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Improving the therapeutic efficacy of neural progenitor cell transplantation following spinal cord injury

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Abstract

Introduction—There have been a wide range of preclinical studies testing cellular therapies to repair the injured spinal cord, yet they remain a challenge to translate because of inconsistencies in efficacy, limited number of patients with acute/subacute SCI and the high costs of clinical trials.

Area covered—This paper focusses on the therapeutic potential of neural precursor cells (NPCs) because they can provide the cellular components capable of promoting repair and enhancing functional improvement following spinal cord injury (SCI). The authors discuss the challenges of NPC transplantation with respect to different populations of NPCs of glial and neuronal lineages, the timing of treatment relative to acute and chronic injury, and the progress in ongoing clinical trials.

Expert commentary—Preclinical research will continue to elucidate mechanisms of recovery associated with NPC transplants, including increasing the partnership with related fields such as spinal atrophies and multiple sclerosis. The clinical trials landscape will grow and include both acute and chronic SCI with increased partnership and strengthened communication between biotechnology, government and academia. There will also be growing effort to develop better biomarkers, imaging and outcome measures for detailed assessment of neurological function and measures of quality of life.

Keywords

Spinal cord injury; neural stem cells; neural progenitors; cell transplantation; axon regeneration; myelination; recovery of function

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Declaration of interest

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1. Introduction

Traumatic spinal cord injury (SCI) is associated with a debilitating set of conditions resulting from the acute primary damage and subsequent secondary injury at subacute and chronic stages. Differences in the anatomical location, type, and severity of injury produce outcomes that vary widely across the patient population with respect to motor, sensory, and autonomic dysfunction [1,2]. The pathophysiology of SCI is complex and multifactorial due to the sequence of events that changes temporally and spatially, the variety of cell types affected by the injury and the diversity of connections disrupted by the injury. Without therapeutic intervention, however, the extent of anatomical and functional plasticity is limited and deficits persist [3–5]. There is little or no long-term recovery post-SCI due to limited (i) anatomical plasticity and axonal growth from injured or spared neurons, (ii) functional myelination of demyelinated and regenerating axons, and (iii) effective recruitment of resident neural stem cells, a population that includes a variety of multipotent and lineage-restricted neural progenitors of the adult spinal cord.

There have been a wide range of preclinical and clinical studies investigating a variety of cellular therapies to repair the injured spinal cord such as stem cells, macrophages, and Schwann cells [6–9]. However, none have yet progressed through all three phases of clinical trials or been approved for patient treatment, including autologous activated macrophages [10,11], neural precursor cells (NPCs) [12,13] and autologous Schwann cells (ClinicalTrials.gov NCT01739023). This is due to significant limitations encountered by all cellular therapies, including inconsistency in therapeutic efficacy, which is usually based on rodent models, the logistical considerations in obtaining donor cells that can be reliably and safely stored for clinical use, the limited pool of patients with acute/subacute SCI and the extensive costs of running clinical trials. We focus our review on the therapeutic potential of NPCs to treat SCI. Pioneering studies in the 1980s [14] revealed that transplanting NPC-rich tissues from the developing fetal spinal cord (FSC) could restore continuity across an injury in the adult spinal cord and improve functional outcome. Scientific advances have enabled more recent studies to use genetic modification of NPCs to track distribution following transplantation [15–18], and to optimize *in vitro* [19,20] and cellular engineering [21,22] methods for greater selectivity over donor cell populations.

2. NPC transplantation

NPC transplantation provides a powerful ‘multi-pronged’ tool to therapeutically target pathological processes at all stages during the temporal evolution of SCI [23,24]. Most directly, NPCs provide a source of new cells to substitute for cells lost or damaged post-SCI (including neurons, oligodendrocytes and astrocytes). Complete cell ‘replacement,’ however, remains a very difficult challenge as it requires specific differentiation, and precise anatomical and functional integration that replicates the uninjured spinal cord. Instead, the goal of NPC transplants is typically to provide the cellular and molecular components capable of promoting repair and enhancing functional improvement. NPC transplants possess important beneficial properties that serve this objective. For example, they can (i) restore structural integrity of the injured spinal cord, bridging the injury site and providing a growth permissive substrate for repair [14,25–33], (ii) produce therapeutic factors (e.g.

trophic factors [34]), which can support tissue preservation, promote axonal plasticity and maintain neuronal excitability, and (iii) modulate neuroinflammatory processes [35]. Transplants can also promote axon sprouting and/or regeneration by reducing the growth inhibitory environment of the injured spinal cord [26] and by forming functional relays [15,36].

We propose that NPC transplantation can be an effective strategy for treatment of SCI that targets multiple facets of injury. In a way, NPCs alone represent an intrinsic combinatorial therapy because of the host of therapeutic properties that they can simultaneously provide; therefore, they are well suited to address the challenging multifactorial nature of SCI pathogenesis. This is especially relevant given the lack of therapeutic benefits observed with therapies targeting single mechanisms in clinical trials for several nervous system disorders or injuries. The capacity of NPCs for persistent integration into the CNS also allows for long-term benefits, and the ability to deliver these cells to select locations provides a powerful tool for local manipulation without unwanted side effects associated with more systemic delivery of other therapeutic agents.

Despite its potential, NPC transplantation is also associated with unique challenges that must be addressed to both maximize its effectiveness and assure safety. Some of these include: (i) choice of appropriate population(s) of NPCs with respect to lineage potential and source of tissue (e.g. spinal cord vs. brain, embryonic vs. adult, allogeneic vs. isogeneic); (ii) expansion and storage of sufficient numbers of cells in a uniform manner and without compromising their beneficial properties; (iii) achieving long-term integration, defined differentiation and functional maturation without the generation of unwanted lineages, persistent proliferation or ectopic localization; (iv) need for noninvasive monitoring of transplanted cells; (v) optimization of the timing and method of delivery; (vi) potential need for long-term immunosuppression in allogeneic transplantation; (vii) invasive nature of transplantation (particularly with direct intraparenchymal injection); and (viii) ethical considerations associated with the source material used for obtaining NPCs (e.g. fetal tissue). To improve therapeutic efficacy with NPC transplantation for the treatment of both SCI and other traumatic CNS disorders, greater effort must be made to address these considerations.

3. Timing of treatment: acute vs. chronic

An essential consideration in transplantation of NPCs is the timing of delivery for treating acute vs. chronic pathophysiology, and the logistics of the procedures with respect to the stability of the patients. While a great deal of experimental work has focused on the acute transplantation of NPCs into the injured spinal cord [15,28,33], the time point used do not typically match the time at which injured people can be treated. To treat patients acutely they must first be transported to a trauma center and be medically stable enough to undergo invasive surgical procedures that are usually required for cell delivery [37]. With a more directed focus on regeneration and repair rather than limiting the early stages of inflammation and damage, delayed transplantation of NPCs has shown therapeutic efficacy in either subacute or chronic injury [26,38–40]. The use of NPCs for subacute or chronic injuries also enables treatment of the majority of people, who are already living with SCI. A

significant challenge to treating the chronically injured spinal cord, however, is that more extensive tissue loss and scarring has occurred, and neuroanatomical reorganization and plasticity have altered underlying circuitry. Ongoing research will need to explore the extent of anatomical and functional plasticity that can occur within a variety of motor and sensory systems and how transplanted NPCs can be used to facilitate delayed repair and contribute to additional plasticity.

4. Using defined populations of progenitors

NPC transplants can be derived from a variety of sources, which define the practical issues related to the isolation and banking of the cells, the use of immunosuppression protocols, concerns about safety, and the potential therapeutic targets (Figure 1.). For example, the use of cells derived directly from fetal tissue presents logistical challenges of obtaining sufficient cells ready for transplantation and defining the relevant population of cells in the fetal tissue, but these cells allow excellent survival and integration of the transplant. More recently, cells used in preclinical studies have been derived from fetal tissues of transgenic animals with markers such as GFP or alkaline phosphatase (AP), allowing to track the distribution of donor cells post-transplantation. In contrast, cells derived from pluripotent sources (ES/iPS cells) allow unlimited expansion, but need specific differentiation protocols and pose potential safety risks for tumor formation [22,41]. It is also possible to isolate, expand, enrich, and bank specific population of NPCs from adult CNS tissue. In all cases, it is essential to generate the appropriate population of cells and to optimize survival and integration of the transplants. One consideration is the inclusion of therapeutic factors (using scaffolds, genetic modification of donor cells, or cotransplantation with other cell populations) to promote survival and integration, thus increasing the efficacy of the transplant [42], but it is also likely that NPC transplantation will be combined with other treatments such as neuroprotective drugs, exercise or electrical stimulation.

4.1. Oligodendrocyte progenitor cells (OPCs)

OPCs remain a leading therapeutic candidate to generate oligodendrocytes for the myelination of host axons – either spared or regenerating [43,44]. There has therefore been a concerted effort to develop methods to differentiate human ESCs into high-purity human OPCs and test their efficacy in models of SCI [45,46], which resulted in a clinical trial by Geron. Furthermore, studies on OPCs and myelination are common with other demyelinating conditions such as multiple sclerosis and leukodystrophies and it is therefore important to consider related studies for treating myelin pathology and gain insight on the extrinsic and intrinsic factors regulating myelination [47]. However, there is evidence that beside myelination, OPCs can also secrete factors relevant for anatomical repair (myelination *and* axon growth) such as clusterin, MCP-1, TIMP-1 and 2, and ApoE [48]. The resulting efficacy of such transplants may depend on the differentiation and/or purification protocols, the number of cells delivered, and the localization of transplants. The assessment of efficacy, however, remains problematic when using human cells in a rodent model (xenograft) as it requires either the use of extensive immunosuppression (e.g. cyclosporine A, tacrolimus) or immune-deficient animals (e.g. T cell-deficient nude rats). These difficulties raise the question of whether the preclinical evidence for functional

remyelination following myelinating cell engraftment into the injured spinal cord support progression to clinical trials [49], and in general how to generate compelling preclinical evidence. In addition, while there is experimental and clinical evidence for primary demyelination (loss of oligodendrocytes, but preservation of underlying axons), it represents only one part of the ongoing neuropathological sequelae that can be therapeutically targeted [50].

4.2. Neuronal restricted precursors (NRPs)

The challenge of directing the differentiation of multipotent neural stem cells toward a neuronal lineage within the injured adult spinal cord has been long recognized to be related to the non-neurogenic environment (typically promotes differentiation toward an astrocytic lineage). One way to resolve this limitation has been the use of progenitors committed to the neuronal lineage prior to transplantation and thus not dependent on host cues for fate choices. A challenge facing transplantation of cultured NRPs alone into the internal environment of a SCI, however, is survival and differentiation. In contrast, NRPs contained within dissociated, uncultured donor FSC tissue exhibit excellent survival and differentiation, thus directing the search for transplantation protocols comparable to FSC tissue: combined transplantation of NRP with glial restricted precursors (GRPs; see below), the inclusion of growth factors and matrix molecules with transplanted NRPs [38,51], cotransplantation with other cell types (e.g. Schwann cells), or delayed transplantation. The possibility of producing graft-derived neurons means that rather than serving as a bridge to repair, transplanted cells can form neuronal relays across the injury site to reconnect disrupted ascending or descending tracts [36], analogous to the polysynaptic circuits – or ‘by-pass’ pathways – that can contribute to neuroplasticity post-SCI. This capability also means that donor neurons can contribute further to ongoing neuronal plasticity within the spinal cord. The ‘relay’ concept began with pioneering FSC studies [14] and continued with NRP/GRP cotransplants and the use of neurotrophic gradients to guide donor axons to their targets [15]. This strategy continues to evolve and has been demonstrated for sensory, motor systems [28,52]. However, anatomical or even electrophysiological evidence does not necessarily translate into optimal functional improvement. There remains a need for greater control of the phenotypic fate of donor NRPs that are capable of more directed growth and synaptic integration. For example, strategies to address this issue include using specific population of neurons with the best properties to form functional connections (e.g. specific subsets of spinal interneurons), adding critical factors or non-neural cellular elements not yet considered, guiding the migration or axonal outgrowth of the transplanted cells toward the target, or combining the transplantation with rehabilitative strategies to facilitate the formation of synaptic connections associated with improved function.

4.3. Astrocytes

Astrocytes play important roles during nervous system development by supporting the formation and function of synapses, modulating the blood–brain barrier (BBB), and regulating metabolic and trophic activity [53]. These properties provide the rationale for using GRPs isolated from the FSC or from pluripotent stem cells to generate immature astrocytes that can stimulate host axon regeneration, promote synapse formation, and modulate the inflammatory environment of the injured spinal cord. Indeed, GRP transplants

have been used as a therapeutic platform in SCI models to promote neuroprotection [54,55] and axon growth [56], to reduce glial scarring [57], and in combination with trophic molecules to enhance migration outside the injury and serve to guide axon growth [58].

Importantly, astrocytes also provide a host of critical functions in the adult CNS, including control of extracellular ionic and neurotransmitter homeostasis via expression of membrane transporter systems, active regulation in synaptic transmission as part of the tri-partite synapse, delivery of energy substrates to neurons, regulation of blood vessel dynamics and BBB properties, expression of neurotrophic factors, and production of extracellular matrix molecules, amongst a number of other roles. It will be important to develop NPC transplantation-based strategies aimed at maintaining these key astrocyte functions after SCI [59]. A number of studies have already begun to address this issue in SCI animal models with success by targeting, for example, restoration of astrocyte glutamate transporter expression using astrocyte progenitor transplants [54,60].

Interestingly, our understanding of astrocyte biology is rapidly advancing to appreciate the importance of astrocyte heterogeneity in the adult CNS with respect to developmental origin, morphology, gene expression profile, cell–cell interactions and function [61]. But not much is known about how this heterogeneity plays into pathogenesis and recovery in diseases such as SCI. It may be necessary to account for this heterogeneity in astrocyte-targeted transplant strategies by using appropriate subtypes of astrocyte progenitors, similar to considerations made when attempting to replace neuronal subpopulations. It will also be important to assure proper maturation of the specific types of astrocytes [54,60] with markers other than just GFAP. In addition, these astrocytes will need to develop appropriate cell–cell interactions with neurons, oligodendrocytes, microglia, and cells associated with the BBB to best achieve restoration of CNS function.

There is now a more nuanced view of astrogliosis and a better appreciation that reactive astrocytes are not just harmful, but play important protective functions by, for example, preventing secondary expansion of the lesion [62] via their intrinsic ability to limit inflammatory cell infiltration [63]. At the same time, these astrocytes may also limit axonal circuit reorganization via the expression of molecules that are inhibitory to axonal growth [64]. Importantly, the response to SCI is not uniform across the astrocyte population; instead, astrocytes respond differentially to the insult based on proximity, timing and a number of other factors that we currently do not fully understand [65]. It will be critical to factor this complexity into NPC transplant strategies aimed at targeting astrocytes.

Last, caution should be exercised when designing astrocyte-focused NPC transplantation approaches. Some studies have demonstrated that such transplants can produce unwanted side effects such as heightened pain transmission in animal models of SCI [66,67], but other studies with astrocyte progenitor transplants have not shown negative effects on pain behavior [55] and have in some instances demonstrated benefit by reducing neuropathic pain after SCI [68]. At any case, considerations such as activation state of the transplanted cells and their potential expression of nociceptive signaling molecules should be factored into design of the transplantation paradigm.

5. Conclusions

The unique significance of NPCs in the context of cellular therapy is their potential to replace cells lost or damaged following SCI. For example, OPCs can be used to generate oligodendrocytes for the remyelination of host axons, and NRPs can be used to generate neurons that will form functional relays across the injury. But NPCs can also produce therapeutic factors to support neuroprotection, modulate neuroinflammation processes, and promote axon sprouting and regeneration by reducing the inhibitory environment of the injury. One can therefore consider NPC transplants as a combinatorial therapy because of the multiple therapeutic properties that they can provide to address the multifactorial nature of SCI pathogenesis. The challenges to maximize the effectiveness of NPC transplants and assure their safety will require efficient methods for preparation, expansion, and storage of the appropriate populations of NPCs, improved protocols for achieving long-term integration, defined differentiation and functional maturation without persistent proliferation or ectopic localization, and optimized timing and method of delivery to minimize the invasive nature of transplantation.

The transition from preclinical research to clinical trials has already started, but the debate of how to demonstrate the robustness of cellular therapies in preclinical studies continues with respect to the use of multiple animal and injury models, the need for independent replication, and defining what is meant by meaningful efficacy. Indeed, there is a concerted effort to develop better biomarkers and outcome measures for detailed assessment of neurological function and measures of quality of life. In the meantime, as the clinical trials landscape grows and struggles with administrative, technical and financial challenges, communication and partnership between academic institutions, biotechnology, and government needs to be strengthened.

6. Expert commentary

Transplanted NPCs have been used in many preclinical studies to treat SCI across a range of animal and injury models [69]. Collectively, preclinical research has shown that donor NPCs survive, integrate with host spinal cord, and can improve functional outcome. This led to the bench-to-bedside translation of NPC transplantation into clinical trials of acute and chronic SCI by StemCell Inc., NeuralStem, Geron and Asterias. While the cost-benefit ratio of developing and testing treatments for SCI – which is considered an ‘orphan disease’ – limits the transition into clinical trials, improved therapeutic efficacy underscores the importance of financial support from federal/state governments, private foundations, industry and academic institutions for ongoing work. It is expected that academia will remain focused on preclinical studies that takes advantage of their basic science expertise, while bio-technology companies and clinical centers will design clinical trials focused on their expertise in clinical grade cell production and management skills.

Most clinical trials are initiated in patients during the acute or ‘subacute’ stages and then expand the treatment window. For example, the original Geron clinical trial tested transplantation of OPCs into patients 7–14 days post-injury, while Asterias has expanded this treatment window to 14–30 days post-injury in the SCiStar trial. Using a different

population of NPCs, StemCells Inc. extended the treatment window to chronic injury, transplanting 1–2 years post-injury in the Pathway™ trial that was, however, recently terminated. It is worth noting that this was not the first attempt to transplant NPCs into the chronically injured spinal cord. In 1997, researchers at the University of Florida transplanted developing human spinal cord tissues into nine patients with post-traumatic syringomyelia [12,13]. This study provided initial clinical evidence for the safety and feasibility of intraspinal NPC transplantation.

7. Five-year view

A consistent preclinical finding with NPC transplantation studies in SCI has been that functional recovery in transplant recipients is not complete, and there can be variability in both anatomical and functional outcomes. Thus, an important goal for future research is to reduce this variability and optimize the extent of functional recovery that can be achieved. Combining NPC transplantation with other therapies may help achieve this. For example, increasing activity within the target motor or sensory systems using rehabilitative training or electrical stimulation may help direct and strengthen appropriate host-donor-host neural circuitry [70]. It is also likely that different motor and sensory systems will benefit from distinctive neuronal and glial progenitor subtypes (e.g. upper extremity motor function may benefit from ipsilaterally projecting, excitatory spinal interneuron progenitors, while treatment of chronic pain may benefit from transplantation of inhibitory interneuronal progenitors). Thus, refining the subtypes of donor neuronal and glial progenitor cells being used for transplantation may also reduce potential variability among recipients [22]. Finally, with mounting evidence for improved functional outcome following NPC transplantation, we predict that there will be considerable progress in translation to clinical trials (<http://www.spinalcord.org/spinal-cord-injury-clinical-trials/>). As technological advances enable greater selection of specific neuronal and glial subtypes, and improved combinatorial strategies with NPC transplantation, we can expect significant improvement in long-term functional outcome after SCI.

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Key issues

- The use of neural precursor cells (NPCs) for cell therapy presents unique advantages because these cells can provide the cellular and molecular components capable of promoting repair and enhancing functional improvement following spinal cord injury (SCI).
- The strategies related to NPC transplantation depend of the use of the specific populations of NPCs of glial and neuronal lineages and the timing of treatment relative to acute and chronic injury.
- Oligodendrocyte progenitor cells (OPC) emerged as a leading therapeutic candidate to promote remyelination of host axons facilitated by the development of methods to differentiate human ESCs into human OPCs, which resulted in a clinical trial by Geron.
- Neuronal restricted precursors (NRP) have been used to obtain cells of neuronal lineage, but transplantation of these cells to form functional relays across the injured spinal cord is still facing challenges related to cell survival and differentiation of specific neuronal phenotypes. However, considerable progress has made in restoring connectivity in both sensory and motors systems.
- The important roles of astrocytes during development and in the adult CNS provide the rationale for using glial restricted precursors (GRPs) isolated from the fetal spinal cord or from pluripotent stem cells to generate immature astrocytes that can stimulate host axon regeneration, promote synapse formation and modulate the inflammatory environment of the injured spinal cord.
- Despite the remarkable progress in research testing a variety of NPCs to repair the injured spinal cord, there are still significant challenges in translating these strategies to the clinics.
- The difficulties in designing clinical trials based on data derived from animal models include inconsistencies in efficacy, difficulties in obtaining reliable and safe sources of donor cells, limited number of patients with acute/subacute SCI and the high costs of clinical trials.
- We anticipate that future research will continue to elucidate mechanisms of recovery associated with NPC transplants and increased collaboration with related fields such as spinal atrophies and multiple sclerosis.
- There will also be growing effort to develop better biomarkers, imaging and outcome measures for detailed assessment of neurological function and measures of quality of life.

- It is likely that the clinical trials landscape will grow and include both acute and chronic SCI with better partnership between biotechnology, government and academia.

Neural Precursor Cells (NPCs) for Spinal Repair

Figure 1. This diagram outlines the basic principles for obtaining and processing neural precursor cells for transplantation

1) Donor cells can be derived from a variety of sources including (a) cells obtained directly from embryonic/fetal or adult neural tissues, which are dissected and dissociated into neural precursors and multipotent stem cells; (b) embryonic stem (ES) cells (pluripotent) and non-neural stem cells or (c) induced pluripotent stem (iPS) cells, which need to be differentiated into neural precursors; (d) somatic cells that can be de-differentiated into iPS cells or directly converted into neural precursors by trans-differentiation. 2) Each of these cell types is then cultured and can be (a) expanded using appropriate growth factors, (b) used to generate a cell line, (c) genetically modified to enhance their therapeutic potential, or (d) used to select distinct subpopulations of glial or neuronal progenitors to increase the purity of the cells of interest such as oligodendrocyte progenitor cells (OPC) or specific types of neuronal progenitors. Cells can be frozen down and stored long-term at any of these stages to create stock for future transplantation experiments. 3) The cells of choice are taken from the frozen stocks, prepared for treatment and injection into the injured spinal cord.