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Cell therapy for the treatment of spinal cord injury with focus on stem cells: A review

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Abstract

Traumatic spinal cord injuries (SCIs) cause physical disruption of axons through the epicenter of the injury site, the release of factors that alter neuronal excitability, and inflammation, leading to deficits in motor, sensory, and autonomic function. Other factors contributing to loss of function include the death of cells and the formation of scar tissue that inhibits regeneration. Current clinical treatments for SCI include performing surgery to stabilize the injury site, administering high doses of corticosteroids to limit secondary injury processes, and providing rehabilitative care. However, no long term cures exist for the treatment of SCI and accordingly more aggressive strategies for repairing the damaged spinal cord have been investigated. A number of different cell therapies have been evaluated in both pre-clinical and clinical trials and here we review these studies that evaluated the following types of cell therapies: neural cells derived from embryonic stem cells (ESCs), oligodendrocyte progenitor cells (OPCs), motor neuron progenitor cells (MNPs), neural stem cells (NSCs), bone marrow-derived stem cells, induced pluripotent stem cells (iPSCs), olfactory unsheathing cells (OECs) and Schwann cells (SCs). We discuss the advantages and disadvantages of each cell type and their specific role in functional improvement. We highlight preclinical versus clinical studies along with discussion of new clinical trials and give suggestions for future areas of study.

Key Words: stem cells, spinal cord injury, embryonic stem cells, neural stem cell, clinical trials, olfactory ensheathing cells, Schwann cells, induced pluripotent stem cells

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Abbreviations:

SCI: spinal cord injury
ASIA: American Spinal Cord Injury Association
ESCs: embryonic stem cells
NSCs: neural stem cells
OECs: olfactory ensheathing cells
SCs: Schwann cells
BBB: blood–brain barrier
CNS: central nervous system
BDNF: brain-derived neurotrophic factor
NT-3: neurotrophin-3
NT-4: neurotrophin-4
PNS: peripheral nervous system
NGF: nerve growth factor
GDNF: glial-cell-line-derived neurotrophic factor
IVF: in vitro fertilization

SCNT: somatic cell nuclear transfer
PGD: pre-implantation genetic diagnosis
PDGFRα: platelet-derived growth factor receptor alpha
NG2: nerve/glial antigen-2
FDA: Food and Drug Administration's
MNPs: Motor neuron progenitors
UMNs: upper motor neurons
LMNs: lower motor neurons
IND: investigational new drug
MSCs: Mesenchymal stem cells
HSCs: hematopoetic stem cells
BMSCs: bone marrow-derived stromal cells
iPSCs: induced pluripotent stem cells
ECM: extra cellular matrix
CST: corticospinal tract

Contents

1. Introduction

Spinal cord injury (SCI) is a devastating neurological disorder affecting thousands of individuals each year [1]. Over the past few decades, enormous progress has been made in our understanding of SCI, but there is yet no cure for repairing the injured spinal cord. SCI is a complex disorder mainly caused by events like trauma caused during a vehicle accident, falling from a significant height, severe twisting of the middle portion of the torso, sport accidents as well as nontraumatic reasons like neoplasms, vascular disease, inflammatory disease and spinal stenosis [1, 2]. When axons of spinal cord are physically disrupted through the epicenter of the injury site, deficits in motor, sensory, and autonomic function are caused. Hence, this disabling neurological disorder usually requires life-long therapy and rehabilitative care [3]. According to the scale designed by American Spinal Cord Injury Association (ASIA), injuries are classified in general terms of being neurologically "complete" or "incomplete" based upon the sacral sparing definition.

Sacral sparing refers to the presence of sensory or motor function in the most caudal sacral segments. A complete injury is defined as the absence of sacral sparing, whereas an incomplete injury is defined as the presence of sacral sparing [4]. Annually, between 250,000 and 500,000 individuals suffer from SCI around the world. The World Health Organization has announced that around 90% of these cases are due to traumatic causes; nevertheless the amount of non-traumatic spinal cord injury appears to be growing [5]. Currently, available treatments for SCI consist of administering high doses of corticosteroids and methylprednisolone for limiting secondary injury processes, surgical interventions to stabilize and decompress the spinal cord and rehabilitative care [6]. Advances in understanding the biology of spinal cord injury can lead to effective therapies for functional restoration [7]. Even restoring some function can result in huge improvements in the quality of life for patients suffering from SCI [8]. However existing treatments do not cure SCI.

Cell and stem cell-based treatments have proved their efficacy in pre-clinical investigations with several therapies making it to clinical trials, such as human embryonic stem cells (ESCs)-derived oligodendrocytes, neural stem cells (NSCs), olfactory ensheathing cells (OECs) and Schwann cells (SCs) [9-11]. This review outlines cell and stem cell-based approaches for treatment of spinal cord injury. Here we present each cell type candidate for SCI, discuss their therapeutic value, review animal studies for different types of cells along a summary given for the future investigation of cell and stem cell based treatments for SCI.

A brief review on different cell based treatments for SCI is summarized at the end in Table 1.

Table 1: A brief review on different cell based treatments for SCI

Type of Cell	Cell extraction source	Animal model	Outcome	Comments	Ref.
ESC	rat, mouse and human ESCs	mouse, rat	survival, integration, remyelination and improving locomotor function	ESCs can be directed to differentiate into specific cells with specific protocols (like 4-/4+ retinoic acid protocol). Tumor formation is a legitimate risk for transplanted ESC-derivates.	[6, 12, 40]

OPC	hESCs	Rat	Enhances remyelination and promotes recovery of motor function	Demyelinating pathology is an important prerequisite for the function of transplanted myelinogenic cells. Therapeutic potential of these cells is demonstrated at eraly time points after SCI.	[59, 159]
MNP	hESCs, mESCs	Rat	promote motor neuron survival and regrowth, assist in regulating the maturation of neuromuscular synapses, increase the release of neurotransmitter	The motor neuron differentiation pathway is largely controlled by sonic hedgehog and retinoic acid and methods have developed to differentiate hESCs to high-purity (>95%) human MNP	[12, 75, 77, 78]
NSC	human NSCs	mouse, rat	Differentiated into myelinating oligodendrocytes and caused integration and functional recovery	Transplanted cells differentiated largely into astrocytes and oligodendrocytes within minimal neuronal differentiation	[66, 88-90]
BMSC	MSCs, HSCs	Rat	Create a more favorable environment by modulating the immune response, limiting damage, expressing growth factors and cytokines, and improving vascularization	They can transdifferentiate along glial and neuronal pathways, though in some studies, the characterization of cell phenotype was limited to the detection of lineage-specific markers with no glial or neuronal cell function apparent	[6, 12, 42, 98]
iPSC	iPSCs-derived NSCs	mouse	- Trilineage neural differentiation, functional recovery - Fail to improve functional recovery of pharmacologically immunosuppressed mice	- No tumor formation observed - Failure may be due to insufficient immunosuppressive effect in combination with immunogenicity of transplanted cells	[113, 114, 120]

OECs	nasal biopsies	Rat	stimulate tissue sparing and neuroprotection, enhance outgrowth of both intact and lesioned axons, activate angiogenesis and remyelinate axons after a range of demyelinating insults	They are supportive in repair processes, but the evidence that they facilitate regeneration of long axonal tracts is limited and it is not yet clear whether they can be expanded in sufficient numbers for use in human cell replacement strategies	[124, 139-141]
SC	SCs	Rat	higher myelin ratio, more axon regeneration into and out of the SC implant and further improved locomotion	Combined transplantation of SCs with methylprednisolone, BDNF, NT-3, BDNF or D15A chondroitinase and elevation of cAMP levels	[151-157]

2. Pathophysiology of SCI

Multiple mechanical forces cause spinal cord injury. The basic mechanical trauma initiates a cascade of events including breakdown of the blood–brain barrier (BBB), influx of peripheral inflammatory factors, activation of glial cells, excitotoxicity, and necrosis [12, 13]. The harshness of the injury can vary depending on the location and severity of the injury [14]. A schematic representation of the injured spinal cord is shown in Figure 1.

Norenberg and coworkers have outlined the pathology of spinal cord injury and potential differences between human and experimental animal models in their review [15]. They have divided human SCI into four groups based on gross findings: solid cord injury, contusion/cavity, laceration, and massive compression. Animal models are extensively used for the study of basic pathological changes that follow SCI. However there are differences in injury type between laboratory-induced SCI and clinical SCI. These differences in injury including anatomical location, laminectomy, anesthesia, laboratory stressors and complications as well as other major reasons for discrepancies between promising animal studies and disappointing clinical trials are reviewed by Akhtar *et al* [16].

Primary lesions occurring at the time of injury are followed by secondary injuries which have different mechanisms that can arise in hours or even years. The primary injuries include physical separation of neuro-glial tissue and vascular destruction, loss of neurons within the grey matter, and loss of myelinating oligodendrocytes in the white matter. This damage happens at the moment of trauma and is followed by microhemorrhages in grey matter spreading out radially and axially in a few hours [1, 6, 7, 15]. The acute phase including edema, hemorrhage, inflammation, axonal swelling/degeneration, and the demise of neurons and oligodendrocytes takes hours to 1-2 days [7, 15, 17]. The “secondary injuries” include collagenous scar formation, traumatic neuromata, Wallerian degeneration, and delayed post-traumatic syringomyelia [18]. A secondary cascade of signaling

events causes boosting the inflammatory cytokines and chemokines repeatedly, which leads to apoptosis, progressive loss of oligodendrocytes (and therefore demyelination), and axonal degeneration [12, 19]. In general, vascular abnormalities, excitotoxicity, oxidative stress, and cell death contribute to secondary damage [7]. Uncontrolled release of excitatory neurotransmitters, like glutamate, occurs, causing the death of neurons and oligodendrocytes [1, 17]. The accumulation of glutamate happens instantly in response to ischemia and membrane depolarization and only takes 15 minutes to reach toxic levels [17, 20]. Damage to the central nervous system (CNS) also can result in the formation of glial scar, which poses a physical obstacle for axonal regeneration [21]. Glial scar includes some inhibitory molecules like myelin associated molecules (Nogo, myelin associated glycoprotein, oligodendrocyte-myelin glycoprotein), chondroitin sulphate proteoglycans, individual proteoglycans (DSD-1/phosphacan, neurocan, versican, brevican, NG2, biglycan and decorin, CS56 antigen) and other molecules (tenascin, CD44) that contribute to blocking the regeneration [21, 22] However, aside detrimental effect of glial scar as an obstacle for regeneration, there are some beneficial effects as well. Enhanced astrocyte migration and premature glial scar formation have shown to facilitate recovery [23].

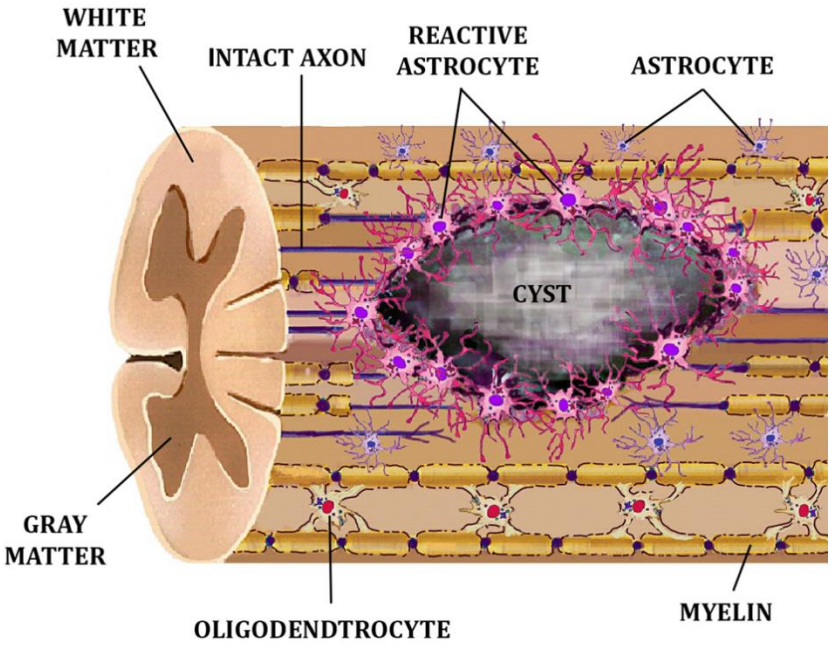

Figure 1: A schematic representation of the injured spinal cord

3. Approaches to functional recovery

An ideal SCI therapy would enhance functional recovery meaning partial or complete return to the normal or proper physiological activity after trauma. Since limiting the progression of injury is much easier than repairing damage, the traditional approach is to stabilizing the patient post injury followed by treatments to limit the damage caused by secondary processes [8]. The formation of glial scar is the

greatest hurdle for regenerating the damaged tissue after SCI. Blocking the effects of glial scar is usually associated with identifying its inhibitory molecules. Some attempts to attenuate these inhibitory components have focused on methods which reduce the synthesis of these components eventually blocking their effect.

Delivering neurotrophic factors for stimulating sprouting axons cross the gap across the lesion is another possible strategy [8, 19]. *In vitro* studies have shown presence of neurotrophins induces oligodendrogliagenesis and enhancement in myelination of ingrowing axons [24]. Administration of brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4 (NT-4) influenced the survival of developing CNS and peripheral nervous system (PNS) neurons and also exerted a neurotropic influence on injured mature CNS neurons by increasing their axonal growth [25]. Treatment using nerve growth factor (NGF), NT-3 and glial-cell-line-derived neurotrophic factor (GDNF), but not BDNF, demonstrated selective regrowth of damaged axons across the dorsal root entry zone and into the spinal cord in adult rats with injured dorsal roots [26].

Using transplanted cells or tissue that support axonal elongation for bridging the gap is another approach for regeneration [19]. The initial studies in this field started to modify axonal growth and provide a surface supporting the growth of new axons [27-29] and regardless of the difficulty of regrowing axons, there has been a remarkable progress in this area [22, 30-39].

Recent studies using cell and tissue transplants for promoting regeneration have focused on stem cells such as ESCs [40] and NSCs [41] as a new regeneration strategy. Stem cells should be incorporated into the injury site in order to restore the lost neuronal functionality, enhance the neuronal plasticity as well as act as support cells that promote regeneration. There are two different ways to utilize stem cells for spinal cord repair: one is to transplant stem cells or cells derived from stem cells to the injured spinal cord, and the other way is taking advantage of the resident stem cells in the spinal cord [42]. Here, we specifically focus on transplanting different types of stem cells as well as two other non-stem cell types that can be used for recovery and discuss their roles in improvement of regeneration, and also the hurdles associated with these cell types

4. Types of cells

4.1. Embryonic Stem Cells (ESCs)

ESCs are pluripotent stem cells isolated from the inner cell mass of blastocysts [43]. Figure 2 illustrates the neural differentiation of ESCs. ESCs utilized in therapeutic research can be derived from embryos made from different methods including *in vitro* fertilization (IVF) [44, 45], somatic cell nuclear transfer (SCNT) [46, 47], altered nuclear transfer (ANT) [48], pre-implantation genetic diagnosis (PGD) [49-51] and parthenogenetic activation of eggs [52]. ESCs have a great potential for cell replacement therapies as they can be propagated *in vitro* almost indefinitely, stably banked, maintain a normal karyotype and differentiation potential even after years of culture and directed to differentiate into diverse cell types [6, 12].

hESCs and mouse ESCs (mESCs) have several morphological and behavioral differences. hESCs have a slower population-doubling rate versus mESCs (~36h population-doubling versus ~12h population-

doubling time) [53]. Both types of ESCs grow colonies but hESC colonies are flat while mESCs form spherical colonies [54]. Both mESCs and hESCs have different signaling networks for maintaining pluripotency as well, and these differences suggest their function and downstream signaling pathways may differ [53].

Although ESCs have great potential and appeal for a therapeutic strategy, there are many concerns regarding their use. Several ethical issues are associated with the use of ESCs as the sourcing of donor embryos can be problematic. Also the possibility of cloning humans from ESCs in the one-step procedure of tetraploid complementation and the risk of their global distribution and being used for reproductive cloning of humans in the future are examples of concerns that need to be put into ethical consideration [55].

hESCs have been directed to differentiate into motor neurons [56], multipotent neural precursors [57, 58], and high purity oligodendrocyte progenitors [59, 60]. mESCs cultured by using a 4-/4+ retinoic acid (RA) protocol have been shown to develop into oligodendrocytes after transplantation into the injured spinal cord [40]; they survived and differentiated primarily into mature oligodendrocytes that were capable of myelinating axons in the demyelination site after transplantation [61]. mESCs predifferentiated with RA also demonstrated better functional outcome after being injected into the lesion epicenter and mESC-treated animals reached the final phase of locomotor recovery[62].

Extensive research has been done on directing ESCs differentiation to produce cells for transplantation after SCI. These cell types include ESC derived neurons, astrocytes and oligodendrocytes. ESC-derived neurons have the ability to survive, integrate and enhance in functional restore after transplantation into injured rat spinal cord [6, 63]. McDonald *et al.* transplanted mESC-derived cells into the spinal cord 9 days after weight drop injury and the cells survived for at least 5 weeks; migrated at least 8 mm away from the site of transplantation; differentiated into astrocytes, oligodendrocytes and neurons without forming tumors; and improved locomotor function [40].

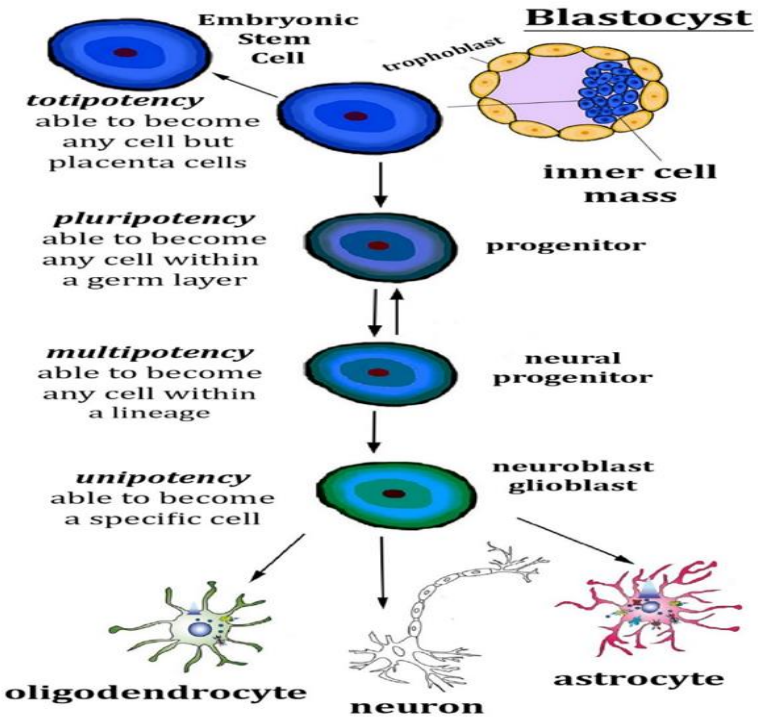

Figure 2: A schematic of embryonic stem cells and their differentiation into the cells of the CNS

4.2. Oligodendrocyte Progenitor Cells (OPCs)

OPCs are scattered in the white matter and gray matter throughout the CNS and represent the main proliferating cell population in the intact spinal cord [64]. OPCs secrete multiple growth factors and oligodendrocyte-myelin permits saltatory conduction, action potential jump from node to node, in axons [12]. They express markers including platelet-derived growth factor receptor alpha (PDGFRα) and nerve/glial antigen-2 (NG2) and are often referred to as NG2-glia, NG2-cells or polydendrocytes [65].

Utilizing endogenous OPCs mainly for remyelination is probably the most practical short-term clinical intention for spinal cord regeneration [66]. Some studies suggested that OPCs have dual lineage potential and besides generating oligodendrocytes they give rise to protoplasmic gray matter astrocytes as well [67, 68]. However, more recent studies have demonstrated that OPCs are restricted to the oligodendrocyte lineage in most situations [69-71].

Keirstead et al. transplanted hESC derived-high purity OPCs into SCI sites of rats 7 days and 10 months after injury and the cells showed survival, redistribution over short distances, and differentiation into oligodendrocytes [59]. In this study, rats receiving OPCs at 7 days after injury exhibited enhanced remyelination and recovery of motor function, while rats that received OPCs after 10 months did not; this indicates that the therapeutic window for this type of treatment is limited to the early post injury period. Sharp et al. transplanted hESC-derived OPCs to thoracic SCI of rats and their experiment resulted in pathotropism (attraction of drugs toward diseased structures) and cells survived and differentiated,

enhanced remyelination, and improved locomotor outcomes without harmful effects [72].

Geron Corporation evaluated therapy using hESC derived-high purity OPCs in Phase I clinical trials. After the U.S. Food and Drug Administration's (FDA) approval of Geron Corporation's clinical trial in 2009, the company started the trial using hESC derived OPCs [73]. A year after initiating the phase I clinical trial, Geron investigators reported encouraging precursory results on safety of cell therapy for four treated patients [11, 74]. However, the trial was surprisingly canceled in November 2011.

4.3. Motor neuron progenitors (MNPs)

In a healthy spinal cord the nerves which lie within the spinal cord are called upper motor neurons (UMNs) and their function is to carry the messages back and forth from the brain to the spinal nerves along the spinal tract. The spinal nerves which branch out from the spinal cord to the other parts of the body are called lower motor neurons (LMNs). The sensory portions of the LMN carry messages about sensation from the skin and other body parts and organs to the brain and the motor portions of the LMN send messages from the brain to the various body parts to initiate actions such as muscle movement. Spinal cord motor neurons synapse with muscle fibers and facilitate muscle contraction by expressing acetylcholine related markers, including choline acetyltransferase and vesicular acetylcholine transferase [12]. MNPs affect endogenous cells by their neurotrophin secretion via several mechanisms, hence they are a good potential therapeutic target. These mechanisms include promotion in motor neuron survival and regrowth and assistance in regulating the maturation of neuromuscular synapses [75-77]. They also increase the release of neurotransmitter and direct synaptic transmission via the Trk family of receptor tyrosine kinases and the p75 neurotrophin receptor[78], enhance neuronal survival, as well as growth of host axons in both normal and injured spinal cords, and improve functional recovery [77, 79, 80].

ESC-derived motor neurons have the ability to populate the embryonic spinal cord, as well as extend axons, and form synapses with target muscles [81]. In 2010, California Stem Cell, Inc. submitted an investigational new drug (IND) application to the FDA to begin phase I clinical trials in infants using human MNPs derived from hESCs for treatment of spinal muscular atrophy, though in 2011 the FDA placed the MotorGraft Program on clinical hold in order to obtain additional data [11].

4.4. Neural stem cells (NSCs)

NSCs can be isolated and expanded from multiple regions of the fetal or adult nervous system including subventricular and subgranular regions [82]. These cells can differentiate into neurons, oligodendrocytes, and astrocytes [83]. NSCs in the lateral ventricle subventricular zone and hippocampal dentate gyrus demonstrate both neuroepithelial and astrocytic properties. They generate transiently amplifying progenitors that can subsequently give rise to neurons. The dentate gyrus neurons stay in the hippocampus, while subventricular zone progeny will migrate to the olfactory bulb [64]. Since NSCs are already committed to a neural fate, it will be easier to differentiate into mature neural phenotypes, and are less likely than ESCs to become neoplastic [6]. Furthermore, NSCs can secrete several neurotrophic factors like BDNF, NGF, and GDNF, *in vitro* and *in vivo* [46]. However, when these cells are transplanted to normal or injured rat spinal cord they have either remained undifferentiated, or differentiated along the

glial lineage [83]. Nevertheless transplantation of these multipotent stem cells, being able to differentiate into a number of cell types but only those of a closely related family of cells, has resulted in integration in regions of tissue damage, differentiation into myelinating oligodendrocytes, and improvement following intraventricular, intravenous, intraspinal, or intraperitoneal delivery to various demyelinating or dysmyelinating animal models [84-87]. Human NSCs transplanted into spinal cord injured mice and rat resulted integration and functional recovery, but the transplanted cells differentiated into astrocytes and oligodendrocytes besides neuronal differentiation [88-91].

Cumming *et al.* reported that long-term engraftment of human NSCs implanted into damaged mouse spinal cord resulted in differentiation into new neurons and oligodendrocytes, leading to locomotor recovery.[88] However, there are risks of side effects if NSCs differentiation after transplantation is not controlled; astrocytic differentiation and aberrant axonal sprouting after NS-cell implantation into injured rat spinal cord may cause hypersensitivity to stimuli that are not normally painful [66]. Transplantation of human ESC-derived NSCs into the injured spinal cord areas of SCI mice resulted in and improvement of motor behavior; grafted cells survived for at least 28 days and differentiated into Tuj1-positive neurons and O4-positive oligodendrocytes at the grafted site [92].

Clinical trials have been undertaken for the use of human fetal NSCs. Seledtsova *et al.* reported positive results on their case study using harvested fetal NSCs in the chronic SCI environment [93]. In 2011 Stem Cells, Inc. initiated the world's first NSC trial in SCI in Switzerland [94]. The NSCs were injected into the spinal cord and migrate to the area of injury to form neurons and oligodendrocytes, critical for remyelinating damaged neuronal axons for recovery of nerve function [11]. A new Phase I safety clinical trial commenced surgeries in September 2014 in the USA by Neuralstem Inc. All patients in the trial will receive six injections of the stem cells directly into or around the injury site. The patients will also receive physical therapy post-surgery as well as immunosuppressive therapy for three months [95]. This study is the first multicenter trial in the United States involving the transplantation of a cellular therapy for SCI since the stoppage of the Geron clinical trial in 2011 [10]. Another NSI-566/acute spinal cord injury Phase I/II trial is also expected to commence in 2014 or early 2015 in Seoul, South Korea by Neuralstem Inc. [95].

4.5. Bone marrow-derived stromal cells

Mesenchymal stem cells (MSCs) and hematopoetic stem cells (HSCs) are two types of stem cells residing in the bone marrow. They are multipotent stem cells that typically form connective tissue and give rise to all the blood cell types in the body and certain immune system cells respectively [43]. HSCs and MSCs can transdifferentiate along glial and neuronal pathways; They are able to form glial and neuronal lineage cells in response to different types of chemical, genetic or physiological induction, howbeit the characterization of cell phenotype was limited to the detection of lineage-specific markers with no glial or neuronal cell function apparent in some studies [12]. These cells are easy to isolate and expand without the concern for technical and ethical problems; they have low immunogenicity which arises from ease of getting donor cells, they don't have the risk of making tumors and can be easily used in autologous transplants. All

these particular features have made them very appealing choice for SCI repair [83].

One of the most important features of those cells is the fact, that though they are often not present in the lesion, they can facilitate SCI recovery via paracrine effect (reduction of inflammatory cytokines, production of bioactive molecules) [96, 97].

Neurally induced bone marrow derived mesenchymal stem cells (NIMSCs) transplanted into rat model of sub-acute spinal cord injury caused both behavioral and histological improvement [98]. MSCs were induced to express neuronal like properties using a modified procedure [99] and locomotor function improvement in NIMSC group was significantly better comparing OECs and control groups [98]. Other studies using genetically modified MSCs showed improvement in axonal regeneration and prevention in hypersensitivity after SCI [100].

Human and rodent bone marrow-derived stromal cells (BMSCs) also have been used in spinal cord injury models as well as initial human clinical trials [101, 102]. There are a number of reports of neuroprotection and even transdifferentiation into neurons, but the generation of neural cells from BMSCs has been questioned. Timing of delivery may be particularly important when using BMSCs, with reports that acute delivery provides more neuroprotection than when cells are transplanted at one week or later [103].

The claims of trans-differentiation of BMSCs into neural lineages have been challenged. Some studies by Sanchez-Ramos *et al.* have shown that human and mouse MSCs can differentiate into NSCs [104, 105], however other studies by Hofstetter *et al.* show lack of multiple neuronal markers and physiological evidence for differentiation of rat MSCs into NSCs *in vivo* and post-SCI transplantation [106]. Though the use of BMSCs has not resulted in exceptional clinical improvements for the injured spinal cord, the majority of recent clinical trials used bone marrow-derived stem cells due to the ease of harvesting and implantation, advantage of using an autologous source of cells and shorter interval required between harvest and transplantation [10]. Overall in phase I trials, BMSCs had no adverse outcomes and also significant changes in functional outcome and statistical improvements were limited [101, 107, 108].

4.6. Induced pluripotent stem cells (iPSCs)-derived cells

Induced pluripotent stem cells (iPSCs) are a type of pluripotent stem cell developed by Japanese Nobel Prize-winning stem cell researcher Shinya Yamanaka, who discovered in 2006 that mature cells can be converted to stem cells. iPSCs are typically derived by reprogramming of somatic cells following introduction of a specific set of reprogramming factors including Oct3/4, SOX2, KLF4 and c-Myc [109, 110]. Since they are patient-specific and can easily be established from patient somatic cells, iPSCs are advantageous for circumventing the ethical issues associated with the harvest of ESCs [111]. However, iPSCs like ESCs have same disadvantages regarding the ability for tumorigenesis, though less than ESCs, due to the introduction of foreign genes into chromosomes and the probability for incomplete reprogramming [109, 110]. The safety of the mouse iPSCs following transplantation and their neural differentiation greatly depend on the somatic cells from which the iPSCs have been derived [112]. iPSCs are a new technology yet an increasing number of experiments are being conducted with iPSC-derived cells in SCI animal models. IPSC-derived neurospheres transplanted into contusive injured spinal cord differentiated into all three neural lineages

without forming teratomas or other tumors. The transplanted cells also participated in remyelination and induced the axonal resulting promotion in locomotor function recovery [113, 114]. These studies also state that detailed evaluations of the cells, including their differentiation potentials and tumorigenic activities before initiation of clinical application is very crucial to establish their safety and effectiveness for therapies. Advanced reprogramming technologies have been developed enabling iPSCs generation with transiently applied synthetic mRNA at high efficiencies, eliminating the need for viral or genomic manipulation and allowing direct clinical translation [114-116]. With this technology neural cells can rapidly be differentiated from pluripotent cells, or even be programmed directly from skin or peripheral blood cells [117, 118]. In a recent study iPSCs-derived NSCs from very aged human cells grafted into adult immunodeficient rats after SCI survived, differentiated into neurons and glia and extended tens of thousands of axons from the lesion site over virtually the entire length of the rat CNS [119]. However, beneficial effects of iPSC-based therapies have been produced mostly using genetically immunodeficient rodents so far. Transplantation of human iPSC-derived neural progenitor cells has failed improve functional recovery of pharmacologically immunosuppressed mice with contusion SCI [120]. Besides, in another study human iPSC-derived neural cells failed to restore function in an early chronic spinal cord injury model in contrast to prior reports in acute and sub-acute injury models [121]. These findings highlight the importance of extensive preclinical studies of transplanted cells needed before the clinical application can be achieved.

4.7. Olfactory ensheathing cells (OECs)

The adult primary olfactory system is a unique part of the nervous system that has maintained the ability to regenerate continuously during adulthood [122]. Dying olfactory receptor neurons are regularly replaced by newborn cells differentiated from a stem cell layer at the base of the epithelium [123]. OECs are found in both the peripheral and central compartments of the primary olfactory system (the olfactory epithelium and bulb) and they participate in supporting olfactory neurogenesis and the retargeting across the PNS/CNS boundary in the olfactory system [124, 125]. OECs, considered to be adult stem cells by some people, are a specialized and highly plastic glial cell that can continuously support the neurogenesis and axonal regeneration of olfactory receptor neurons [126]. These cells enhance outgrowth of both intact and injured axons, stimulate tissue sparing and neuroprotection, activate angiogenesis and remyelinate axons after a range of demyelinating insults [124]. They utilize different molecular signals that stimulate repair including neurotrophins (NGF, BDNF and NT-4/5)[127-129], extra cellular matrix (ECM) molecule (laminin [130, 131], collagen type IV [132, 133], L1CAM, Gro1, Timp2 [134, 135]) and other growth factors such as FGF-2 [136-138] which acts as a mitogen for OECs. They support and guide constant olfactory axons in the PNS and hence hold great promise for SCI medication as they are able to create a permissible microenvironment across the lesion for regeneration of axons [83]. Many rodent model studies have demonstrated the potential of OECs to promote re-myelination and long-distance regrowth of axons within the injured spinal cord, and thus to facilitate improvement of locomotor performance following SCI [139-141].

Huang *et al.* harvested OECs from aborted fetuses and grafted them directly to the SCI lesion cavity. A Phase I clinical trial demonstrated that there were no adverse effects, but patients needed immunosuppression for the transplant which elevated the possibility of morbidity. Later autologous OECs have been transplanted alternatively to overcome this deficiency [10]. In a recent study led by Geoffrey Raisman, autologous OECs have been successfully used to enable a paralyzed patient to walk again [142]. The transplant, which was carried out by surgeons in Poland involved taking OECs from the patient's own olfactory bulbs, and then grafting these cells at the site of injury, where they promote nerve cell growth to bridge the gap and restore function. An added advantage in using the patient's own cells is that it avoids the problem of rejection by their immune system. OECs are an alternative cell type for transplant, however they bear limitations of graft morbidity and also limitations in the small neural cell stock derived from nasal mucosa.

4.8. Schwann Cells (SCs)

Schwann cells (SCs) are the principal glial cells of the PNS, producing the myelin sheaths that surround the PNS axons. Moreover, due to their diverse abilities regarding secretion of a variety of growth factors, expression on the membrane surface of adhesive molecules and production of extracellular matrix molecules that support axon growth, they have a crucial role in promoting peripheral nerve regeneration after injury [143-145]. The first experiment involving the transplantation of purified rat SCs for SCI treatment in 1981 [146] and since then, they have become one of the most widely transplanted neural cells in SCI. When implanted in the injured spinal cord, SCs support regeneration of axons, myelinate or ensheathe regenerated axons, reduce cyst formation in the injured tissue, reduce secondary damage of tissue around the initial injury site, and modestly improve limb movements [147]. Although they can facilitate regeneration in different ways, some axons, such as those of the corticospinal tract (CST), remain unaffected by SC grafts [148]. Besides, the environment they create is often so permissive that axons are reluctant to leave SC grafts and that limits clinical applicability [149, 150]. When SCs transplantation is combined with additional treatments, further improved regeneration is acquired. Studies regarding transplantation of SCs with methylprednisolone [151], SCs plus BDNF and NT-3 [152, 153], SCs transduced to secrete BDNF or encode D15A (a molecule with BDNF and NT-3 activity) [154, 155] and SCs plus OECs and chondroitinase [38, 156] or elevation of cAMP levels [157] have all resulted in higher myelin ratio, more axon regeneration into and out of the SC implant and further improved locomotion. In a study by Saberi *et al.* autologous transplantation of SCs in just four SCI patients did not reveal any serious complications up to 1 year after the surgery yet, no functional outcome was reported [158]. A new clinical trial based on safety of autologous human SCs transplantation in subjects with sub-acute SCI is currently recruiting participants by University of Miami (NCT01739023). Results should be available by November 2015.

5. Summary

SCI is a complex disorder mainly caused by a physical disruption of spinal cord axons through the epicenter of injury and leads to deficits in motor, sensory, and autonomic function. The initial mechanical trauma to the spinal cord results breakdown of the BBB, influx of peripheral inflammatory factors, activation of glial

cells, excitotoxicity, and necrosis. A secondary cascade of signaling events leads to the cyclic increase in inflammatory cytokines and chemokines, leading to apoptosis, progressive loss of oligodendrocytes which leads to demyelination, and axonal degeneration. The inflammatory response results in fluid accumulation and the influx of immune cells facilitated by their expression of matrix metalloproteinases. Macrophages can aid nerve regrowth by phagocytosing myelin debris, which is known to inhibit axonal regeneration and may release protective cytokines, however, the cytokines and chemokines produced by immune cells can also propagate the inflammatory response, inducing a reactive process of secondary apoptosis in the tissue that surrounds the injury site. The injured spinal cord eventually becomes gliotic. Gliosis is beneficial for the reestablishment of physical and chemical integrity of the CNS since absence of the glial scar has been associated with impairments in the repair of the BBB. However it makes a physical obstacle preventing neuroregeneration. During gliosis, astrocytes and oligodendrocyte progenitors are activated within the injury site and secrete inhibitory molecules that prevent physical and functional recovery of the injured CNS. Hence, SCI is characterized by a highly reactive environment that presents significant obstacles for repair, as well as for the survival and integration of transplanted cells.

The traditional approach to repair the damage has been limiting the secondary injury that follows trauma. Other approaches comprise delivery of neurotrophic factors to stimulate sprouting axons cross the gap across the lesion, using transplanted cells or tissue that supports axonal elongation for bridging the gap is another approach for regeneration and specifically stem cell based strategies for treatment of SCI.

With all the current effort for treatment, there is yet no long term cure for repairing the injured spinal cord. Cellular therapies have shown promising results in animal models. Stem cells can replace damaged or diseased cells, provide a cell-based electrical 'relay' between neurons above and below the injury, facilitate regeneration by providing neuroprotective or growth factors, and play other indirect roles such as promoting neovascularization or providing a permissive substrate for regeneration of endogenous cells. Stem cells used in different studies for SCI treatment can be categorized among ESCs, OPCs, MNPs, NSCs, BMSCs, and iPCSs.

ESCs have great potential and appeal for a therapeutic strategy though there are many concerns regarding their tumorigenicity and ethical issues. Utilizing OPCs mainly for remyelination is the most practical short-term clinical intention for spinal cord regeneration. Transplanted OPCs SCI sites showed survival, redistribution over short distances, and differentiation into oligodendrocytes, however the therapeutic window for this type of treatment is limited to the early post injury period. ESC-derived motor neurons have the ability to populate the embryonic spinal cord, as well as extend axons, and form synapses with target muscles. Transplantation of NSCs into the injured spinal cord areas of SCI mice resulted in improvement of motor behavior; survival and differentiation into Tuj1-positive neurons and O4-positive oligodendrocytes. BMSCs showed no adverse outcomes in phase I clinical trial but significant changes in functional outcome and statistical improvements were limited. IPSCs are the very recent promising strategy with an increasing number of

experiments being conducted in the area of SCI. They are advantageous for circumventing popular ethical issues associated with the harvest of ESCs as well as immunological rejection problem. However, regardless of their promising results future work needs to focus on the specific hiPSC-derivatives, co-therapies and their safety in order to start clinical trials. OECs are an alternative option for transplant consideration, however they bear limitations of graft morbidity and also limitations in the small neural cell stock derived from nasal mucosa. Of all the cells used for SCI, SCs have the longest history of transplantation. SCs support regeneration of axons, myelinate or ensheathe regenerated, reduce cyst formation in the injured tissue, reduce secondary damage of tissue around injury site, and improve limb movements. Although using SCs alone as a treatment is accompanied with some limitations, combined transplantation of SCs with additional treatments acquires further improved regeneration.

Animal models have shown some positive results including improved locomotor function and remyelination, validating scientific principles and strategies. Results from these studies have proved their efficacy in pre-clinical investigations with several therapies making it to clinical trials.

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