REVIEW

Novel innovations in cell and gene therapies for spinal cord injury [version 1; peer review: 3 approved]

Mohammad-Masoud Zavvarian¹,², Amirali Toossi¹, Mohamad Khazaei¹, James Hong¹,², Michael Fehlings¹-⁴

¹Krembil Research Institute, University Health Network, Toronto, Canada
²Institute of Medical Science, University of Toronto, Toronto, Canada
³Department of Surgery, University of Toronto, Toronto, Canada
⁴Spinal Program, Toronto Western Hospital, University Health Network, Toronto, Canada

Abstract
Spinal cord injury (SCI) leads to chronic and multifaceted disability, which severely impacts the physical and mental health as well as the socio-economic status of affected individuals. Permanent disabilities following SCI result from the failure of injured neurons to regenerate and rebuild functional connections with their original targets. Inhibitory factors present in the SCI microenvironment and the poor intrinsic regenerative capacity of adult spinal cord neurons are obstacles for regeneration and functional recovery. Considerable progress has been made in recent years in developing cell and molecular approaches to enable the regeneration of damaged spinal cord tissue. In this review, we highlight several potent cell-based approaches and genetic manipulation strategies (gene therapy) that are being investigated to reconstruct damaged or lost spinal neural circuits and explore emerging novel combinatorial approaches for enhancing recovery from SCI.

Keywords
Spinal Cord Injury; Gene Therapy; Neuroregeneration; Stem Cells; Combinatorial Treatments
Corresponding author: Michael Fehlings (Michael.Fehlings@uhn.ca)

Author roles: Zavvarian MM: Conceptualization, Data Curation, Formal Analysis, Investigation, Visualization, Writing – Original Draft Preparation, Writing – Review & Editing; Toossi A: Conceptualization, Data Curation, Formal Analysis, Investigation, Writing – Original Draft Preparation, Writing – Review & Editing; Khazaei M: Conceptualization, Data Curation, Formal Analysis, Writing – Original Draft Preparation, Writing – Review & Editing; Hong J: Data Curation, Formal Analysis, Project Administration, Resources, Writing – Review & Editing; Fehlings M: Conceptualization, Investigation, Project Administration, Resources, Supervision, Writing – Review & Editing

Competing interests: No competing interests were disclosed.

Grant information: This work was supported by the Canadian Institutes of Health Research (CIHR to MGF). MGF was supported by the Halbert Chair in Neural Repair and Regeneration and the DeZwirek Foundation. MZ was awarded the Ontario Graduate Scholarship (OGS), and AT was supported by the Krembil Postdoctoral and Clinical Research Fellowship Award.

The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Copyright: © 2020 Zavvarian MM et al. This is an open access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

How to cite this article: Zavvarian MM, Toossi A, Khazaei M et al. Novel innovations in cell and gene therapies for spinal cord injury [version 1; peer review: 3 approved] F1000Research 2020, 9(F1000 Faculty Rev):279 https://doi.org/10.12688/f1000research.21989.1

First published: 22 Apr 2020, 9(F1000 Faculty Rev):279 https://doi.org/10.12688/f1000research.21989.1
Introduction

Traumatic spinal cord injury (SCI) can prompt debilitating autonomic and sensorimotor impairments, severely limiting the patient’s independence, quality of life, and socioeconomic status. The prevalence of SCI ranges between 250 and 906 cases per million, with life expectancy typically spanning several decades from the time of injury. This leads to a prolonged life with disabilities and a staggering cost of care (estimated between 1.2 and 5.1 million US dollars for the patient’s lifespan). In addition, concurrent complications, such as respiratory difficulty, autoimmune dysfunction, neuropathic pain, and autonomic dysreflexia, further exacerbate the patient’s health and wellbeing. While many regenerative treatments have been investigated over the years, an effective therapy does not yet exist in the clinic. This paper reviews several emerging regenerative strategies for SCI and the knowledge gaps associated with them. These treatment strategies are 1) regenerative genetic manipulation (gene therapy) approaches, 2) cellular transplantation therapy, and 3) combinatorial approaches to enhance functional outcomes.

Pathological hallmarks of spinal cord injury

The traumatic fracture and dislocation of the vertebral column introduces laceration, compression, and contusive damage to the spinal cord, impairing local neurons and the supporting glial and vascular cells. The primary mechanical shock to the neural tissue and disruption of the cell membrane during the primary injury permeabilizes the cells, resulting in a cascade of molecular and signaling pathways that initiate a series of secondary injuries to the spinal cord. The formation of free radicals and oxidative stress as a consequence of secondary injuries result in more neuronal and glial death, mainly due to apoptosis. This also results in activation of local microglia and astrocytes to produce proinflammatory signals for a greater immune response. In parallel, the acute vascular damage and permeabilization will increase hemorrhage, hypoxia, and the infiltration of reactive immune cells to the injury epicenter. Despite the adaptive role of the introduced immune cells in debris clearance, the prolonged activity of immune cells leads to swelling and further damages the local cells.

The injured spinal cord tissue can be divided into three distinct histological compartments, including a non-neural lesion core, a surrounding astroglial border, and a preserved reactive neural tissue. Each compartment is composed of a unique cellular makeup and poses a distinct barrier to functional recovery. First, the lesion core is the site of the fibrotic scar and cystic cavity. Cavitation results from debris clearance but limits axonal growth and neurogenesis. The surrounding astroglial scar is formed from newly differentiated astrocytes, which encapsulate the reactive immune cells within the damaged tissue zone. The functional role of astrocytic production and the integration of inhibitory molecules within the astroglial border continues to be under investigation. While genetic knockout studies demonstrate the adaptive role of astrocyte scar formation, the prevailing paradigm suggests that this border constitutes a major impediment to neural regeneration. In the perilesional zone, synaptic damage and demyelination result in circuit inactivation. Circuit reorganization can be either maladaptive—leading to neuropathic pain, muscle spasticity, and autonomic dysreflexia—or adaptive, as it can restore function after incomplete SCI.

During both primary and secondary injury, the secretion of inhibitory molecules is a critical barrier to regeneration. For instance, disintegration of myelin and demyelination releases potent inhibitory extracellular molecules, such as myelin-associated glycoprotein (MAG), oligodendrocyte-myelin glycoprotein (OMgp), and neurite outgrowth inhibitor A (Nogo A). Furthermore, reactive glia secrete tenasin as well as chondroitinase sulfate proteoglycans (CSPGs), which include brevican, phosphacan, neurocan, versican, and neural/glial antigen 2 (NG2) proteoglycans. These molecules lead to the activation of the Rho–ROCK signaling pathway, which intrinsically inhibits neuronal repair and regeneration. Hence, despite the wide distribution of oligodendrocyte progenitor cells (OPCs) and the localization of neural progenitor cells (NPCs) along the ependymal layers of the spinal central canal, limited endogenous neural regeneration occurs in the injured spinal cord. Numerous therapeutics aimed at mitigating these barriers to regeneration have been examined over the past few decades in both clinical and preclinical studies (Figure 1). This review provides a concise overview of the newly developed regenerative gene and cell therapies following traumatic SCI.

![Figure 1. Spinal cord injury pathology and regenerative therapeutics.](image-url)
Regenerative gene therapy

Gene therapy is the introduction of new genetic material to modify maladaptive transcription in a cell or to introduce downregulated or novel genes\(^9\). The advent of CRISPR/Cas9 genome editing approaches and recombinant replication-defective viral constructs enables targeted interventions in the injured spinal cord, mitigating the risk of potential adverse off-target effects. Gene therapy has gained promising advancement in the past decade, as six therapies have gained clinical approval for conditions such as spinal muscular atrophy or Leber’s congenital amaurosis\(^8\). Two potential applications of gene therapy for SCI include *in vivo* gene delivery to the spinal cord or *ex vivo* transduction of cells for subsequent transplantation into the spinal cord. The advancement of *in vivo* gene delivery via non-integrating adeno-associated viral (AAV) constructs allows durable and sustained episomal expression of a therapeutic gene or a gene silencer (Figure 2)\(^9\). Thus far, preclinical investigations reveal the applications of *in vivo* gene therapy in the injured spinal cord to 1) enhance the expression of pro-regenerative factors, 2) molecularly modulate neural circuits, 3) block the expression of detrimental proteins, and 4) introduce matrix-modifying enzymes for the degradation of inhibitory particles.

Expression of pro-regenerative factors

The injured axons at the lesion core possess limited regenerative ability. The expression of pro-regenerative factors can increase the regenerative potential of damaged neurons. Krüppel-like factors (KLFs) are a family of transcriptional factors which are crucial for axonal regeneration and plasticity. Although KLF4 inhibits axon regeneration, KLF6 and KLF7 are important promoters of axon regeneration\(^8,12\). Therapeutically induced KLF7 overexpression stimulates axonal sprouting\(^7\). Another pro-regenerative gene therapy target is SOX11, which is a transcriptional factor actively involved in neurogenesis. SOX11 overexpression via an AAV-mediated strategy promotes axonal sprouting in preclinical SCI models\(^12\). There is growing evidence that combined overexpression of growth factors will have a greater effect on axonal regeneration. Multiple genes can be combined into the same viral construct, which ease their therapeutic administration. The combined AAV-induced overexpression of osteopontin, insulin-like growth factor 1 (IGF1), ciliary-derived neurotrophic factor (CNTF), fibroblast growth factor 2 (FGF2), glial-derived neurotrophic factor (GDNF), and epidermal growth factor (EGF) suggests a 100-fold increase in axonal growth\(^11\).

Expression of circuit-modifying factors

The majority of SCI patients suffering from complete functional loss (classified as grade A by the American Spinal Injury Association) continue to possess anatomically preserved neural tissue around the lesion core, which remains dormant after injury\(^17,35\). Neural circuit modulation utilizes the neuroplastic nature of local synapses to reform functional “bypass” circuits around the lesion core, hence activating the dormant preserved neural tissue\(^36\). The staggered double hemisection (SDH) SCI model enables the examination of local relay circuits, as it interrupts all supraspinal inputs while sparing contralateral relay connections in the spinal cord\(^37\). Although advances in rehabilitative training and epidural stimulation have shown incremental progress in stimulating dormant circuits, these therapies can be strengthened and supplemented with molecular modulators of relay circuits to maximize their effects. Recent pharmacological screening in mouse SDH has identified chloride potassium symporter 5 (KCC2) as an important modulator of neural circuits\(^38\). KCC2 plays an important role in inhibitory neurotransmission at the synaptic cleft and subsequently balances the excitatory/inhibitory (E/I) ratio. Although pharmacological KCC2 agonists can improve behavioral recovery after SCI, this improvement diminishes

![Figure 2. Gene therapy applications investigated in preclinical spinal cord injury models. Adeno-associated viruses (AAVs) introduce non-integrating genetic material, which can express 1) pro-regenerative factors, 2) circuit-modifying factors, 3) gene silencers for inhibitory factors, and 4) matrix-modifying enzymes.](image-url)
upon cessation of daily drug administration\textsuperscript{38}. Gene therapy is an effective tool for continuous expression of neuro-modulatory factors, such as KCC2, and circumvents the continuous modulation required for modification of the spinal neural circuit. AAV-mediated KCC2 overexpression, under the influence of a synapsin promoter, is shown to improve functional recovery without the risk of adverse off-target effects associated with pharmacological strategies\textsuperscript{40}.

\textbf{Suppression of inhibitory molecules}

Transcriptional suppression of intrinsic inhibitory molecules can circumvent the inability of adult neurons to regenerate across the injured spinal cord. Short-hairpin RNA (shRNA) constructs are capable of therapeutically silencing the expression of inhibitory factors. For instance, phosphatase and tensin homolog (PTEN) is a tumor suppressor, which converts phosphatidylinositol-3,4,5-triphosphate (RO3P) to phosphatidylinositol-4,5-bisphosphate (RI2P). PTEN blocks the growth and extension of adult neurons, as it is known to inhibit axonal protein synthesis through negative regulation of the mechanistic target of rapamycin (mTOR)\textsuperscript{40}. The downregulation of mTOR both in adulthood and after axonal injury limits the regenerative potential of damaged neurons\textsuperscript{40}. PTEN deletion via an AAV-mediated Cre–LoxP system enables axonal regeneration in the mouse corticospinal tract following injury\textsuperscript{39,40}. Additionally, shRNA-mediated suppression of PTEN increases the regrowth of the corticospinal tract injury\textsuperscript{41}.

\textbf{Enzymatic degradation of the glial scar}

The secretion of CSPGs by reactive astrocytes and other glial and non-neural cells in the glial scar is one of the major barriers to axonal outgrowth and regeneration\textsuperscript{41}. Enzymatic degradation of CSPGs, using the bacterial enzyme chondroitinase ABC (ChABC), has been proven in preclinical studies to improve regeneration and functional recovery after SCI\textsuperscript{42,43}. However, ChABC has a low half-life and limited thermal stability, which requires its repetitve administration to the spinal cord\textsuperscript{44}. Gene therapy is one of the delivery options for ChABC, as it avoids the need for repetitive invasive enzymatic infusion while still promoting neuroplasticity and functional improvement. The application of lentiviral constructs allows temporal control of ChABC expression under inducible promoters (e.g. TetON promoters), suggesting that long-term expression of ChABC is critical for the recovery of fine motor movement after cervical SCI\textsuperscript{45}. This paves the way for a future clinical trial for ChABC gene therapy in SCI patients.

\textbf{Regenerative cellular therapies}

Cellular approaches hold promise as a regenerative therapy for SCI, as they address multiple facets of the injury pathophysiology concurrently\textsuperscript{46–48}. Transplanted cells can replace lost neurons and glial cells, immunomodulate local and systemic environments, secrete critical neurotrophic factors, and produce a growth-permissive extracellular matrix to influence both cell survival and differentiation\textsuperscript{46,47}. While numerous cell types, including mesenchymal stem cells, olfactory ensheathing cells, and Schwann cells, have been studied\textsuperscript{49}, the stem/progenitor cells with the potential to differentiate into neural cell lineages (neurons, astrocytes, and oligodendrocytes) are uniquely poised to regenerate the injured spinal cord. As a general term, these cells are referred to as neural stem/progenitor cells (NSPCs) or simply NPCs. NPCs are self-renewing, tripotent stem cells capable of differentiating into synaptically integrating neurons, myelinating oligodendrocytes, and astrocytes after transplantation into SCI\textsuperscript{49,50–53} (Figure 3). There are also studies that have used bipotent or unipotent cells that are lineage restricted\textsuperscript{44}. Oligodendrocytes and neurons differentiated from NPCs are capable of remyelinating denuded axons and can re-establish interrupted neuronal pathways via intra- and trans-segmental relay circuits\textsuperscript{50}. Our lab and others have shown that the two major mechanisms for functional recovery following NPC transplantation are 1) integration of NPC-derived neurons into disconnected circuits to relay neural signals and 2) myelination of denuded axons by NPC-derived oligodendrocytes\textsuperscript{53}\textsuperscript{55–57}.

NPCs have historically been derived from adult tissue sources (e.g. subventricular zone of the forebrain, subgranular zone of the dentate gyrus, and central canal in the spinal cord) or by differentiation of embryonic stem cells (ESCs)\textsuperscript{47,48}. ESC-derived NPCs present ethical challenges, and the clinical derivation of NPCs from adult tissues for autologous transplantation is not feasible. However, exciting advances have facilitated safe NPC derivation from translationally relevant human induced pluripotent stem cells (hiPSCs). This is particularly advantageous, as iPSCs can be made from easily accessible autologous somatic cells (e.g. skin or blood) using non-viral techniques, which provides a clinically attractive approach to cell therapy\textsuperscript{47,48}.

\textbf{Remyelination of the denuded axons}

While recent work suggests the degree of endogenous remyelination by oligodendrocyte progenitor cells is higher than previously reported\textsuperscript{50}, it remains widely accepted that the functional benefits from these populations are limited owing to high rates of apoptosis and poor proliferation\textsuperscript{49}. Fortunately, it has been shown that transplanted NSPCs can differentiate to oligodendrocytes in the injured spinal cord to remyelinate denuded axons\textsuperscript{56,59} and concurrently promote preservation of endogenous myelin\textsuperscript{50,60}. Importantly, we have found that this neurobehavioral effect is lost when adult NPCs are derived from myelin basic protein (MBP)-deficient \textit{Shiverer} mice incapable of producing functional myelin\textsuperscript{61,62}. Recognizing these preclinical discoveries, Lineage Cell Therapeutics is currently undertaking a phase I/II clinical trial for SCI employing human ESC-derived OPCs (clinicaltrials.gov identifier: NCT02302157). While this is an important first-in-human study, several limitations exist. First, the use of allogeneic ES-derived cells brings ethical, technical, and safety concerns. Human iPSC-derived cells would instead allow for the potential generation of autologous cell lines whilst avoiding ethical issues. Second, the bipotent OPCs cannot efficiently differentiate into neurons and, therefore, have limited potential to restore lost neuronal populations. In contrast, the proportion of mature oligodendrocytes that differentiate from typical tripotent NPCs is low in the injured spinal cord microenvironment, limiting functional recovery. To address this challenge, our lab has generated myelinating oligodendrogenic tripotent NPC (oNPCs)\textsuperscript{53}. ONPCs transplanted into rodents with SCI...
Neural progenitor cells (NPCs) are self-renewing, tripotent cells capable of differentiating into synaptically integrating neurons, myelinating oligodendrocytes, and supportive astrocytes. NPCs can be derived from adult or embryonic tissue sources or pluripotent cells like embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs).

The transplanted cells also showed significant migration along the rostrocaudal axis and proportionally greater differentiation into oligodendrocytes. The oNPCs promoted perilesional tissue sparing and axonal remyelination, which resulted in motor function recovery. Our findings showed that biasing NPC differentiation along an oligodendroglial lineage represents a promising approach to promote tissue sparing, axonal remyelination, and neural repair post-SCI.

Restoring the interrupted neuronal pathways

NPC-derived neurons have the potential to integrate into endogenous neural networks and re-establish interrupted neuronal pathways. Despite recent progress, the level of graft-host integration and the degree of intra- and trans-segmental relay circuits regenerated by the transplanted neurons has been modest. This is partly because of suboptimal differentiation of transplanted NPCs in the injured cord microenvironment to non-neuronal cells and partly because of the difference in the identity of transplanted NPCs within the spinal cord niche. Spinal cord trauma initiates a cascade of cellular and molecular changes that drastically alter the composition of factors and extracellular matrix proteins in the local niche. These perturbations affect the fate determination of transplanted cells and impair their ability to effectively integrate with host tissue. In the post-injury niche, transplanted tripotent NPCs predominantly differentiate to astrocytes. When endogenous adult NPCs proliferate in response to injury, the vast majority of the newly generated cells are glial fibrillary acidic protein-positive (GFAP+) astrocytes. Similarly, when different types of NPCs are transplanted to a lesioned spinal cord microenvironment, they mainly differentiate into cells with an astrocytic phenotype. Although differentiation to astrocytes may be important for regeneration to improve functional recovery after transplantation, it is important that NPC grafts can also differentiate into synaptically integrating neurons.

In addition to suboptimal differentiation, another reason for the poor integration of transplanted cells is a mismatch in cell identity. Native NPCs, along the entire rostrocaudal neural axis, possess a unique region-specific identity (e.g. forebrain, midbrain, cervical, thoracic, etc.) which is accompanied by distinct neural differentiation in terms of channel composition, axonal projection pattern, and neurotransmitter phenotype. These distinct characteristics allow proper integration during development and in adulthood. Most of the NPCs currently used in preclinical and clinical studies possess a cortical brain identity, which is poorly suited for the spinal cord niche. These cells terminally differentiate into neuronal cell subtypes (e.g. cortical, subcortical, or deep nuclear neurons), which do...
following a T9 contusive injury, rats undergoing treadmill training paradigms to improve the SCI study model and simulate clinical conditions more closely 

strategies, studies of combinatorial treatments are still rare. 

tially synergistic mechanisms with other regenerative treatment alone are often limited. Despite the prevalence of rehabilitation training in clinical treatment protocols for SCI and its potential complexity warrants the development of combinatorial and personalized treatment strategies. Over the years, multiple combinatorial approaches involving gene and cell therapies have been investigated for SCI. Here, we review the most recent findings employing rehabilitation training, neuromodulation, and biomaterials to enhance regenerative treatments and ultimately SCI recovery.

Rehabilitation training is the cornerstone of clinical interventions for SCI and has been demonstrated to improve the functional recovery of people with SCI. Mechanisms behind the obtained recovery include the upregulation of neurotrophins (e.g., BDNF), activity-dependent neuroplastic changes of the spared networks, increased regeneration, and axonal sprouting. While rehabilitation offers functional benefits to individuals with SCI, improvements with this intervention alone are often limited. Despite the prevalence of rehabilitation training in clinical treatment protocols for SCI and its potentially synergistic mechanisms with other regenerative treatment strategies, studies of combinatorial treatments are still rare. Future preclinical studies should incorporate rehabilitation training paradigms to improve the SCI study model and simulate clinical conditions more closely.

Recently, a few studies have investigated combined rehabilitation and stem cell transplantation. One study demonstrated that following a T9 contusive injury, rats undergoing treadmill training (TT) and transplantation of NPCs showed superior functional recovery, graft survival, and remyelination when treatments were combined. In another study, following a T9 contusion injury in rats, transplantation of NPCs in combination with TT and ChABC enhanced functional recovery in the chronic phase of injury. Further investigations are needed to optimize such combinatorial treatment strategies.

Biomaterial scaffolds
Biomaterial scaffolds have long been investigated as tools for the localized and prolonged delivery of drugs, bridging the injury site with a hospitable microenvironment for the regeneration of endogenous networks, and the introduction of exogenous stem cells into a spinal microenvironment promoting cell survival, growth, and plasticity. A large range of biomaterials have been investigated for these goals such as the fibrin matrix, hyaluronan methylcellulose (HAMC), and polyethylene glycol–gelatin methacrylate (PEG–GelMA).

In applications related to stem cell therapies, design parameters such as the material, geometrical dimensions, shape, and mechanical properties of the scaffold and the scaffold–stem cell interactions can influence the outcome. For instance, Leipzig and Shoichet investigated the effect of scaffold stiffness on the differentiation profile and proliferation of NPCs. They demonstrated that cultures in scaffolds with high stiffness were more oligodendrogenic compared with soft scaffolds that favored astrocytic and neuronal fates. Recent advances in the 3D printing of biomaterial scaffolds have made their precise morphological design possible. For instance, 3D-printed PEG–GelMA scaffolds can mimic the spinal cord morphology with microchannels located in its white matter region. Transplantation of such scaffolds seeded with NPCs into the spinal cord of rats with transection SCIs resulted in the columnar growth of exogenous NPC axons throughout the scaffold microchannels. Evidence of endogenous axonal growth into the scaffold was also reported.

Biomaterials are also promising tools for combining appropriate cell and drug treatment strategies. For instance, the combined transplantation of olfactory ensheathing cells and a bridge scaffold containing Schwann cells along with intrathecal administration of ChABC resulted in greater functional recovery compared to cell transplantation alone. These functional improvements were evident by both behavioral and neuroanatomical assessments. More recently, Nori and colleagues combined the transplantation of oligodendroglialogenic NPCs with biomaterial delivery of ChABC in a clip-contusion model of chronic thoracic SCI in rats. In this study, ChABC was delivered using a crosslinked methylcellulose (XMC) hydrogel capable of sustained ChABC release for 7 days. This combinatorial strategy led to superior functional recovery from SCI compared with stem cell transplantation alone. Fuhrmann and colleagues took this a step further and used biomaterials to deliver both stem cells and drugs into the spinal cord parenchyma. In this study, OPCs were delivered using injectable methylcellulose hydrogels conjugated with platelet-derived growth factor-A (PDGF-A) in a clip compression rat model of thoracic SCI. This strategy resulted in enhanced early survival of the grafted cells.
Conclusions
The rate of SCI has been on the rise in the past few decades, and this condition currently constitutes the second leading cause of paralysis worldwide. Restoring sensorimotor and autonomic function for people with SCI can significantly improve their quality of life. Recent innovations in effective gene- and cell-based therapies have vastly improved the technical ability to induce regeneration in the spinal cord. However, successful clinical translation of these techniques requires further optimization. Combinatorial strategies can greatly enhance the implementation and functional outcomes of these regenerative treatments.

Abbreviations
AAV, adeno-associated viral; ChABC, chondroitinase ABC; CNTF, Ciliary-derived neurotrophic factor; CSPG, chondroitin sulfate proteoglycans; EGF, Epidermal growth factor; ESC, embryonic stem cell; FGF2, Fibroblast growth factor 2; GDNF, glial-derived neurotrophic factor; HAMC, Hyaluronan methylcellulose; hiPSC, human induced pluripotent stem cell; IGF1, Insulin-like growth factor 1; KCC2, chloride potassium symporter 5; KLF, Krüppel-like factor; Ompg, Oligodendrocyte-myelin glycoprotein; MAG, Myelin-associated glycoprotein; MBP, Myelin basic protein; NG2, Neural/Glial antigen 2; Nogo A, Neurite outgrowth inhibitor A; NPC, neural progenitor cell; NSPC, neural stem/progenitor cell; oNPC, oligodendrogenic tripotent neural progenitor cell; OPC, oligodendrocyte progenitor cells; PEG–GelMA, polyethylene glycol–gelatin methacrylate; PTEN, phosphatase and tensin homolog; SCI, spinal cord injury; SDH, staggered double hemisection; shRNA, short-hairpin RNA; XMC: Crosslinked methylcellulose.

Acknowledgements
The figures were created with BioRender.com. The authors would like to thank Dr. Tim Worden (University Health Network, Canada) for copyediting the manuscript.

References
1. James SL, Theadom A, Ellenbogen RG, et al.: Global, regional, and national burden of traumatic brain injury and spinal cord injury, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 2019; 18(1): 56–87. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
2. Ahuja CS, Wilson JR, Nor S, et al.: Traumatic spinal cord injury. Nat Rev Dis Primers. 2017; 3: 17018. Published Abstract | Publisher Full Text
3. Singh A, Tetreault L, Katto-Ryan S, et al.: Global prevalence and incidence of traumatic spinal cord injury. Clin Epidemiol. 2016; 8: 309–314. Published Abstract | Publisher Full Text | Free Full Text
4. National Spinal Cord Injury Statistical Center: Spinal Cord Injury, Facts and Figures at a Glance. University of Alabama at Birmingham. 2020. Reference Source
5. Warren PM, Steiger SC, Dick TE, et al.: Rapid and robust restoration of breathing long after spinal cord injury. Nat Commun. 2018; 9(1): 4843. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
6. Schwab JM, Zhang Y, Kopp MA, et al.: The paradox of chronic neuroinflammation, systemic immune suppression, autoimmunity after traumatic chronic spinal cord injury. Exp Neurol. 2014; 258: 121–9. Published Abstract | Publisher Full Text | Free Full Text
7. Celik EC, Erhan B, Lakse E: The clinical characteristics of neuropathic pain in patients with spinal cord injury. Spinal Cord. 2012; 50(6): 585–9. Published Abstract | Publisher Full Text
8. Sharif H, Hou S: Autonomic dysreflexia: A cardiovascular disorder following spinal cord injury. Neurol Regen Res. 2017; 12(6): 1390–402. Published Abstract | Publisher Full Text | Free Full Text
9. Fleming JC, Norengberg MD, Ramsay DA, et al.: The cellular inflammatory response in human spinal cords after injury. Brain. 2006; 129(Pt 12): 3249–69. Published Abstract | Publisher Full Text | Free Full Text
10. Tator CH, Fehlings MG: Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg. 1991; 75(1): 15–26. Published Abstract | Publisher Full Text | Free Full Text
11. Burda JE, Sofroniew MV: Reactive gliosis and the multicellular response to CNS damage and disease. Neuron. 2014; 81(2): 229–48. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
12. O'Shea TM, Burda JE, Sofroniew MV: Cell biology of spinal cord injury and repair. J Clin Invest. 2017; 127(9): 3259–70. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
13. Sofroniew MV: Dissecting spinal cord regeneration. Nature. 2018; 557(7705): 343–50. Published Abstract | Publisher Full Text | F1000 Recommendation
14. Dias DO, Kim H, Holl D, et al.: Reducing Pericyte-Derived Scarring Promotes Recovery after Spinal Cord Injury. Cell. 2018; 173(1): 153–165.e22. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
15. Anderson MA, Burda JE, Chen Y, et al.: Astrocyte scar formation aids central nervous system axon regeneration. Nature. 2016; 532(7598): 195–200. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
16. Bradbury EJ, Bumsude ER: Moving beyond the glial scar for spinal cord repair. Nat Commun. 2019; 10(1): 3879. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
17. Blesch A, Tuszynski MH: Spinal cord injury: plasticity, regeneration and the challenge of translational drug development. Trends Neurosci. 2009; 32(1): 41–7. Published Abstract | Publisher Full Text | Free Full Text
18. Tsimou M, Dalamagkati K, Seifalian A: Advances in regenerative therapies for spinal cord injury: A biomaterials approach. Neural Regen Res. 2015; 10(5): 726–42. Published Abstract | Publisher Full Text | Free Full Text
19. Schwab ME, Strittmatter SJt: Nogo limits neural plasticity and recovery from injury. Curr Opin Neurobiol. 2014; 27: 53–60. Published Abstract | Publisher Full Text | Free Full Text
20. Jones LL, Yamaguchi Y, Stallicup 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(7): 2792–803. Published Abstract | Publisher Full Text | Free Full Text
21. Forgione N, Fehlings MG: Rho-ROCK inhibition in the treatment of spinal cord injury. World Neurosurg. 2014; 82(3–4): e526–9. Published Abstract | Publisher Full Text | Free Full Text
22. Barnabé-Heider F, Göritz C, Sabelström H, et al.: Glycogen synthase kinase-3β promotes autophagy and cell survival in neurons. EMBO J. 2014; 33(17): 2237–49. Published Abstract | Publisher Full Text | Free Full Text
23. Meletis K, Barnabé-Heider F, Carlén M, et al.: Spinal cord injury reveals multilineage differentiation of ependymal cells. PLoS Biol. 2008; 6(7): e182. Published Abstract | Publisher Full Text | Free Full Text
24. Dias DO, Kwon BK, Okon EB, Plunet W, et al.: NG2 inclusions promote the neuroprotective actions of GDNF in vivo. J Neurosci. 2014; 34(5): 1589–910. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
25. Karon BK, Okon EB, Plunet W, et al.: A systematic review of directly applied biologic therapies for acute spinal cord injury. J Neurotrauma. 2011; 28(8): 1589–910. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
26. Curt A, Hsieh J, Schubert M, et al.: Safety and Preliminary Efficacy of Allogeneic Neural Stem Cell Transplantation in Chronic Spinal Cord Injury: A Translational Phase IIa Trial. 2019. Reference Source
27. Kwon BK, Okon EB, Plunet W, et al.: NG2 inclusions promote the neuroprotective actions of GDNF in vivo. J Neurosci. 2014; 34(5): 1589–910. Published Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation
28. Curt A, Hsieh J, Schubert M, et al.: Safety and Preliminary Efficacy of Allogeneic Neural Stem Cell Transplantation in Chronic Spinal Cord Injury: A Translational Phase IIa Trial. 2019. Reference Source
Cells as a Therapeutic Strategy for Spinal Cord Injury: Opportunities and Challenges. J Clin Med. 2020; 9(11): 37-65. PubMed Abstract | Publisher Full Text | Free Full Text

49. Tetzlaff W, Oken EB, Karimi-Abdolrezaee S, et al.: A systematic review of cellular transplantation therapies for spinal cord injury. J Neurotrauma. 2011; 28(8): 1611–62. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

50. Assink P, Duncan GJ, Piemel JR, et al.: Myelinogenic Plasticity of Oligodendrocyte Precursor Cells following Spinal Cord Contusion Injury. J Neurosci. 2017; 37(6): 6355-68. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

51. Kadoya K, Lu P, Nguyen K, et al.: Spinal cord reconstitution with homologous neural grafts enables robust corticospinal regeneration. Nat Med. 2016; 22(5): 479–87. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

52. 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(4): 789–96. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

53. Nagoshi N, Khazaei M, Ahtiors JE, et al.: Human Spinal Oligodendroglial Neurogenic Progenitor Cells Promote Functional Recovery After Spinal Cord Injury by Axonal Remyelination and Tissue Sparing. Stem Cells Transl Med. 2018; 7(11): 806–18. PubMed Abstract | Publisher Full Text | Free Full Text

54. Lepore AC, Fischer I: Lineage-restricted neural precursors survive, migrate, and differentiate following transplantation into the injured adult spinal cord. Exp Neurol. 2005; 194(1): 40–52. PubMed Abstract | Publisher Full Text | Free Full Text

55. Karimi-Abdolrezaee S, Eftekharpoor E, Wang J, et al.: Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurorecovery after spinal cord injury. J Neurosci. 2008; 26(13): 3377–89. PubMed Abstract | Publisher Full Text | Free Full Text

56. Salewski RP, Mitchell RA, Shen C, et al.: Transplantation of neural stem cells clonally derived from embryonic stem cells promotes recovery after murine spinal cord injury. Stem Cells Dev. 2015; 24(1): 36–50. PubMed Abstract | Editor Full Text | Free Full Text | F1000 Recommendation

57. Adler AF, Lee-Kubli C, Kumamaru H, et al.: Comprehensive Monosynaptic Rabies Virus Mapping of Host Connectivity with Neural Progenitor Grafts after Spinal Cord Injury. Stem Cell Reports. 2017; 8(6): 1525–33. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

58. Hawryluk GW, Sparo S, Chew D, et al.: An examination of the mechanisms by which neural precursors augment recovery following spinal cord injury: a key role for remyelination. Cell Transplant. 2014; 23(3): 365–80. PubMed Abstract | Publisher Full Text

59. Eftekharpoor E, Karimi-Abdolrezaee S, Wang J, et al.: Myelination of congenitally dysmyelinated spinal cord axons by adult neural precursor cells results in formation of nodes of Ranvier and improved axonal conduction. J Neurosci. 2007; 27(13): 3416–28. PubMed Abstract | Publisher Full Text | Free Full Text

60. Salewski RP, Mitchell RA, Li L, et al.: Transplantation of Induced Pluripotent Stem Cell-Derived Neural Stem Cells Mediate Functional Recovery Following Thoracic Spinal Cord Injury Through Remyelination of Axons. Stem Cells Transl Med. 2015; 4(7): 743–54. PubMed Abstract | Publisher Full Text | Free Full Text

61. Hunt M, Lu P, Tuszynski MH: Myelination of axons emerging from neural progenitor grafts after spinal cord injury. Exp Neurol. 2017; 296: 69–73. PubMed Abstract | Publisher Full Text

62. Khazaei M, Ahuja CS, Fehlings MG: Generation of Oligodendroglial Spinal Neural Progenitor Cells From Human Induced Pluripotent Stem Cells. Curr Protoc Stem Cell Biol. 2017; 42(10): 20.2.1-20.2.14. PubMed Abstract | Publisher Full Text

63. Kumamaru H, Saiwai H, Kubota K, et al.: Therapeutic activities of engrafted neural stem/precursor cells are not dormant in the chronically injured spinal cord. Stem Cells. 2013; 31(8): 1535–47. PubMed Abstract | Publisher Full Text

64. Bonner JF, Stewart O: Repair of spinal cord injury with neuronal relays: From feto grafts to neural stem cells. Brain Res. 2015; 1619: 115–23. PubMed Abstract | Publisher Full Text | Free Full Text

65. Tischert A, Heidemann M, Kleinlogl S, et al.: Embryonic Cell Grafts in a Culture Model of Spinal Cord Lesion: Neuronal Relay Formation Is Essential for Functional Regeneration. Stem Cell Reports. 2016; 6(3): 220. PubMed Abstract | Publisher Full Text | Free Full Text

66. Charnakhin M, Eftekharpoor E, Karimi-Abdolrezaee S, et al.: Genome-wide expression profiling of staged response in a spinal cord clip compression injury model. BMC Genomics. 2013; 14: 583. PubMed Abstract | Publisher Full Text | Free Full Text

67. De Biasse A, Knoblaich SM, Di Giovanni S, et al.: Gene expression profiling of experimental traumatic spinal cord injury as a function of distance from
impact site and injury severity. Physiol Genomics. 2005; 22(3): 368–81.

69. Chen J, Leong SY, Schachner M: Differential expression of cell fate determinants in neurons and glial cells of adult mouse spinal cord after compression injury. Eur J Neurosci. 2005; 22(8): 1895–906. PubMed Abstract | Publisher Full Text

70. Dyck SM, Alizadeh A, Santhosh KT, et al.: Chondroitin Sulfate Proteoglycans Negatively Modulate Spinal Cord Neural Precursor Cells by Signaling Through LAR and RPTP and Modulation of the RhoROCK Pathway. Stem Cells. 2015; 33(8): 2550–63. PubMed Abstract | Publisher Full Text

71. Setoguchi T, Nakashima K, Takizawa T, et al.: Treatment of spinal cord injury by transplantation of fetal neural precursor cells engineered to express BMP inhibitor. Exp Neurol. 2004; 189(1): 33–44. PubMed Abstract | Publisher Full Text

72. Wang B, Xiao Z, Chen B, et al.: Nogo-66 promotes the differentiation of neural progenitors into astroglial lineage cells through mTOR-STAT3 pathway. PLoS One. 2008; 3(3): e1856. PubMed Abstract | Publisher Full Text | Free Full Text

73. Namiki J, Tator CH: Cell proliferation and nestin expression in the epidymida of the adult rat spinal cord after injury. J Neuropath Exp Neurol. 1999; 58(5): 489–98. PubMed Abstract | Publisher Full Text

74. Cao QL, Zhang YP, Howard RM, et al.: Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. Exp Neurol. 2001; 167(1): 48–58. PubMed Abstract | Publisher Full Text

75. Faulkner JR, Herrmann JE, Woo MJ, et al.: Reactive astrocytes protect tissue and preserve function after spinal cord injury. J Neuropathol Exp Neurol. 2004; 24(2): 2143–55. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

76. Khazaie M, Ahuja CS, Nakashima H, et al.: GDN stores the fate of neural progenitor grafts by attenuating Notch signals in the injured spinal cord in rodents. Sci Transl Med. 2020; 12(325): eaau3538. PubMed Abstract | Publisher Full Text

77. Kirkey A, Grealish S, Wolf DA, et al.: Generation of regionally specified neural progenitors and functional neurons from human embryonic stem cells under defined conditions. Cell Rep. 2012; 1(6): 702–14. PubMed Abstract | Publisher Full Text

78. Tsuji O, Sugai K, Yamaguchi R, et al.: Concise Review: Laying the Groundwork for a First-In-Human Study of an Induced Pluripotent Stem Cell-Based Intervention for Spinal Cord Injury. Stem Cells. 2019; 37(1): 8–13. PubMed Abstract | Publisher Full Text

79. Butts JC, McCreedy DA, Martinez-Vargas JA, et al.: Differentiation of V2a interneurons from human pluripotent stem cells. Proc Natl Acad Sci U S A. 2017; 114(3): 4969–74. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

80. Zhuludeva LV, Iyer N, Qiang L, et al.: Transplantation of Neural Progenitors and V2a Interneurons after Spinal Cord Injury. J Neurotrauma. 2018; 35(24): 2883–902. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

81. Liu SJ, Wang Q, Tang HH, et al.: Heterogeneity among traumatic spinal cord injuries at the thoracolumbar junction: helping select patients for clinical trials. Spinal Cord. 2019; 57(11): 972–8. PubMed Abstract | Publisher Full Text

82. Behrmann AL, Harkema SJ: Physical rehabilitation as an agent for recovery after spinal cord injury. Phys Med Rehabil Clin N Am. 2007; 18(2): 183–202, v. PubMed Abstract | Publisher Full Text

83. Yang JF, Muesmer KE: Training to achieve over ground walking after spinal cord injury: a review of who, what, when, and how. J Spinal Cord Med. 2013; 36(5): 293–304. PubMed Abstract | Publisher Full Text | Epub Ahead of Print

84. Vayman S, Gomez-Pinilla F: License to run: exercise impacts functional plasticity in the intact and injured central nervous system by using neurotrophins. Neurorehabil Neural Repair. 2005; 19(4): 283–95. PubMed Abstract | Publisher Full Text

85. Fouad K, Tetzlaff W: Rehabilitative training and plasticity following spinal cord injury. Exp Neurol. 2012; 235(1): 91–9. PubMed Abstract | Publisher Full Text

86. Lovely RG, Gregor RJ, Roy RR, et al.: Effects of training on the recovery of full- weight-bearing stepping in the adult spinal cat. Exp Neurol. 1986; 92(2): 421–35. PubMed Abstract | Publisher Full Text

87. Dobkin BH: Neurobiology of rehabilitation. Ann N Y Acad Sci. 2004; 1038: 148–70. PubMed Abstract | Publisher Full Text | Free Full Text

88. Fouad K, Pedersen V, Schwabe ME, et al.: Cervical sprouting of corticospinal fibers after thoracic spinal cord injury accompanies shifts in evoked motor responses. Curr Biol. 2001; 11(22): 1766–70. PubMed Abstract | Publisher Full Text | Free Full Text

89. Torres-ESPN A, Forero J, Ferrich KK, et al.: Eliciting inflammation enables successful rehabilitative training in chronic spinal cord injury. Brain. 2018; 141(7): 1946–62. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

90. Hwang DH, Shin HY, Kwon MJ, et al.: Survival 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(38): 12786–900. PubMed Abstract | Publisher Full Text | Free Full Text

91. Ziemba AM, Gilbert RJ: Biomaterials for Local, Controlled Drug Delivery to the Injured Spinal Cord. Front Pharmacol. 2017; 8: 5. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

92. Yao S, Yu S, Cao Z, et al.: Hierarchically aligned fibrin nanofiber hydrogel accelerated axonal regrowth and locomotor function recovery in rat spinal cord injury. Int J Nanomedicine. 2018; 13: 2983–90. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

93. Yao L, Daly W, Newland B, et al.: Improved axonal regeneration of transplanted spinal cord mediated by multichannel collagen conduits functionalized with neurotrophin-3 gene. Gene Ther. 2013; 20(12): 1149–57. PubMed Abstract | Publisher Full Text

94. Brock JH, Graham L, Staufenberg E, et al.: Bone Marrow Stromal Cell Intraspinal Transplants Fail to Improve Motor Outcomes in a Severe Model of Spinal Cord Injury. J Neurotrauma. 2016; 33(12): 1103–14. PubMed Abstract | Publisher Full Text | Free Full Text

95. Lu P, Wang Y, Graham L, et al.: Long-distance growth and connectivity of neural stem cells after severe spinal cord injury. Cell. 2012; 150(6): 1264–73. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

96. Mezhe AJ, Tam RY, Zahr T, et al.: Repair of the injured spinal cord by transplantation of neural stem cells in a hyaluronan-based hydrogel. Biomaterials. 2013; 34(15): 3775–83. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

97. Koffler J, Zhu W, Ou X, et al.: Biomimetic 3D-printed scaffolds for spinal cord injury repair. Nat Med. 2019; 25(2): 263–9. PubMed Abstract | Publisher Full Text | Free Full Text | F1000 Recommendation

98. Smeal RM, Tresco PA: The influence of substrate curvature on neurite outgrowth is cell type dependent. Exp Neurol. 2008; 213(2): 281–92. PubMed Abstract | Publisher Full Text

99. Lezpiz SG, Schoichet MS: The effect of substrate stiffness on adult neural stem cell behavior. Biomaterials. 2009; 30(36): 8667–78. PubMed Abstract | Publisher Full Text

100. Fouad K, Schnell L, Bunge MB, et al.: Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. J Neurosci. 2005; 25(6): 1169–78. PubMed Abstract | Publisher Full Text | Free Full Text

101. Palkoiska MM, Vulic K, Schoichet MS: Affinity-based release of chondroitinase ABC from a modified methyccellulose hydrogel. J Control Release. 2013; 171(1): 11–6. PubMed Abstract | Publisher Full Text

102. Führmann T, Tam RY, Ballarin B, et al.: Injectable hydrogel promotes early survival of induced pluripotent stem cell-derived oligodendrocytes and attenuates longterm teratoma formation in a spinal cord injury model. Biomaterials. 2016; 83: 23–36. PubMed Abstract | Publisher Full Text
Open Peer Review

Current Peer Review Status: ✓ ✓ ✓

Editorial Note on the Review Process
F1000 Faculty Reviews are written by members of the prestigious F1000 Faculty. They are commissioned and are peer reviewed before publication to ensure that the final, published version is comprehensive and accessible. The reviewers who approved the final version are listed with their names and affiliations.

The reviewers who approved this article are:

**Version 1**

1. **Michael A Lane**
   Division of Respiratory Research, The Marion Murray Spinal Cord Research Center, Drexel University, Philadelphia, PA, USA
   **Competing Interests:** No competing interests were disclosed.

2. **Zhigang He**
   Department of Neurology, Harvard Medical School, Boston, MA, USA
   **Competing Interests:** No competing interests were disclosed.

3. **Elizabeth J Bradbury**
   Institute of Psychiatry, Psychology & Neuroscience, King's College London, London, UK
   **Competing Interests:** No competing interests were disclosed.

The benefits of publishing with F1000Research:

- Your article is published within days, with no editorial bias
- You can publish traditional articles, null/negative results, case reports, data notes and more
- The peer review process is transparent and collaborative
- Your article is indexed in PubMed after passing peer review
- Dedicated customer support at every stage

For pre-submission enquiries, contact research@f1000.com