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

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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.

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+ leading to excitotoxic injury in nearby neurons and reuptake failure by astrocytes 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
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 cistic cavitating, 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, PTPs [20, 21]. Furthermore, existing myelin- and neuron-related signals neurite outgrowth inhibitor (NOGO) [22], oligodendrocyte myelin glycoprotein, semaphorin 3A, semaphorin 4D [23], and myelin-associated glycoprotein [24] bind to NOGO receptor (NgR) (or neuropilin-1/plexin-A1 for semaphorin 3A, semaphorin 4D [23], and myelin-associated glycoprotein [24]) to activator 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. Denuclearized 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 (NCT01997518) 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-α (TNF-α), and interleukin-1β (IL-1β). Preclinical studies of acute minocycline treatment showed decreased inflammatory cell infiltration, reduced cystic cavitating, 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 results have been followed up by the phase III Multicentric trial 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 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.
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-α and IL-1β 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.

**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.
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

| 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 |
| MAP ≥ 85 mm Hg | Decreases cord ischemia by increasing perfusion pressure | MAPS (n = 100a; 2017b) | N/A |
| In phase III trial | | | |
| Minocycline | Inhibits microglial activation and downregulates COX-2, TNF-α, and IL-1β | MASC (n = 248b; 2018c) | N/A |
| Riluzole | Sodium-channel blocker, indirectly inhibits presynaptic glutamate release and potentiates reuptake | RISCIS (n = 351d; 2017e) | N/A |
| G-CSF | Reduces glutamate-induced apoptosis and TNF-α/IL-1β expression | G-SPIRIT (n = 8f; 2018g) | N/A |
| In phase II trial | | | |
| VX-210 (Cethrin) | Inactivation of the ROCK pathway | Cethrin in Acute Cervical Spinal Cord Injury (2016h) | N/A |
| Mg-PEG 3350 | Antiexcitotoxic and antiapoptotic | 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 |

*aProjected enrollment per https://clinicaltrials.gov. 
*bProject 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.
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 antiecitotoxic 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 [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-oligodendroglialogenic [72, 73], pro-neuronogenic [74], immunomodulatory [75, 76], and/or antigliotic [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 iPSC 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 prosurvival 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 axons. 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, high-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 transpluriotent 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...
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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REFERENCES

1. Spinal cord injury facts and figures at a glance. J Spinal Cord Med 2014;37:117–118.
2. Christopher and Dana Reeve Foundation. One degree of separation: Paralysis and spinal cord injury in the United States. 2010. Available at http://www.christopherreeve.org/site/c.3d1FKRNNoFjG/b.5091685/k.5880/One_Degree_of_Separation.htm. Accessed March 31, 2016.
3. LaPlaca MC, Simon CM, Prado GR et al. CNS injury biomechanics and experimental models. Prog Brain Res 2007;161:13–26.
4. Choo AM, Lu J, Lam CK et al. Contusion, dislocation, and distraction: Primary hemorrhage and membrane permeability in distinct mechanisms of spinal cord injury. J Neurosurg Spine 2007;6:255–266.
5. Whetstone WD, Hsu JY, Eisenberg M et al. Blood-spinal cord barrier after spinal cord injury: Relation to revascularization and wound healing. J Neurosci Res 2003;74:227–239.
6. Mautes AE, Weinzierl MR, Donovan F et al. Vascular events after spinal cord injury: Contribution to secondary pathogenesis. Phys Ther 2000;80:673–687.
7. Waxman SG. Demyelination in spinal cord injury. J Neurosci 1989;9:1–14.
8. Li S, Stys PK. Mechanisms of ionotropic glutamate receptor-mediated excitotoxicity in isolated spinal cord white matter. J Neurosci 2000;20:1190–1198.
9. Guha A, Tator CH, Rochon J. Spinal cord blood flow and systemic blood pressure after experimental spinal cord injury in rats. Stroke 1989;20:372–377.
10. Guha A, Tator CH. Acute cardiovascular effects of experimental spinal cord injury. J Trauma 1988;28:481–490.
11. Milhorat TH, Capocelli AL Jr, Anzilli AP et al. Pathological basis of spinal cord cavitation in syringomyelia: Analysis of 105 autopsy cases. J Neurosurg 1995;82:802–812.
12. Yuan YM, He C, The glial scar in spinal cord injury and repair. Neurosci Bull 2013;29:421–435.
13. Snow DM, Lemmon V, Carrino DA et al. Sulfated proteoglycans in astrogial barriers inhibit neurite outgrowth in vitro. Exp Neurol 1993;119:131–139.
14. Höke A, Silver J. Proteoglycans and other repulsive molecules in glial boundaries during development and regeneration of the nervous system. Prog Brain Res 1996;108:149–163.
15. Becker T, Amliker B, Becker CG et al. Tenascin-R inhibits regrowth of optic fibers in vitro and persists in the optic nerve of mice after injury. Glia 2000;29:330–346.
16. Silver J. Inhibitory molecules in development and regeneration. J Neurobiol 1994;24:suppl(1):S22–S24.
17. Butt AM, Duncan A, Hornby MF et al. Cells expressing the NG2 antigen contact nodes of Ranvier in adult CNS white matter. Glia 1999;26:84–91.
18. Jones LL, Yamaguchi Y, Stalciup WB et al. NG2 is a major chondroitin sulfate proteoglycan produced after spinal cord injury and is expressed by macrophages and oligodendrocyte progenitors. J Neurosci 2002;22:2792–2803.
19. McKeon RJ, Schreiber RC, Rudge JS et al. Reduction of neurite outgrowth in a model of glial scarring following CNS injury is correlated with the expression of inhibitory molecules on reactive astrocytes. J Neurosci 1991;11:3398–3411.
20. Forgione N, Fehlings MG. Rho-ROCK inhibition in the treatment of spinal cord injury. World Neurosurg 2014;82:e353–e359.
21. Chen MS, Huber AB, van der Haar ME et al. Nogo-A is a myelin-associated neurite outgrowth inhibitor and an antigen for monoclonal antibody 11-N. Nature 2000;403:434–439.
22. Moreau-Fauvarque C, Kumanogoh A, Camand E et al. The transmembrane semaphorin SemaphorinCD100, an inhibitor of axonal growth, is expressed on oligodendrocytes and upregulated after CNS lesion. J Neurosci 2003;23:9229–9239.
23. Cafferty WB, Duffy P, Huebner E et al. MAG and Olig2 synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. J Neurosci 2010;30:6825–6837.
24. Nashmi R, Fehlings MG. Mechanisms of axonal dysfunction after spinal cord injury: With an emphasis on the role of voltage-gated potassium channels. Brain Res Brain Res Rev 2001;38:165–191.
25. Nashmi R, Fehlings MG. Changes in axonal physiology and morphology after chronic compressive injury of the rat thoracic spinal cord. Neuroscience 2001;104:235–251.
26. Bhatt JM, Gordon PH. Current clinical trials in amyotrophic lateral sclerosis. Expert Opin Investig Drugs 2007;16:1197–1207.
27. Aebischer P, Norgaard-Pedersen B, Spina M et al. Intraspinal gene transfer in human amyotrophic lateral sclerosis: a phase I trial. Neurology 2003;60:1071–1077.
28. Aebischer P, Norgaard-Pedersen B, Spina M et al. Intraspinal gene transfer in human amyotrophic lateral sclerosis: a phase I trial. Neurology 2003;60:1071–1077.
29. Aebischer P, Norgaard-Pedersen B, Spina M et al. Intraspinal gene transfer in human amyotrophic lateral sclerosis: a phase I trial. Neurology 2003;60:1071–1077.
30. Aebischer P, Norgaard-Pedersen B, Spina M et al. Intraspinal gene transfer in human amyotrophic lateral sclerosis: a phase I trial. Neurology 2003;60:1071–1077.
31. Aebischer P, Norgaard-Pedersen B, Spina M et al. Intraspinal gene transfer in human amyotrophic lateral sclerosis: a phase I trial. Neurology 2003;60:1071–1077.
32. Aebischer P, Norgaard-Pedersen B, Spina M et al. Intraspinal gene transfer in human amyotrophic lateral sclerosis: a phase I trial. Neurology 2003;60:1071–1077.
33. Aebischer P, Norgaard-Pedersen B, Spina M et al. Intraspinal gene transfer in human amyotrophic lateral sclerosis: a phase I trial. Neurology 2003;60:1071–1077.
quality of evidence and strength of recommenda-
tions. BMJ 2008;336:924–926.
43 Jaeschke R, Guyatt GH, Dellingier P et al. Use of GRADE grid to reach decisions on clinical practice guidelines when consensus is elusive. BMJ 2008;337:a744.
44 Schünemann HJ, Oxman AD, Brozek J et al. Grading quality of evidence and strength of recommendations for diagnostic tests and strategies. BMJ 2008;336:1106–1110.
45 Hypothermia after Cardiac Arrest Study Group. Mild therapeutic hypothermia to improve the neurologic outcome after cardiac arrest. N Engl J Med 2002;346:549–556.
46 Papile LA, Barry JE, Benitz W et al. Hypothermia and neonatal encephalopathy. Pediatrics 2013;134:1114–1150.
47 Kwon BK, Mann C, Sohn HM et al. Hypothermia for spinal cord injury. Spine J 2008;8:359–374.
48 Levi AD, Green BA, Wang MY et al. Clinical application of modest hypothermia after spinal cord injury. J Neurotrauma 2009;26:407–415.
49 The Miami Project to Cure Paralysis. Neuroprotection—therapeutic hypothermia. 2014. Available at http://www.themiamiproject.org/research/what-are-clinical-trials/clinical-trials/therapeutic-hypothermia-acute/. Accessed October 15, 2015.
50 Fehlings MG, Vaccaro A, Wilson JR et al. Early versus delayed decompression for traumatic spinal cord injury: Results of the Surgical Timing in Acute Spinal Cord Injury Study (STASCIS). PLoS One 2012;7:e32037.
51 Wilson JR, Singh A, Craven C et al. Early versus late surgery for traumatic spinal cord injury: The results of a prospective Canadian cohort study. Spinal Cord 2012;50:840–843.
52 Dvorak MFNV, Noonan VK, Fallah N et al. The influence of time from injury to surgery on motor recovery and length of hospital stay in acute traumatic spinal cord injury: an observational Canadian cohort study. J Neurotrauma 2015;32:645–654.
53 Wallner S, Peters S, Pitzer C et al. The granulocyte-colony-stimulating factor has a dual role in neuronal and vascular plasticity. Front Cell Dev Biol 2015;3:48.
54 Vishw N, Miyata M, Kamada T et al. Granulocyte colony-stimulating factor attenuates neuronal death and promotes functional recovery after spinal cord injury in mice. J Neurotraumatol Exp Neurol 2007;66:724–731.
55 Takahashi H, Yamazaki M, Okawa A et al. Neuroprotective therapy using granulocyte colony-stimulating factor for acute spinal cord injury: A phase II/III clinical trial. Eur Spine J 2012;21:2580–2587.
56 Kamiya K, Koda M, Furuya T et al. Neuroprotective therapy with granulocyte colony-stimulating factor in acute spinal cord injury: A comparison with high-dose methylprednisolone as a historical control. Eur Spine J 2015;24:963–967.
57 Wilson JR, Forgione N, Fehlings MG. Emerging therapies for acute traumatic spinal cord injury. CMAJ 2013;185:485–492.
58 Resnick DK. Updated guidelines for the management of acute cervical spine and spinal cord injury. Neurosurgery 2013;72(suppl 2):1–2.
59 Eng BC, Gaspard E, Beskonaki E, Solanoglu I et al. Magnesium sulfate treatment in experimental spinal cord injury: Impact on vascular changes and early clinical results. Neurosurg Rev 2003;26:283–287.
60 Siddiqui AM, Khazaie M, Fehlings MG. Translating mechanisms of neuroprotection, regeneration, and repair to treatment of spinal cord injury. Proc Brain Res 2015;238:15–54.
61 Bradbury EJ, Moon LDF, Popat RJ et al. Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 2002;416:636–640.
62 Jones LL, Sajid D, Tuszyński MH. Axonal regeneration through regions of chondroitin sulfate proteoglycan deposition after spinal cord injury: A balance of permissiveness and inhibition. J Neurosci 2003;23:9276–9288.
63 Ikegami T, Nakamura M, Yamane J et al. Chondroitinase ABC combined with neural stem/progenitor cell transplantation enhances graft cell migration and outgrowth of growth-associated protein-43-positive fibers after rat spinal cord injury. Eur J Neurosci 2005;22:3036–3046.
64 Carter LM, McMahon SB, Bradbury EJ. Delayed treatment with chondroitinase ABC reverses chronic atrophy of rubrospinal neurons following spinal cord injury. Exp Neurol 2011;228:149–156.
65 Bartus K, James ND, Dianeglos A et al. Large-scale chondroitin sulfate proteoglycan digestion with chondroitinase ABC gene therapy leads to reduced pathology and modulates macrophage phenotype following spinal cord contusion injury. J Neurosci 2014;34:4822–4836.
66 Niederöst B, Oertle T, Fritsche J et al. Nogo-A and myelin-associated glycoprotein mediate neurite growth inhibition by antago-nistic regulation of RhoA and Rac1. J Neurosci 2002;22:10368–10376.
67 Freund P, Schmidlin E, Wanner T et al. Nogo-A-specific antibody treatment enhances sprouting and functional recovery after cerebral lesion in adult primates. Nat Med 2006;12:790–792.
68 Gonzenbach RR, Schwab ME. Inhibi-tion of neurite growth to repair the injured adult CNS: Focus on Nogo. Cell Mol Life Sci 2008;65:161–176.
69 Liebscher T, Schnell L, Schnell D et al. Nogo-A antibody improves regeneration and locomotion of spinal cord-injured rats. Ann Neurol 2005;58:706–719.
70 Meieringer V, Pradat P-F, Cosse A et al. Safety, pharmacokinetic, and functional effects of the Nogo-A monoclonal antibody in amyo-trophic lateral sclerosis: A randomized, first-in-human clinical trial. PLoS One 2014;9:e97803.
71 Fehlings MG, Theodore N, Harrop J et al. A phase II/III clinical trial of a recombinant Rho protein antagonist in acute spinal cord injury. J Neurotrauma 2011;28:777–796.
72 Bai L, Lennon DP, Eaton V et al. Human bone marrow-derived mesenchymal stem cells induce Th2-polarized immune response and promote endogenous repair in animal models of multiple sclerosis. J Neurosci 2008;28:1202–1203.
73 Arriola A, Kiel ME, Shi Y et al. Adjunctive MSCs enhance myelin formation by xenogenic oligodendrocyte precursors transplanted in the retina. Cell Res 2010;20:728–734.
74 Zhang J, Wang B, Xiao Z et al. Olfactory ensheathing cells promote proliferation and in-hibition of pathological activation of neural progenitor cells through activation of Notch signaling. Neurosurgery 2008;153:406–413.
75 Wang L, Shi J, van Ginkel FW et al. Neural stem/progenitor cells modulate immune responses by suppressing T lymphocytes with nitric oxide and prostaglandin E2. Exp Neurol 2009;216:177–183.
76 Okamura RM, Lebkowski J, Au M et al. Immunological properties of human embryonic stem cell-derived oligodendrocyte precursor cells. J Neuroimmunol 2007;192:134–144.
77 Jäderstad J, Jäderstad LM, Li J et al. Communication via gap junctions underlies early functional and beneficial interactions between grafted neural stem cells and the host. Proc Natl Acad Sci USA 2010;107:5184–5189.
78 Curt ACS, Fehlings M, Huhn S. Phase II/II clinical trial of HuCNS-SC cells in chronic thora-cic spinal cord injury—interim analysis. 2014. http://www.stemcellinc.com/Presentations/ASIA_FINAL.pdf. Accessed October 15, 2015.
79 Hawryluk GW, Fehlings MG. The center of the spinal cord may be central to its repair. Cell Stem Cell 2008;3:230–232.
80 Martens DJ, Seaberg RM, van der Kooi D. In vivo infusions of exogenous growth factors into the fourth ventricle of the adult mouse brain increase the proliferation of neural progenitors around the fourth ventricle and the central canal of the spinal cord. Eur J Neurosci 2002;16:1045–1057.
81 Espinosa-Jeffrey A, Oregel K, Higgins L et al. Strategies for endogenous spinal cord re-pair: HPMA hydrogel to recruit migrating endogenous stem cells. Adv Exp Med Biol 2012;760:25–52.
82 Babona-Pilipos R, Popovic MR, Morshed CM. A galvanotaxis assay for analysis of neural precursor cell migration kinetics in an externally applied direct current electric field. J Vis Exp 2012.
83 Draper JS, Moore HD, Ruban LN et al. Cellu-lar and characterization of human embryonic stem cells. Stem Cells Dev 2004;13:325–336.
84 Draper JS, Smith K, Gokhale P et al. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. Nat Biotechnol 2004;22:53–54.
85 Brambrink T, Foreman R, Welsted GG et al. Sequential expression of pluripotency markers during direct reprogramming of mouse somatic cells. Cell Stem Cell 2008;2:151–159.
86 Stadtfeld M, Nagaya M, Utikal J et al. Induced pluripotent stem cells generated without viral integration. Science 2008;322:945–949.
87 Woltjen K, Michael IP, Mohseni P et al. piggyBac transposition reprograms fibroblasts to induced pluripotent stem cells. Nature 2009;458:767–770.
88 Salewski RP,öffekharpour E, Fehlings MG. Are induced pluripotent stem cells the fu-ture of cell-based regenerative therapies for spinal cord injury? J Cell Physiol 2010;222:515–521.
89 Hu BY, Weick JP, Yu J et al. Neural differ-entiation of human induced pluripotent stem cells follows developmental principles but with variable potency. Proc Natl Acad Sci USA 2010;107:4335–4340.
90 Feng Q, Lu S-J, Klimanskaya I et al. Hemangioblastic derivatives from human indi-cated pluripotent stem cells exhibit limited expansion and early senescence. STEM CELLS 2010;28:704–711.
Neuroregeneration and Neuroprotection in SCI

91 Vaskova EA, Stekleneva AE, Medvedev SP et al. “Epigenetic memory” phenomenon in induced pluripotent stem cells. Acta Naturae 2013;5:15–21.
92 Darabi VR, Veeravalli KK, Dinh DH. Mesenchymal stem cells in the treatment of spinal cord injuries: A review. World J Stem Cells 2014;6:120–133.
93 Swartlander MD, Blakney AK, Amer LD et al. Immunomodulation by mesenchymal stem cells combats the foreign body response to cell-laden synthetic hydrogels. Biomaterials 2011;32:706–713.
94 Bessout R, Sémont A, Démargay C et al. Mesenchymal stem cell therapy induces guco- corticoid synthesis in colonic mucosa and suppresses radiation-activated T cells: New insights into MSC immunomodulation. Mucosal Immunol 2014;7:656–669.
95 Lim JH, Kim JS, Yoon IH et al. Immunomodulation of delayed-type hypersensitivity responses by mesenchymal stem cells is associated with bystander T cell apoptosis in the draining lymph node. J Immunol 2010;185:4022–4029.
96 Quertainmont R, Cantinieaux D, Botman O et al. Mesenchymal stem cell graft improves recovery after spinal cord injury in adult rats through neurotrophic and pro-angiogenic actions. PLoS One 2012;7:e39500.
97 Kim JW, Ha KY, Molon JN et al. Bone marrow-derived mesenchymal stem cell transplantation for chronic spinal cord injury in rats: Comparative study between intrasural and intravenous transplantation. Spine 2013;38:E1065–E1074.
98 Jarocha D, Miliczarek O, Kawecki Z et al. Preliminary study of autologous bone marrow nucleated cells transplantation in children with spinal cord injury. STEM CELLS TRANSLATIONAL MEDICINE 2014;3:395–404.
99 Windus LC, Lineburg KE, Scott SE et al. Lamellipodia mediate the heterogeneity of central olfactory ensheathing cells. Cell Mol Life Sci 2010;67:1735–1750.
100 Silva NA, Cooke MJ, Tam RY et al. The effects of peptide modified gellan gum and olfactory ensheathing glia cells on neural stem/progenitor cell fate. Biomaterials 2012;33:6345–6354.
101 Liu J, Chen P, Wang Q et al. Meta-analysis of olfactory ensheathing cell transplantation promoting functional recovery of motor nerves in rats with complete spinal cord transection. Neuro Regen Res 2014;9:1850–1858.
102 Li L, Adnan H, Xu B et al. Effects of transplantation of olfactory ensheathing cells in chronic spinal cord injury: A systematic review and meta-analysis. Eur Spine J 2015;24:919–930.
103 Nistor GJ, Totoiu MO, Haque N et al. Human embryonic stem cells differentiated into oligodendrocytes in high purity and myelinate after spinal cord transplantation. Glia 2005;49:385–396.
104 Tetzlaff W, Okon EB, Karimi-Abdolrezae S et al. A systematic review of cellular transplantation therapies for spinal cord injury. J Neurotrauma 2011;28:1611–1622.
105 Gil JE, Woo D-H, Shin J-H et al. Vitroneuralization and oligodendrocyte differentiation during neurogenesis of human embryonic stem cells. FEBS Lett 2009;583:561–567.
106 Lu P, Woodruff G, Wang Y et al. Long-distance axonal growth from human induced pluripotent stem cells after spinal cord injury. Neuron 2014;83:789–796.
107 Bregman BS, Kunkel-Bagden E, Reier PJ et al. Recoveries after spinal cord injury: mechanisms underlying transplant-mediated recovery of function differ after spinal cord injury in newborn and adult rats. Exp Neurol 1993;123:3–16.
108 Jakeman LB, Reier PJ. Axonal projections between fetal spinal cord transplants and the adult rat spinal cord: A neuroanatomical tracing study of local interactions. J Comp Neurol 1991;307:311–334.
109 Torper O, Pfisterer U, Wolf DA et al. Generation of induced neurons via direct conversion in vivo. Proc Natl Acad Sci USA 2013;110:7038–7043.
110 Hu B-Y, Zhang S-C. Differentiation of spinal motor neurons from pluripotent human stem cells. Nat Protoc 2009;4:1295–1304.
111 Raper J, Mason C. Cellular strategies of axonal pathfinding. Cold Spring Harb Perspect Biol 2010;2:a001933.
112 van den Pol AN, Kim WT. NILE/L1 and NCAM-polysialic acid expression on growing axons of isolated neurons. J Comp Neurol 1993;332:237–257.
113 Zhang S, Xia YY, Lim HC et al. NCAM-mediated locomotor recovery from spinal cord contusion injury involves neuroprotection, axon regeneration, and synaptogenesis. Neurochem Int 2010;56:919–929.
114 Wolman MA, Sittaramane VK, Essner JI et al. Transient axonal glycoprotein-1 (TAG-1) and laminin-alpha1 regulate dynamic growth cone behaviors and initial axon direction in vivo. Neural Dev 2008;3:6.
115 Blackmore M, Letourneau PC, LI, beta1 integrin, and cadherins mediate axonal regeneration in the embryonic spinal cord. J Neurobiol 2006;66:1564–1583.
116 Zhang L, Kaneko S, Kikuchi K et al. Rewiring of regenerated axons by combining treadmill training with semaphorin3A inhibition. Mol Brain 2014;7:14.
117 Kennedy TE, Wang H, Marshall W et al. Axon guidance by diffusible chemottractants: A gradient of netrin protein in the developing spinal cord. J Neurosci 2006;26:8866–8874.
118 Karimi-Abdolrezae S, Eftekharpour E, Wang J et al. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci 2006;26:3377–3389.
119 Karimi-Abdolrezae S, Eftekharpour E, Wang J et al. Synergistic effects of transplanted adult neural stem/progenitor cells, chondroitinase, and growth factors promote functional repair and plasticity of the chronically injured spinal cord. J Neurosci 2010;30:1657–1673.
120 Liu WG, Wang ZY, Huang ZS. Bone marrow-derived mesenchymal stem cells expressing the bFGF transgene promote axon regeneration and functional recovery after spinal cord injury in rats. Neurol Res 2011;33:686–693.
121 Hong SR, Kwon MJ, Lee HG et al. Hepatocyte growth factor reduces astrocytic scar formation and promotes axonal growth beyond glial scars after spinal cord injury. Exp Neurol 2012;233:312–322.
122 Tobias CA, Shumsky JS, Shibata M et al. Delayed grafting of BDNF and NT-3 producing fibroblasts into the injured spinal cord stimulates sprouting, partially rescues axotomized red nucleus neurons from loss and atrophy, and provides limited regeneration. Exp Neurol 2003;184:97–113.
123 Zhang YJ, Zhang W, Lin CG et al. Neurotrophin-3 gene modified mesenchymal stem cells promote remyelination and functional recovery in the demyelinated spinal cord of rats. J Neurol Sci 2012;313:64–74.
124 Sasaki M, Radtke C, Tan AM et al. BDNF-hypersecreting human mesenchymal stem cells promote functional recovery, axonal sprouting, and protection of corticospinal neurons after spinal cord injury. J Neurosci 2009;29:14932–14941.
125 Blesch A, Tsuzynski MH. Cellular GDNF delivery promotes growth of motor and dorsal column sensory axons after partial and complete spinal cord transections and induces remyelination. J Comp Neurol 2003;467:407–417.
126 Rooney GE, McMahon SS, Ritter T et al. Neurotrophic factor-expressing mesenchymal stem cells survive transplantation into the con- spined spinal cord without differentiating into neural cells. Tissue Eng Part A 2009;15:3049–3059.
127 Volic K, Shoichet MS. Tunable growth factor delivery from injectable hydrogels for tissue engineering. J Am Chem Soc 2012;134:882–885.
128 Mayfield AE, Tilkoo EE, Latham N et al. The effect of encapsulation of cardiac stem cells within matrix-enriched hydrogel capsules on cell survival, post-ischemic cell retention and cardiac function. Biomaterials 2014;35:133–142.
129 Lippens E, Swennen I, Giroius J et al. Cell survival and proliferation after encapsulation in a chemically modified Pluronic(R) F127 hydrogel. J Biomat Appl 2013;27:828–839.
130 Hwang DH, Shin HY, Kwon MJ et al. Sur- vival of neural stem cell grafts in the lesioned spinal cord is enhanced by a combination of treadmill locomotor training via insulin-like growth factor-1 signaling. J Neurosci 2014;34:12788–12800.
131 Gupta D, Tator CH, Shoichet MS. Fast- gelling injectable blend of hyaluronan and methylcellulose for intraheal, localized delivery to the injured spinal cord. Biomaterials 2006;27:2370–2379.
132 Itohaka H, Kuroda S, Shichinohe H et al. Fibrin matrix provides a suitable scaffold for bone marrow stromal cells transplanted into in- jured spinal cord: A novel material for CNS tis- sue engineering. Neuropathology 2009;29:248–257.
133 Tang YD, Lavik EB, Qu X et al. Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proc Natl Acad Sci USA 2002;99:3024–3029.
134 Stolkos S, Tuszynski MH. Freeze-dried agarose scaffolds with uniaxial channels stimulate and guide linear axonal growth following spinal cord injury. Biomaterials 2006;27:443–451.

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135 Gros T, Sakamoto JS, Blesch A et al. Regeneration of long-tract axons through sites of spinal cord injury using templated agarose scaffolds. Biomaterials 2010;31:6719–6729.

136 Tsai EC, Dalton PD, Shoichet MS et al. Matrix inclusion within synthetic hydrogel guidance channels improves specific supraspinal and local axonal regeneration after complete spinal cord transection. Biomaterials 2006;27:519–533.

137 Mothe AJ, Tam RY, Zahir T et al. Repair of the injured spinal cord by transplantation of neural stem cells in a hyaluronan-based hydrogel. Biomaterials 2013;34:3775–3783.

138 Taylor SJ, McDonald JW 3rd, Sakiyama-Elbert SE. Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. J Control Release 2004;98:281–294.

139 Ansorena E, De Berdt P, Ucakar B et al. Injectable alginate hydrogel loaded with GDNF promotes functional recovery in a hemisection model of spinal cord injury. Int J Pharm 2013;455:148–158.

140 Shen YH, Shoichet MS, Radisic M. Vascular endothelial growth factor immobilized in collagen scaffold promotes penetration and proliferation of endothelial cells. Acta Biomater 2008;4:477–489.

141 Leipzig ND, Xu C, Zahir T et al. Functional immobilization of interferon-gamma induces neuronal differentiation of neural stem cells. J Biomed Mater Res A 2010;93:625–633.

142 Jain A, Kim YT, McKeon RJ et al. In situ gelling hydrogels for conformal repair of spinal cord defects, and local delivery of BDNF after spinal cord injury. Biomaterials 2006;27:497–504.

143 Taylor SJ, Rosenzweig ES, McDonald JW 3rd, Sakiyama-Elbert SE. Delivery of neurotrophin-3 from fibrin enhances neuronal fiber sprouting after spinal cord injury. J Control Release 2006;113:226–235.

144 Caplan MR, Schwartzfarb EM, Zhang S et al. Effects of systematic variation of amino acid sequence on the mechanical properties of a self-assembling, oligopeptide biomaterial. J Biomater Sci Polym Ed 2002;13:225–236.

145 Segers VF, Lee RT. Local delivery of proteins and the use of self-assembling peptides. Drug Discov Today 2007;12:561–568.

146 Liu Y, Ye H, Satkunendarajah K et al. A self-assembling peptide reduces glial scarring, attenuates post-traumatic inflammation and promotes neurological recovery following spinal cord injury. Acta Biomater 2013;9:8075–8088.

147 Tyseling-Mattiace VM, Sahni V, Niece KL et al. Self-assembling nanofibers inhibit glial scar formation and promote axon elongation after spinal cord injury. J Neurosci 2008;28:3814–3823.

148 Iwasaki M, Wilcox JT, Nishimura Y et al. Synergistic effects of self-assembling peptide and neural stem/progenitor cells to promote tissue repair and forelimb functional recovery in cervical spinal cord injury. Biomaterials 2014;35:2617–2629.

149 Cadotte DW, Fehlings MG. Will imaging biomarkers transform spinal cord injury trials? Lancet Neurol 2013;12:843–844.

150 Pouw MH, Hosman AJ, van Middendorp JJ et al. Biomarkers in spinal cord injury. Spinal Cord 2009;47:519–525.