

## Concise Review: Bridging the Gap: Novel Neuroregenerative and Neuroprotective Strategies in Spinal Cord Injury

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**Key Words.** Spinal cord injury • Stem cell • Trauma • Biomaterial • Neuroprotection • Neuroregeneration

### ABSTRACT

**Spinal cord injuries (SCIs) result in devastating lifelong disability for patients and their families. The initial mechanical trauma is followed by a damaging secondary injury cascade involving proapoptotic signaling, ischemia, and inflammatory cell infiltration. Ongoing cellular necrosis releases ATP, DNA, glutamate, and free radicals to create a cytotoxic postinjury milieu. Long-term regeneration of lost or injured networks is further impeded by cystic cavitation and the formation of an inhibitory glial-chondroitin sulfate proteoglycan scar. In this article, we discuss important neuroprotective interventions currently applied in clinical practice, including surgical decompression, blood pressure augmentation, and i.v. methylprednisolone. We then explore exciting translational therapies on the horizon, such as riluzole, minocycline, fibroblast growth factor, magnesium, and hypothermia. Finally, we summarize the key neuroregenerative strategies of the next decade, including glial scar degradation, Rho-ROCK inhibition, cell-based therapies, and novel bioengineered adjuncts. Throughout, we emphasize the need for combinatorial approaches to this multifactorial problem and discuss relevant studies at the forefront of translation. We conclude by providing our perspectives on the future direction of SCI research.** *STEM CELLS TRANSLATIONAL MEDICINE 2016;5:1–11*

### SIGNIFICANCE

Spinal cord injuries (SCIs) result in devastating, lifelong disability for patients and their families. This article discusses important neuroprotective interventions currently applied in clinical practice, including surgical decompression, blood pressure augmentation, and i.v. methylprednisolone. Translational therapies on the horizon are discussed, such as riluzole, minocycline, fibroblast growth factor, magnesium, and hypothermia. The key neuroregenerative strategies of the next decade are summarized, including glial scar degradation, Rho-ROCK inhibition, cell-based therapies, and novel bioengineered adjuncts. The need for combinatorial approaches to this multifactorial problem is emphasized, relevant studies at the forefront of translation are discussed, and perspectives on the future direction of SCI research are presented.

### INTRODUCTION

#### The Acute Injury and Postinjury Milieu

Traumatic spinal cord injuries affect 1.4 million North Americans, a disproportionate number of whom are younger than 30 years. Direct lifetime costs are staggering, at \$1.1 to \$4.6 million per patient [1, 2]. The initial mechanical insult is followed by a secondary injury cascade that generates further permanent damage. Promising neuroprotective strategies to mitigate the secondary injury, and neuroregenerative approaches to restore function, are discussed herein.

Acute cell dysfunction and death occur via cell permeabilization and initiation of proapoptotic signaling cascades and because of ischemia due to destruction of the sensitive microvascular supply

[3, 4]. Furthermore, disruption of the blood-spinal cord barrier exposes the cord to inflammatory cells, cytokines, and vasoactive peptides [5, 6]. In the subsequent hours, hemorrhage and progressive edema cyclically add to the harsh postinjury milieu. Ongoing cellular necrosis releases ATP, DNA, and  $K^+$ , which activate microglia to secrete proinflammatory cytokines. As a result, dramatic numbers of macrophages, microglia, and polymorphonuclear leukocytes infiltrate the injury site [7]. Engaged phagocytes generate reactive free radicals and cytotoxic by-products that further contribute to cell death. Moreover, release of glutamate via neurons and reuptake failure by astrocytes lead to excitotoxic injury in nearby neurons [8, 9]. At a systemic level, loss of CNS-mediated sympathetic vascular tone results in profound

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hypotension. Furthermore, impaired local autoregulation makes the cord particularly susceptible to ongoing postinjury ischemia [10, 11] (Fig. 1). Each step in this injury cascade is an important target for combinatorial neuroprotective strategies.

### Barriers to Recovery

Regeneration after spinal cord injury (SCI) requires targeted axon growth and remyelination of long tracts. Loss of the cord's structural framework, including cystic cavitation, impairs directed axonal regrowth and free cell migration [12]. In addition to architectural disruption, uncontrolled reactive astrogliosis generates inhibitory glial scarring by creating a physical barrier of irregular mesh-like astrocytic processes in the perilesional zone [13]. Extracellular matrix in the glial scar is predominantly composed of chondroitin sulfate proteoglycans (CSPGs) [14, 15], tenascin [16, 17], and neural/glial antigen 2 (NG2) proteoglycans [18, 19], which further restrict axonal regeneration and inhibit neurite outgrowth through membrane tyrosine phosphatase, PTP $\sigma$  [20, 21]. Furthermore, existing myelin- and neuron-related signals neurite outgrowth inhibitor (NOMO) [22], oligodendrocyte myelin glycoprotein, semaphorin 3A, semaphorin 4D [23], and myelin-associated glycoprotein [24] bind to NOMO receptor (NgR) (or neuropilin-1/plexin-A1 for semaphorin 3A) to activate Rho GTPase and its downstream effector, Rho-associated protein kinase (ROCK) [21]. Together, these result in growth cone collapse and potent inhibition of regeneration [20].

In addition to reforming neural circuits, myelination is an important component of regeneration. Demyelination of axons by oligodendroglial apoptosis along with minimal oligodendrocyte precursor cell (OPC) proliferation after injury contribute to poor functional recovery. Denuded axons lose rapid saltatory conduction and are particularly vulnerable to nonfunctional electrogenesis [25, 26]. Preserving and regenerating functional, myelinated circuits is key to SCI recovery.

### Neuroprotection

#### Sodium Channel Blockade

Riluzole is a U.S. Food and Drug Administration (FDA)-approved benzothiazole antiepileptic used in amyotrophic lateral sclerosis (ALS) [27]. Riluzole selectively blocks tetrodotoxin-sodium channels associated with injured neurons (Table 1). It also inhibits presynaptic glutamate release and increases reuptake to potentially mitigate excitotoxicity [28]. Its approval by regulatory bodies and its demonstrated safety in ALS make it a particularly appealing drug for translation in SCI. In preclinical studies, treatment with riluzole has resulted in dramatic sensorimotor improvements functionally and electrophysiologically [29, 30]. A consortium (led by senior author M.F. and including AOSpine, the North American Clinical Trials Network, the Rick Hansen Institute, and the Ontario Neurotrauma Foundation) is conducting a phase II/III randomized controlled trial (NCT01597518) entitled "Riluzole in Spinal Cord Injury Study" (RISCIS) to assess the effects of riluzole using the American Spinal Injury Association (ASIA) Impairment Scale (AIS), Spinal Cord Independence Measure, and brief pain inventory outcomes [31]. The trial is recruiting patients with C4–C8 level injuries and is expected to conclude in December 2018.

#### Anti-Inflammatory Drugs

Minocycline is a CNS-penetrating tetracycline antibiotic that inhibits microglial activation and downregulates proinflammatory

cyclooxygenase-2, tumor necrosis factor- $\alpha$  (TNF- $\alpha$ ), and interleukin-1 $\beta$  (IL-1 $\beta$ ). Preclinical studies of acute minocycline treatment showed decreased inflammatory cell infiltration, reduced cystic cavitation, and improved behavioral outcomes [32]. A phase II randomized controlled trial (RCT) ( $n = 52$ ) examining the effects of 7 days of i.v. minocycline versus placebo demonstrated safety, stable cerebrospinal fluid (CSF) drug levels, and a trend toward improved motor scores [33]. These exciting results have led to the phase III Minocycline in Acute Spinal Cord Injury trial (NCT01828203) in which 7 days of i.v. minocycline is compared with placebo in 248 patients. Completion of the trial is expected in 2018 [34].

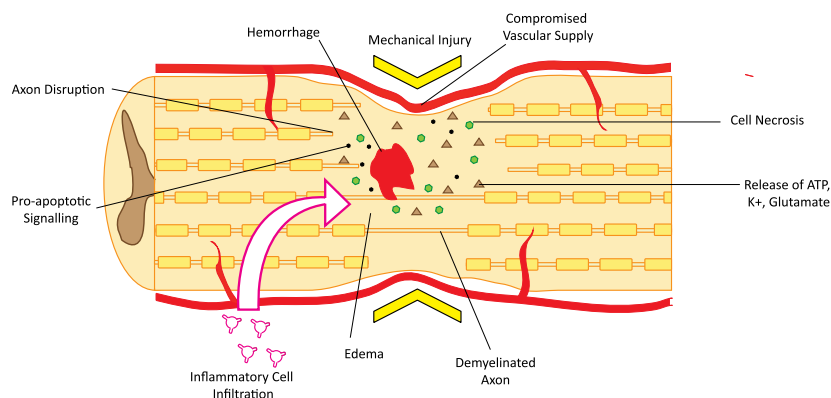
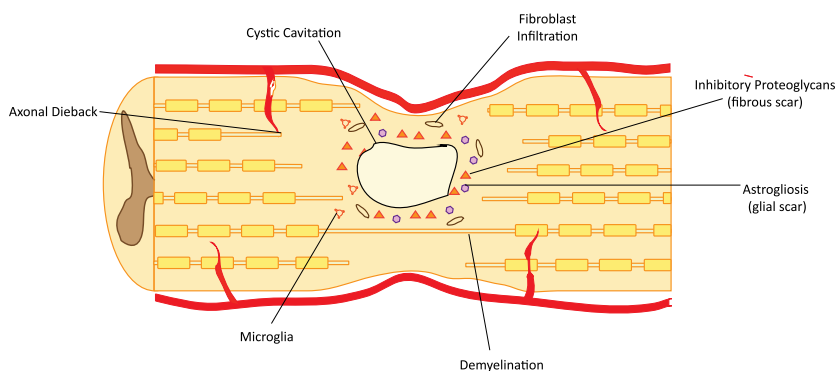
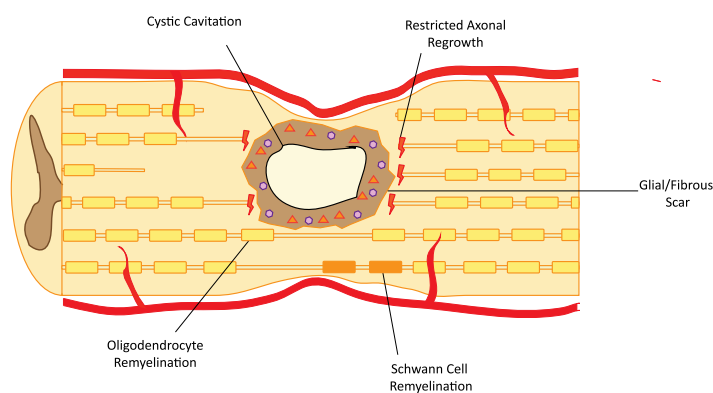
Methylprednisolone (MPSS) is a synthetic glucocorticoid that acts on cytoplasmic receptors to upregulate anti-inflammatory factors and interfere with the actions of proinflammatory cytokines, arachidonic acid metabolites, and adhesion proteins. In animal models, MPSS has also been shown to mitigate oxidative stress and enhance oligodendrocyte and motor neuron survival [35]. A series of clinical trials and meta-analyses over the last 3 decades have collectively demonstrated improvements in motor scores for patients administered i.v. MPSS within 8 hours of injury [36–38]. Providing MPSS to this subset of patients will be recommended in the upcoming 2016 AOSpine guidelines, developed by an international and interdisciplinary committee of expert physicians, allied health workers, patients, and independent consultants applying the rigorous Grading of Recommendations Assessment, Development and Evaluation (GRADE) tool [39–44].

#### Therapeutic Hypothermia

Therapeutic hypothermia has been successfully used for neuroprotection in patients after resuscitated cardiac arrest [45] and neonatal hypoxic-ischemia encephalopathy [46]. Hypothermia significantly decreases the basal metabolic rate of the CNS and diminishes the systemic inflammatory response [47]. In SCI, pilot studies of systemic hypothermia have demonstrated that it may show promise as a neuroprotective intervention [48]. The Acute Rapid Cooling Therapy for Injuries of the Spinal Cord (ARCTIC) phase II/III trial, which aims to evaluate the safety and efficacy of cooling within 6 hours of injury, is pending approval [49].

#### Surgical Decompression

After injury, progressive edema and hemorrhage generate mechanical pressure on the confined spinal cord. Surgical decompression relieves this pressure to mitigate further secondary injury. The Surgical Timing in Acute Spinal Cord Injury (STASCIS) trial, published in 2012, was a prospective observational study of 222 patients undergoing early (<24 hours) versus late (>24 hours) decompression. Patients receiving early surgical intervention were twice as likely to improve by 2 or more AIS grades at 6 months [50]. A prospective Canadian cohort study similarly demonstrated that a significantly greater proportion of patients who underwent early decompression improved by two or more AIS grades [51]. Furthermore, Dvorak et al. reported shorter lengths of hospital stay after early decompression for patients with ASIA A (complete) or ASIA B (complete motor; incomplete sensory) injuries [52]. Early decompression in acute SCI is now a widely adopted practice that we strongly advocate.

**Acute (0-48 Hours)****Subacute (2–14 Days)****Intermediate (2 Weeks–6 Months) / Chronic (>6 Months)**

**Figure 1.** Pathophysiology of spinal cord injury in the acute, subacute, and chronic setting. Acute traumatic injury causes cell death through ischemia, release of cytotoxic molecules, initiation of apoptotic cascades, hemorrhage, edema, and infiltration of inflammatory cells. In the subacute phase, cystic cavities begin to coalesce and become surrounded by reactive astrocytes, fibroblasts, and inflammatory cells. Inhibitory proteoglycans are secreted into the extracellular matrix. Degeneration/dieback of damaged and denuded axons occurs. In the intermediate/chronic phase, encompassing most patients, mechanical and chemotactic barriers restrict axon regeneration. Limited remyelination by oligodendrocytes and Schwann cells may portend small functional gains during this period.

**Other Neuroprotective Strategies**

Numerous other neuroprotective approaches have been translated into recently concluded or ongoing clinical trials. Granulocyte colony-stimulating factor (G-CSF) is a signaling glycoprotein that has been shown to enhance the survival of ischemic CNS cells, protect against glutamate-induced apoptosis, and reduce TNF- $\alpha$  and

IL-1 $\beta$  expression in vivo [53, 54]. Two phase I/IIa nonrandomized trials have demonstrated improved AIS scores after G-CSF administration, without significant adverse events [55, 56]. A phase III RCT of i.v. G-CSF, the G-CSF-Mediated Spinal Cord Injury Recovery Induction Trial (G-SPiRIT;  $n = 88$ ) is recruiting patients with acute cervical SCI in Japan. The study began in May 2015 and is expected to conclude in 2018.

**Table 1.** Current neuroprotective strategies for spinal cord injury

Treatment	Mechanism of action	Relevant trial (n; Year)	Key findings
In current practice			
Surgical decompression	Reduces mechanical pressure on the confined cord to mitigate ischemia and pressure-related cell death	STASCIS (n = 313; 2012)	Patients undergoing early surgery (<24 hours) were twice as likely as those who underwent late surgery (>24 hours) to improve their AIS motor scores by 2 or more at 6-month follow-up
MPSS	Upregulates anti-inflammatory factors and interferes with the actions of proinflammatory cytokines, arachidonic acid metabolites, and adhesion proteins	NASCIS II (n = 487; 1990)	No difference in overall analysis. Post hoc analysis demonstrated improved AIS scores for patients given MPSS within 8 hours of injury
		NASCIS III (n = 499; 1997)	No difference in overall analysis. Post hoc analysis demonstrated improved AIS scores for patients given 48 hours MPSS regimen within 3–8 hours of injury
MAP $\geq$ 85 mm Hg	Decreases cord ischemia by increasing perfusion pressure	MAPS (n = 100 <sup>a</sup> ; 2017 <sup>b</sup> )	N/A
In phase III trial			
Minocycline	Inhibits microglial activation and downregulates COX-2, TNF- $\alpha$ , and IL-1 $\beta$	MASC (n = 248 <sup>a</sup> ; 2018 <sup>b</sup> )	N/A
Riluzole	Sodium-channel blocker, indirectly inhibits presynaptic glutamate release and potentiates reuptake	RISCIS (n = 351 <sup>a</sup> ; 2017 <sup>b</sup> )	N/A
G-CSF	Reduces glutamate-induced apoptosis and TNF- $\alpha$ /IL-1 $\beta$ expression	G-SPIRIT (n = 8 <sup>a</sup> ; 2018 <sup>b</sup> )	N/A
In phase II trial			
VX-210 (Cethrin)	Inactivation of the ROCK pathway	Cethrin in Acute Cervical Spinal Cord Injury (2016 <sup>b</sup> )	N/A
Mg-PEG 3350	Antixcitotoxic and antiapoptotic NMDA-receptor antagonism	A Study of AC105 in Patients With Acute Traumatic Spinal Cord Injury (n = 16; 2015)	Trial discontinued
bFGF/FGF2	Decreases oxyradical generation and excitotoxic cell death; promotes angiogenesis	Study to Evaluate the Efficacy, Safety, and Pharmacokinetics of SUN13837 Injection in Adult Subjects With Acute Spinal Cord Injury (ASCI) (n = 62; 2015)	Trial discontinued
In phase I trial/pilot studies			
Therapeutic hypothermia (32°C–34°C)	Decreases basal metabolic rate and inflammatory response	ARCTIC (pending approval)	N/A

<sup>a</sup>Projected enrollment per <https://clinicaltrials.gov>.

<sup>b</sup>Project study completion date.

Abbreviations: AIS, American Spinal Injury Association Impairment Scale; ARCTIC, Acute Rapid Cooling Therapy for Injuries of the Spinal Cord; bFGF, basic fibroblast growth factor; COX, cyclooxygenase; FGF2, fibroblast growth factor 2; G-CSF, granulocyte colony-stimulating factor; G-SPIRIT, G-CSF mediated spinal cord injury recovery induction trial; IL, interleukin; MAP, mean arterial pressure; MAPS, Mean Arterial Blood Pressure Treatment for Acute Spinal Cord Injury; MASC, Minocycline in Acute Spinal Cord Injury; MPSS, methylprednisolone sodium succinate; N/A, not applicable; NASCIS, National Acute Spinal Cord Injury Study; NMDA, N-methyl-D-aspartate receptor; PEG, polyethylene glycol; RISCIS, Riluzole in Spinal Cord Injury Study; ROCK, Rho-associated, coiled-coil protein kinase; STASCIS, Surgical Timing in Acute Spinal Cord Injury Study; TNF, tumor necrosis factor.

Vascular compression, intraluminal thrombosis, loss of autoregulation, and system hypotension contribute to ongoing post-injury cord ischemia. Trials of blood pressure augmentation to reduce ischemia have demonstrated improved ASIA grade outcomes for patients with mean arterial pressures (MAPs) held

above 85–90 mm Hg [57]. The American Association of Neurological Surgeons and Congress of Neurological Surgeons provide level III recommendations to maintain MAP for 7 days after injury [58]. Building on this, the Canadian Multicentre CSF Monitoring and Biomarker Study (CAMPER; NCT01279811) is

recruiting participants to assess the effects of cord perfusion pressure (MAP minus intrathecal CSF pressure) on AIS scores and neuropathic pain inventories. CAMPER will also provide insight into the temporal profiles and prognostic value of CSF biomarkers after SCI [34].

Magnesium is an *N*-methyl-D-aspartate receptor antagonist with antiexcitotoxic and antiapoptotic properties successfully used in the neuroprotection of animals after traumatic brain injury, myocardial infarction, and SCI [53–56, 59]. Sustaining sufficiently high CSF levels of Mg requires an excipient such as polyethylene glycol (PEG). A phase I/II placebo-controlled RCT ( $n = 40$ ) of AC105 (Acorda Therapeutics, Ardsley, NY, <http://www.acorda.com>) was started but subsequently discontinued [34].

Basic fibroblast growth factor (bFGF) is a heparin-binding protein involved with wound healing, angiogenesis, embryogenesis, and the *in vitro* maintenance of stem cell pluripotency. It has also been shown to decrease oxyradical generation and excitotoxic cell death in preclinical models of neurodegenerative diseases and SCI [60]. A phase I/II RCT ( $n = 164$ ) of an FGF analog, SUN13837 (Asubio Pharmaceuticals, Edison, NJ, <http://www.asubio.co.jp>), was discontinued early [34].

## NEUROREGENERATION

### The Glial Scar

#### CSPGs in the Glial Scar

Chondroitinase ABC (ChABC) is a bacterial enzyme shown to effectively degrade CSPGs, including NG2, promoting functional gains in mouse models after intrathecal administration using an osmotic minipump [61, 62]. Evidence also shows that coadministration of ChABC with neural precursor cells enhances transplant survival and remyelination of host axons [63, 64]. More recently, large-scale CSPG digestion by direct lentiviral ChABC gene delivery into rat spinal cords demonstrated reduced cavitation volume and enhanced axon preservation. Treated rats also displayed improved sensorimotor function on behavioral and electrophysiological assessments [65]. ChABC is an exciting therapy for which the optimal modality for delivery remains to be elucidated. Future avenues of research may include exploration of human CNS-specific analogs of ChABC and development of novel, safe, and effective delivery techniques.

#### Anti-NOGO/RhoA-ROCK

Another promising field of study is the NOGO-A/RhoA-Rock pathway. Neurite outgrowth inhibitor A (NOGO-A) is an integral membrane protein in oligodendrocytes that binds NgR. NgR phosphorylates the small GTPase RhoA, which subsequently activates ROCK to inhibit neurite outgrowth and collapse the growth cone [66]. Blocking the function of myelin protein NOGO-A with NOGO-receptor antagonists, anti-NOGO-A antibodies, or inhibition of downstream RhoA-ROCK has been shown to enhance neurite growth and axonal regeneration in animal studies [67–69]. A phase II clinical trial of a monoclonal NOGO-A antibody is now under way in ALS, the results of which may portend a trial in SCI [70]. Furthermore, VX-210 (Cethrin; BioAxone BioSciences, Cambridge, MA, <http://bioaxonebio.com>) is a Rho GTPase antagonist that demonstrated significant motor improvement and no safety concerns in a phase I/IIa trial

(NCT00500812) in SCI [71]. A phase IIb trial, supported by Vertex Pharmaceuticals (Boston, MA, <http://www.vrtx.com>), is expected to begin in the near future to further quantify efficacy and safety. The results of these trials will be important in determining the course of investigation of these pathways as therapeutic approaches for SCI.

## CELL-BASED APPROACHES

Cell therapies using pluripotent sources are an exciting strategy based on the grafts' ability to be pro-oligodendroglial [72, 73], pro-neuronogenic [74], immunomodulatory [75, 76], and/or antiapoptotic [77]. Furthermore, transplanted cells may be capable of modifying the microenvironment and regenerating/remyelinating damaged circuits. However, to successfully use these strategies, we must optimize the cell source, differentiation protocols, and graft survival.

Stem Cell Inc. (Newark, CA, <http://www.stemcellsinc.com>) is conducting an international phase I/II clinical trial (NCT02163876) of human CNS stem cell injections for cervical SCI that is expected to conclude in 2017. Ongoing follow-up for a similar phase I/II thoracic injury trial (NCT01321333) has demonstrated patient improvement in multiple sensory modalities with no safety concerns thus far [78]. Neuralstem (Germantown, MD, <http://www.neuralstem.com>) began a phase I safety trial (NCT01772810) at the University of California San Diego of NSI-566 neural stem cell transplants for chronic thoracic SCI in 2014, with expected completion in February 2016. These are important clinical proof-of-concept steps in the path to widespread translation of cell therapies.

### Cell Sources

Endogenous neural stem cells may be mobilized from local reservoirs, particularly the ependymal layer of the spinal cord central canal [79]. Techniques are being developed to achieve this using growth factor infusions [80], transplanted hydrogels [81], or electrical fields [82]. In parallel, grafts of exogenous human embryonic- and induced pluripotent stem (iPS)-derived cells are being investigated. Human embryonic stem (ES) cells allow consistent differentiation compared with human iPS cells but present ethical challenges, possible karyotypic instability, and are in limited supply [83, 84]. Additionally, the prospect of an autologous pluripotent cell therapy with induced pluripotent stem cell (iPSC) technology is enticing in SCI, where the immune response is always at the forefront. Human iPSC technology offers a highly translatable and potentially unlimited source of pluripotent cells; however, logistical issues surrounding low reprogramming efficiency and insertion mutagenesis exist with viral derivation [85, 86]. Nonviral iPSC generation, such as using the piggyBac transposon, affords an effective and reproducible alternative [87, 88]. iPSC technology continues to evolve as potential early senescence and the variable yield of neural progeny of iPS compared with ES cells are investigated [89, 90]. Furthermore, the effect of residual epigenetic memory in DNA methylation and histone modification remains to be fully understood [77, 91].

Several other important cell types are being investigated for SCI, including mesenchymal stem cells (MSCs), olfactory ensheathing cells (OECs), and bone marrow nucleated cells (BMNCs). MSCs are multipotent stromal cells capable of

differentiating along connective tissue lineages, allowing them to play a key role in tissue repair [92]. They are also capable of potentially modulating the local and systemic immune response [93–95]. In preclinical models, MSCs have been associated with neural tissue sparing, increased levels of pro-survival trophic factors, and neovascularization [96, 97]. Several phase I and II trials of autologous MSCs, transplanted into the parenchyma or intrathecal space, are ongoing worldwide. Two phase III trials have also been registered (NCT02481440, NCT01676441), with results expected in the next 1–2 years [34]. BMNCs are being studied for their similar supportive properties. They have been shown to positively modulate the microenvironment in SCI, possibly mediated by the small fraction (0.01%–0.001%) of MSCs in BMNCs. A recent study of intraparenchymal and intravenous autologous BMNC administration in children with chronic SCI showed no significant adverse events [98]. Further preclinical and clinical work is required to better understand the mechanism of action of BMNCs.

OECs are glia that ensheath olfactory neurons in a manner similar to the way Schwann cells ensheath peripheral neurons. They support exposed olfactory cells in the nasal mucosa by phagocytosing bacteria and debris, secreting neurotrophic factors, and facilitating axon regeneration after loss [99, 100]. OECs are harvested from the olfactory bulb or mucosa and, when transplanted into the injured cord, promote remyelination, axonal regeneration, and improve behavioral outcomes [101]. At least 10 studies of patients with chronic SCI treated with OECs have been described (cumulative  $n = 1,193$ ). A recent meta-analysis found no significant increase in adverse events after OEC transplant; however, higher-quality studies are still required to define efficacy [102].

### Remyelinating the Injured Cord

Oligodendrocytes are particularly vulnerable to traumatic injury, leaving behind demyelinated, dysfunctional axons. Exogenous intraparenchymal injections of neural precursor cells (NPCs) and OPCs have been shown to produce well-differentiated, myelinating oligodendrocytes *in vivo*. Moreover, rodents transplanted with human OPCs and NPCs weeks after SCI have shown significant functional recovery [103, 104]. However, differentiation protocols can be complex and provide heterogeneous results. Evolving differentiation protocols include Noggin (bone morphogenetic protein inhibition), SB431542 (downstream Smad inhibition), and Sonic hedgehog [105]. Protocol refinement and better molecular characterization of the cells being generated are critical advancements on the path to definitive translation (Fig. 2).

### Axon Regeneration

Human neural stem cells have shown mature neuronal differentiation in animal models with long-distance axonal growth and integrated connectivity with the host CNS [106]. Host axons have been shown to reciprocally connect with transplanted neural stem cells, creating relay circuits that can potentially bridge disrupted tracts [107, 108]. Emerging *in vitro* and *in vivo* protocols for generating direct induced and transpluripotent pathway mature neurons hold the potential to rebuild host circuits [109, 110]. However, mechanisms to direct axonal growth and synapse development in functionally targeted areas are lacking. This represents a critical barrier to recovery. Axon pathfinding strategies have predominantly focused on the role of chemotactic and adhesive cues in guiding the neuronal growth

cone [111]. *In vitro* and *in vivo* studies demonstrating the importance of cell adhesion molecules, including nerve growth factor-inducible large external glycoprotein [112], neural cell adhesion molecule [112, 113], transient axonal glycoprotein-1 (TAG-1) [114], calcium-dependent cadherins [115], semaphorin 3A [23, 116], and netrin [117] have advanced our understanding of embryonic development of the CNS [111]. Further discovery and exploitation of the underlying molecular pathways may yield potent adjunctive methods of generating functional neuronal circuits through chemotactic signaling of transplanted cells (Fig. 2).

### Improving Cell Survival

Transplanted xenograft cells have poor survival rates in animal SCI models. Continued progress in the field will require improvements in cell survival by modifying cells, the injured cord milieu, and the host animals. Growth factors (platelet-derived growth factor, FGF2, and epidermal growth factor) and anti-inflammatory agents (minocycline) have been shown to improve cell survival but can be logistically challenging in animal models (e.g., osmotic minipumps) [118, 119]. Alternate interventions to increase growth factor levels or decrease the immune response will be required to continue improving cell survival. One approach is the genetic modification of grafted cells to inducibly express the necessary proteins. Fibroblasts and mesenchymal stem cells have been successfully modified to secrete bFGF [120], hepatocyte growth factor [121], NT3 [122, 123], brain-derived neurotrophic factor (BDNF) [122, 124], and glial cell-derived neurotrophic factor (GDNF) [125, 126] *in vivo*. Safe and efficient methods of transducing human ES/iPS cells in a similar fashion are being studied (Fig. 2).

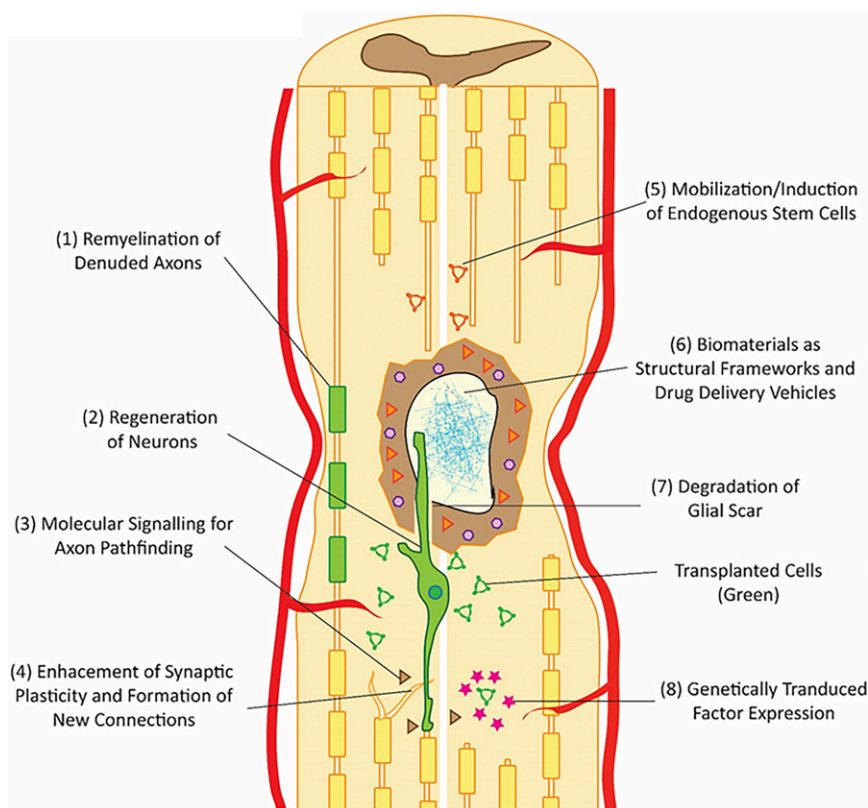
Other strategies of interest are the development of bioengineered cell transplant vehicles to gradually deliver signaling proteins either spontaneously or after an exogenous stimulus. This has been achieved with success with fibrin matrices [106], hyaluronan/methylcellulose [127], and other bioengineered materials. Growth factor-secreting hydrogels have been shown to decrease cavity volume and improve behavioral measures after injury. Furthermore, hydrogels can be used to mitigate immunorejection of transplanted cells through encapsulation to form a temporary physical barrier to the immune response [128, 129].

An often overlooked but critical additional method is the mobilization of endogenous growth factors through noninvasive interventions such as physical rehabilitation. While rehabilitation is an integral component of the care provided to patients with SCI, it is often overlooked in preclinical trials. Host animals that undergo treadmill locomotor training postinjury show significantly enhanced NPC survival mediated by insulin-like growth factor-1 signaling [130]. This finding highlights the necessity of adjunctive therapies in SCI and underscores the importance of reciprocal knowledge exchange between the clinical and preclinical worlds.

## BIOMATERIALS

### Drug-Eluting Hydrogels and Self-Assembling Peptides

Transplantable hydrogel polymers are an attractive medium to fill cavitation defects. Their porous construction allows cell migration and nutrient diffusion [131]. Hydrogels can also function



**Figure 2.** Schematic highlighting promising primary and adjunctive neuroregenerative strategies in spinal cord injury. Transplanted auto/allogenic cells can be differentiated to (1) oligodendrocytes to remyelinate denuded axons or (2) neurons for restoration of functional neural circuits. (3) Promoting this is likely to require molecular signaling techniques for axon guidance. (4) Enhancement of synaptic plasticity with formation of new connections may be a key mechanism of recovery. (5) Another important approach is induction of endogenous neural stem cells, particularly from the central canal. (6) The disruption of the structural architecture of the cord can be overcome by cotransplants of biomaterials that can form a framework for growth. (7) The glial scar barrier can be degraded by enzymes such as chondroitinase ABC. (8) Finally, transplanted cells can be genetically modified to secrete prosurvival, promigration, or other important factors.

as cell-delivery vehicles to improve graft survival and migration [132, 133]. Furthermore, engineered needle-injectable hydrogels can promote cell differentiation and form a barrier against the immune response. Multiple biomaterial substrates have been evaluated in SCI, including agarose [134, 135], collagen [136], hyaluronan/methylcellulose [137], fibrin [138], and alginate [139], all of which have shown promising results in supporting regeneration. Further modification to integrate growth factors [140] or immunomodulatory drugs [141] provides additional high-yield combinatorial opportunities for exploration (Fig. 2). This has been successfully performed for BDNF [142], NT-3 [143], and GDNF [139].

Synthetic self-assembling peptide (SAP) hydrogels are a unique class of engineered proteins that can associate into highly stable organized tissue scaffolds in situ [144, 145]. While liquid at ambient temperature, when exposed to the mammalian body, they begin to assemble into a biocompatible nanofiber structure similar to native extracellular matrix [146]. SAPs grafted into injured cords have demonstrated reduced astrogliosis and cell death with enhanced axonal regeneration [147]. Furthermore, cotransplants with neural stem cells have been shown to promote injury repair and functional recovery of the forelimbs in cervical SCI [148]. The technology behind drug-eluting hydrogels and SAPs is rapidly evolving and we

foresee this becoming an increasingly important adjunct to cell-based therapies moving forward.

#### FUTURE DIRECTIONS

The multifactorial nature of SCI and neural repair necessitates a combinatorial approach if we are to translate experimental therapeutics into significant functional gains for patients. While acute neuroprotective interventions are crucial to mitigate secondary injury, therapeutic neuroregenerative approaches are required to help the millions of patients living with chronic postinjury disability. Pluripotent cell-based therapies will play a central role but require further advancements in genetic engineering, biomaterials, and a deeper understanding of SCI at a molecular level. Furthermore, the development of less-toxic immunosuppressive drugs or consistent methods of generating autologous iPS cells is an important milestone on the path to translation. Careful targeting of these treatment strategies to individual subsets of patients is an important avenue of further investigation requiring a better understanding of injury heterogeneity. In defining these groups, a critical need exists for validated biomarkers of injury severity and recovery trajectory through magnetic resonance imaging [149] and serum/CSF biochemistry [150].

Successful future therapies will require these and other synergistic approaches to address the persistent barriers to regeneration, including the glial scar, the loss of structural framework, and immunorejection. Ongoing clinical trials underscore the excitement and progress we have made in investigating therapeutic approaches to SCI and highlight the importance of the work being done by thousands of scientists in regenerative medicine.

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#### AUTHOR CONTRIBUTIONS

C.S.A.: manuscript writing; M.F.: manuscript writing, final approval of manuscript.

#### DISCLOSURE OF POTENTIAL CONFLICTS OF INTEREST

M.F. is an investigator in the Stem Cells Inc. phase I/II clinical trial (NCT02163876) of human CNS stem cell injections for cervical SCI and the lead investigator in the upcoming phase IIb trial, supported by Vertex Pharmaceuticals Inc. The other author indicated no potential conflicts of interest.

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