ESCs and have limited differentiation capacity in comparison [162].

In treating SCI, ESCs demonstrate great potential in promoting axon regeneration and functional recovery, but the lack of full characterization of cell safety, efficacy, and phenotype has significantly limited their clinical applications. Furthermore, the use of ESCs in cervical SCI models is limited to less than a handful of peer-reviewed studies. As cervical injuries are becoming more prevalent, future studies must be designed that better replicate clinical injuries in order to more accurately test cell therapeutic strategies.

4. Induced pluripotent stem cells (iPSCs)

4.1. Derivation of iPSCs

Induced pluripotent stem cells (iPSCs) are created by reprogramming adult somatic fibroblasts to revert to a pluripotent stem cell state initially via retroviral delivery of Oct3/4, Sox2, c-Myc, and Klf4 [163–165]. Now iPSCs can be generated via multiple processes, each with its own merits and limitations. Viral transduction is easy to use and reproducible, yields iPSCs efficiently, and is controlled. However there is an increased risk of insertional mutagenesis and transgene reactivation, incomplete splicing, and clone-to-clone variation [166–168]. Reprogramming factors can also be fused to cell-penetrating peptides or introduced through plasmids, which requires no genomic modification but is also a time consuming and potentially inefficient process [169]. Finally iPSCs can be induced via mRNA introduction, which is a highly effective and rapid method; it also requires no genomic modification and is deemed safe due to the transient nature of mRNA. However, repeated transfections are typically required [170,171].

While MSCs are hindered by their limited potency and the harvesting of ESCs is subject to ethical constraints, the pluripotency and source of iPSCs circumvent some of these issues, therefore making them a promising alternative in cellular transplantation therapies. iPSCs share many similarities with ESCs and provide a comparative alternative in that they have the same morphology, gene markers, and potential to form teratomas (ability to differentiate into all three germ layers) [163,165]. Furthermore, the use of iPSCs opens up new possibilities for clinical consideration—ethical concerns are diminished and, in the case of potential transplants, cells can be harvested directly from the patient, therefore avoiding the need for immunosuppression.

4.2. Cell reprogramming technologies for controlled differentiation

There has been a strong motivation to create iPSC differentiation protocols that drive stem cells toward the three main neural lineages *in vitro*. Methods to generate functional neurons have been of particular interest so as to study the differences in neuronal networks in both healthy and impaired states [172–177]. In one report, mature human fibroblasts were directly programmed into synaptically active functional neurons via a cocktail of miR-124, BRN2, and MYT1L [178]. An additional group found that Ascl1 (which has pioneer factor properties) in conjunction with BRN2, and MYT1L, successfully drove murine fibroblasts into

neuronal cells with appropriate morphology, expression, and formation of functional synapses [179]. One report demonstrated that the overexpression of neurogenin 2 efficiently transformed iPSCs into functional neurons that were able to spontaneously form excitatory synaptic networks. Furthermore, these networks both synaptically integrate once transplanted into the mouse brain and exhibit plasticity [180]. The majority of research utilizes iPSCs driven toward a neural progenitor state [181,182]; however iPSC differentiation has also been useful in disease modeling. As an example, midbrain dopaminergic (DA) neuron phenotypes have been generated, which has been particularly useful in studying Parkinson's disease (PD), typically characterized by the loss of these DA neurons [183–186]. In one study by the Pera group, a stable iPSC line was derived from a PD patient that carried the most common PD-associated genetic mutation and differentiated into midbrain DA neurons. These iPSC-derived DA neurons exhibited classic hallmarks of PD-related damage including accumulation of α-synuclein and oxidative stress, susceptibility to H_2O_2-induced CASP3 activation, and sensitivity to 6-OHDA and proteasome inhibition. Additionally, other groups have shown that iPSCs can be successfully driven toward glutamatergic, GABAergic, motor, and retinal neuron phenotypes [187–199]. While not specific to SCI, these results demonstrate that developing differentiation protocols capable of generating specific neural subtypes can open up new research avenues in understanding and creating therapies for neuropathologies.

There is also interest in reliably driving iPSCs toward functional glial subtypes, as glial cells are heavily affected in the process of neurodegeneration. In a study by Krencik and Zhang, exogenous patterning molecules were used to transform iPSCs into a neuroepithelial phenotype. From there, administration of mitogens allowed for the generation of astroglial progenitors, which could then be further differentiated into functional astrocytes via ciliary neurotrophic factor [200]. Another protocol utilized the forced expression of Sox10, Olig2, and Zfb536 to directly reprogram rodent fibroblasts into oligodendrocyte precursor cells. The resulting population of precursors exhibited typical morphology and gene expression and gave rise to mature oligodendrocytes that could ensheath dorsal root ganglion cells *in vitro* and form myelin *in vivo* [201].

The intended goal behind SCI therapies is to ameliorate damage and restore the circuitry within the CNS. Cellular transplantation offers an innovative means in accomplishing this, but is obviously extremely dependent upon the characteristics and capabilities of the transplanted cell type. Driving human iPSCs toward neuronal lineages via reproducible and robust differentiation protocols represents a practical interface between developmental neurobiology and SCI research; it may be possible to tailor iPSCs toward a more developmentally appropriate, specific neuronal cell type capable of restoring CNS connectivity rather than the uncharacterized progenitor populations previously used with limited functional recovery.

4.3. Treating cervical SCI with iPSCs

Similar to MSC and ESC-focused SCI therapies, there is a scarcity of targeted preclinical therapies for SCI using iPSC transplantation. Therapies do show positive outcomes yet they are limited in number; to date there are only five published studies using either rodent or

simian models of thoracic SCI [202]. In these studies, iPSCs were driven toward neural stem spheres [203], neural stem cells (NSCs) [202,204], and neurospheres [205,206] with all except one study experiencing amelioration of the inhibitory nature of the lesion site, synaptic integration of transplanted cells, and significant functional improvement in transplanted animals.

Therapies targeting cervical SCI are equally as limited. There are four published studies to date that have examined acute, subacute, and chronic iPSC transplantation following cervical SCI. Li and colleagues evaluated respiratory function following transplantation of iPSC-derived astrocytes engineered to overexpress GLT1, an astroglial glutamate transporter [207]. Both rats and mice underwent a C4 contusion injury resulting in chronic diaphragm dysfunction and phrenic motor neuron deterioration. Immediately post injury, subjects received two separate intraspinal injections rostral and caudal to the lesion within the ventral horn. At time points ranging from 2 days to 4 weeks post transplant, it was demonstrated that transplanted grafts survived and differentiated into GFAP-positive astrocytes, were not tumorigenic, and had less than 10% proliferation (evidenced by Ki67 staining). Furthermore, lesion area and volume were reduced within 1 mm rostral and caudal to the lesion epicenter and innervation of the diaphragm neuromuscular junction was preserved in animals that had received iPSC-derived astrocyte transplants that overexpressed GLT1. Through analysis of spontaneous electromyogram (EMG) activity, GLT1-overexpressing astrocyte transplants significantly magnified EMG amplitude in the dorsal region of the hemidiaphragm, further demonstrating preservation of diaphragmatic respiratory function.

Another study by Lu and colleagues examined the effect of human iPSC-derived NSCs harvested from an elderly donor in a C5 lateral hemisection rat model [208]. While the chosen cell population was minimally characterized, *in vitro* analysis demonstrated reduced expression of Tra1-81 and SSEA4 (pluripotency markers) and maintained expression of nestin and Sox2 (NSC-associated markers). Two weeks post injury, NSCs were intraspinally co-transplanted with a fibrin matrix and a raft of growth factors. By 3 months post transplantation, there was evidence that the grafted cells had survived, distributed throughout the lesion, and integrated with host axons. The majority of grafted cells expressed NeuN and mature neuronal markers MAP2 and Tuj1 alongside mature astrocytic marker GFAP, suggesting preferential differentiation into neuronal and astrocytic lineages. There was also evidence of proliferation and spinal motor neuron identity within a small percentage of transplanted cells via the expression of Ki67 and ChAT, respectively. Most notably, a remarkable amount of axonal growth was present extending from the lesion site toward the olfactory bulbs and lumbar spine sections. Despite robust axonal growth, no behavioral recovery was observed.

In consideration of the substantial lack of existing chronic cervical SCI data, Nutt and colleagues investigated an early chronic injury model mimicking the deficits seen in human injury [209]. Four weeks following a cervical contusion injury at C4, iPSC-derived neural progenitor cells and fibroblasts were co-transplanted rostral and caudal to the lesion in a rat model. Immunohistochemical analysis suggested the differentiation of transplanted cells into mature neurons as well as the intermingling of the host CNS with transplanted cells, as evidenced by NeuN/FOX-3 labeling. Despite interactions between host and donor cells,

transplanted cells did not express glutamate receptors. Furthermore, transplanted cells were not positive for serotonin but did express GABA and were shown co-localized with host positive choline acetyltransferase. Behavioral recovery was weak; grasping and weight bearing were only slightly improved by transplants.

	MSCs	ESCs	iPSCs
Source	Bone marrow, adipose, umbilical cord	Fetal tissue	Somatic (adult) cells
Lineage differentiation	Mesodermal lineage	All three germ layers—endoderm, mesoderm, and ectoderm	All three germ layers—endoderm, mesoderm, and ectoderm
Derivation	Purified by surface markers from adult tissue	Embryonic (inner cell mass of blastocyst)	Induced or reprogrammed to "stemness"
Ease of isolation	Easily accessible sources	Difficult; isolated from fetal tissue	Easily accessible sources (e.g., skin)
Differentiation potential	Multipotent	Pluripotent	Pluripotent
Ethical issues	Minimal; cells can be isolated from the patient	Strong concerns	Minimal; even skin cells can be induced to be pluripotent
Axonal regrowth	Yes; by tissue sparing and neuroprotection	Yes; by transdifferentiation	Yes; by transdifferentiation
Functional outcome	Mild to moderate	Moderate	Mild to moderate
Immunomodulatory	Yes	Low	Low
Immunogenicity/autologous	Low; safe for autologous transplantation	High; often requires immunosuppression	Low; safe for autologous transplantation
Tumorigenicity	No tumor formation	Teratoma formation	Teratoma formation
Clinical trial	Advanced to Phase III	Advanced to Phase II	Preclinical only

Table 2. A comparative scheme of the characteristics of MSCs, ESCs and iPSCs described in this chapter.

In contrast, Kobayashi and colleagues examined the safety and efficacy of iPSC-derived NSC transplants in a simian model of cervical SCI [210]. Human iPSCs were cultured and induced to form neurospheres and passaged a secondary and tertiary time prior to transplantation. Adult female marmosets were given a moderate contusion at the C5 level and received an intraspinal injection of cultured iPSC-neurospheres 9 days post injury at the lesion site. By 12 weeks post transplant, hematoxylin-eosin staining revealed that the grafted cells survived and were positive for NeuN, GFAP, and Olig 1 indicating differentiation into all three neural subtypes. Additionally, animals that received transplants had reduced cystic cavity size, no evidence of tumorigenicity, increased angiogenesis, and a higher amount of neurofilaments and descending motor axons at the lesion center. Severe demyelination was evident surrounding the lesion site in both transplanted and control groups; however, the amount was

significantly exacerbated in animals that did not receive cellular transplantation. These findings were further supported by MRI and myelin mapping in which myelin sparing was more evident in the transplanted group and an intramedullary high-signal intensity area in the lesion site of the control group. Calcitonin-generated peptide fibers, which are involved in spinal pain mechanisms, did not differ between transplanted and control groups. In nonhuman primates, contusion at the C5 level in a severed central cord injury model leads to tetraplegia with an expected gradual improvement in motor function. By 8 weeks post injury, there were significant differences in the open field test, bar grip strength test, and cage climbing tests between transplanted and control groups, which stayed consistent throughout the study indicating some level of functional recovery due to transplantation. Promising results from the various cell-based therapies have demonstrated varying degrees of axonal regeneration and functional recovery. **Table 2** provides a summary of the characteristics of MSC, ESC, and iPSC types and also notes their functional outcomes, tumorigenicity, and clinical trial stage to date.

5. Summary and conclusions

Great care and consideration must be taken when choosing an optimal stem cell type as a potential cellular transplantation treatment for cervical SCIs. Of the three main stem cell types discussed here, there are distinct advantages and disadvantages to each. The use of MSCs in treating nervous system injuries remains a hotly debated topic due to their limited survival, differentiation potential, and functional recovery outcomes. Nevertheless, their immunomodulatory properties and growth factor secretion make them potentially beneficial for use in combinatorial strategies especially if delivered noninvasively. ESCs offer significantly more differentiation potential for neural applications than adult stem cells and have the added benefit of promoting functional recovery in cervical SCI models. However, the lack of detailed cell characterization, need for immunosuppression, and overall ethical concerns have led to only a single cervical SCI clinical trial. Moreover, the use of ESCs in preclinical cervical SCI studies is limited to only two ESC-derived phenotypes (OPCs and pMNs). Significant research must still be performed to fully explore alternative appropriate cell types that can potentially promote functional regeneration. Finally iPSCs, the newest technology in stem cell sources, propose an interesting alternative to fate-limited MSCs and ethically restricted ESCs in treating cervical SCI. Promising preclinical data has indicated iPSC-based therapies can improve functional outcomes after injury; however, their recent discovery highlights the need for careful characterization and exploration of secure differentiation protocols. Further studies must still be completed before iPSCs can be approved for clinical applications.

While stem cell transplantation therapies have shown promise in promoting post-injury regeneration, both anatomical and functional recovery still remain imperfect; no preclinical or clinical study to date has dramatically restored significant recovery in patients. Experimental evidence has shown that native regeneration and plasticity occur in limited amounts following injury. These innate processes can be enhanced via the addition of neurotrophic and immunomodulatory factors, the removal of lesion-associated inhibitory factors, and injury-

appropriate rehabilitation regimens and physical activity. It would be of great interest to determine whether combinatorial approaches utilizing stem cell transplantation in conjunction with the strategies described above provides a synergistic effect within the living system. Furthermore, the vast majority of cell transplantation studies utilize cell populations driven toward immature final phenotypes. The pluripotent capabilities of ESCs and iPSCs provide the freedom to drive these cell types toward numerous definitive lineages or ages. This, however, will be defined by developing appropriate differentiation protocols that can be used in both preclinical and clinical settings. It is possible that transplanting more mature cells results in the establishment and integration of meaningful circuitry within the host nervous system to restore and promote functional recovery.

The broad scope of stem cell therapies offers a myriad of therapeutic potential. However, due to the limited number of preclinical and clinical studies, extensive logistical questions remain regarding how to optimize their usage. Nonetheless, the great strides made in designing and improving effective stem cell therapies for enhancing function promises an exciting future for the field of spinal cord injury repair.

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