JOURNAL OF NEUROTRAUMA 28:1611–1682 (August 2011)

DOI: 10.1089/neu.2009.1177

A Systematic Review of Cellular Transplantation Therapies for Spinal Cord Injury

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Abstract

Cell transplantation therapies have become a major focus in pre-clinical research as a promising strategy for the treatment of spinal cord injury (SCI). In this article, we systematically review the available pre-clinical literature on the most commonly used cell types in order to assess the body of evidence that may support their translation to human SCI patients. These cell types include Schwann cells, olfactory ensheathing glial cells, embryonic and adult neural stem/progenitor cells, fate-restricted neural/glial precursor cells, and bone-marrow stromal cells. Studies were included for review only if they described the transplantation of the cell substrate into an *in-vivo* model of traumatic SCI, induced either bluntly or sharply. Using these inclusion criteria, 162 studies were identified and reviewed in detail, emphasizing their behavioral effects (although not limiting the scope of the discussion to behavioral effects alone). Significant differences between cells of the same "type" exist based on the species and age of donor, as well as culture conditions and mode of delivery. Many of these studies used cell transplantations in combination with other strategies. The systematic review makes it very apparent that cells derived from rodent sources have been the most extensively studied, while only 19 studies reported the transplantation of human cells, nine of which utilized bone-marrow stromal cells. Similarly, the vast majority of studies have been conducted in rodent models of injury, and few studies have investigated cell transplantation in larger mammals or primates. With respect to the timing of intervention, nearly all of the studies reviewed were conducted with transplantations occurring subacutely and acutely, while chronic treatments were rare and often failed to yield functional benefits.

Key words: animal model; cell transplantation therapies; spinal cord injury; systematic review; translational research

Introduction

Cellular transplantations for the treatment of spinal cord injuries (SCI) have been the subject of many pre-clinical studies over the past two decades. Various cell types have been championed based on their potential to form myelin, promote and guide axonal growth, and bridge the site of injury. In addition, it cannot be overlooked that many cells secrete trophic factors, which may have neuroprotective effects and/or promote plasticity in the spared spinal cord. Hence, the beneficial effects of these cellular therapies are multifactorial and often difficult to attribute to one single mechanism.

The purpose of this review is to describe, for the most-studied and best-understood cell types used in SCI research, the current body of pre-clinical literature that might support the translation of such treatments into human clinical trials. Given the explosion of interest in therapeutic approaches to SCI and the large number of cell candidates that are reportedly beneficial in animal models of SCI, it is difficult – if not

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impossible – to cover the field comprehensively. We felt a need to limit this review to the "best studied cell types," since unlike a specific pharmacologic agent, a given "cell type" can vary considerably from laboratory to laboratory due to differences in the source materials (age, gender, and species from which the cells are taken, or progenitors from which the cells are generated), cell purity and contamination with other cell types, culture conditions (such as number of passages), and variability of media used – to list only a few confounding factors. Hence, a "cell type" has become an umbrella term for several subtypes of cells, and a larger body of data from several laboratories is needed to build consensus regarding the identity, benefits, risks, and translational potential of a given cell as a therapeutic candidate for SCI.

Most cell transplantations are delivered directly into the site of injury or adjacent to it by injecting a few microliters of cell suspension (with several hundred thousand cells) via fine needles or glass capillaries. Attempts have been made to deliver cell substrates to the injured cord via intrathecal injection or even systemically via intravenous infusions. With a few exceptions, rodent models of SCI are used, and the transplantation is typically performed 1–2 weeks after the injury (herein referred to as "subacute" treatment, since transplantations performed immediately after injury – "acutely" – generally yield poor results due to the robust inflammatory response initiated at the time of injury). Only a few chronic studies have been reported, which is a disquieting issue for patients with chronic SCI who are often the most ardent consumers of information regarding cell transplant technologies. While the demonstration of neurologic benefit in rodent models is viewed as evidence that the cell therapy in question may have a "therapeutic" potential in human SCI, it is important to point out that the majority of such studies employ rodent or mouse cells that would be impossible to implant into humans. The number of studies in which human cells are actually tested in such rodent models is remarkably low.

Our strategy, therefore, was to select cell therapies that have reasonable "translational potential" by virtue of the fact that they had been under extensive pre-clinical investigation. While it was not the intention to attempt to cover the entire SCI transplantation field, our goal was to apply the tenets of systematic review to the specific cell therapies that met these conditions. By performing a systematic review of these cellular therapies, we hoped to provide the field with an overview of the body of pre-clinical evidence that supports (or fails to support) the translation of the therapy into human trials.

Methodology

In the summer of 2008, a PubMed search was conducted on "spinal cord injury" and the cell type of interest (e.g., "Schwann cell" or "neural stem cell"). We performed the PubMed search with the "cell type," recognizing that this would be an umbrella search term for possible subtypes (e.g., "neural stem cells," which might come from adult or embryonic sources).

From the list of studies generated through this fairly indiscriminant search, we applied the following criteria to systematically review the pre-clinical literature on these therapies. The inclusion criteria for these studies were:

- Studies that evaluated the cell therapy in an *in-vivo* model of traumatic SCI. Such models employed either blunt contusion or compression SCI injuries (e.g., weight drop, force- or displacement-controlled electromagnetic impactors, clip compression, defined weight placements, balloon compression) or sharp injuries (e.g., full transection, partial section, as well as electrolytic lesions).
- Studies that included a control group for the cell transplantation experiment.
- The presence of at least two peer-reviewed publications available on the "cell type" from independent laboratories.

Exclusion criteria for this systematic review were:

- Studies using non-traumatic local or global ischemia models, photothrombotic models, demyelination models.
- Studies of injury to the cauda equina or conus.
- Studies of root avulsions or injuries to the dorsal root entry zone.
- Studies that evaluated the cell therapy exclusively *in vitro*.
- Studies with $n = 3$ or fewer animals in a rodent experiment.
- Studies with reportedly greater than >30% loss of animals.
- Studies with fewer than 7 days survival time.
- Studies in which there were single reports from one laboratory only on a given "umbrella cell type."
- Studies describing the experimental application of a cell substrate into human SCI patients. As this was a review of pre-clinical literature, such human studies, while important, could not be included.

The data from those studies that fitted the criteria were then extracted into a table format to depict the animal model, injury model, the treatment's dose and timing, the experimental groups tested in the study and the "*n* per group", and the reported behavioral and non-behavioral outcomes (e.g., histological, biochemical, or physiologic outcomes). A summary statement about the body of literature was then generated.

Results

Using this selection process, we identified the following cell "types" and grouped the studies according to the following "umbrella" cell type: Schwann cells, olfactory ensheathing glial cells, neural stem/progenitor cells (adult and embryonic), mesenchymal stem cells (most from bone marrow). The heterogeneity of each cell type is reflected in the tables, as the studies within each cell type were further organized according to the origin of the cells, which represents arguably the largest confounding factor in interpreting the translational potential of the cell type (Table 1).

The PubMed searches on these therapies were conducted in the spring/summer of 2008 by SCI researchers across Canada (plus one from the United States). By applying the previously described criteria (essentially, *in-vivo* animal studies utilizing a traumatic model of SCI), the following publications were selected, and the tables for each of these respective cell therapies are listed below.

Schwann cells and their combinations (Table 2)

Schwann cells (SCs) are the myelin-forming cells of the peripheral nervous system, and have been shown not only to myelinate (remyelinate) axons after transplantation into the

Table 1. Types of Cell Transplants

Cell types	*Cell subtypes (based on source – species – age)*	*Number of studies*
Schwann Cells (SCs)	1. Schwann cells from nerves of humans	2
n = 43	2. Schwann cells from nerves of adult rat	39
	3. Schwann cells from nerves of newborn rodents	4
	4. Schwann cells derived from other sources (Skin, bone marrow)	2
Olfactory Ensheathing Cells (OECs)	1. OECs (or OEC-like cells) from humans	1
N = 26	2. OECs form large mammals primates & pigs	2
	3. OECs from olfactory bulb of adult rodents	14
	4. OECs from olfactory bulb and lamina propria of prenatal or newborn rodents	6
	5. OECs from pieces of lamina propria (olfactory nasal mucosa) of adult rats	3
	6. OECs from immortalized cell lines	1
Neural Stem/Progenitor Cells (NSPCs)	1. from prenatal/embryonic humans (heNSPCs)	1
n = 37	2. from human immortalized cell lines	4
	3. from prenatal/embryonic rodents (reNSPCs)	12
	4. from neonatal rodents	1
	5. from adult rodents	12
	6. from rodent immortalized cell lines	5
	7. from embryonic stem cells (ESCs)	2
Fate-Restricted Neural and Glial Precursors (NRPs and GRPs)	1. neural restricted precursors	1
	2. glial restricted precursors	4
	3. combination of neural and glial restricted precursors	4
n = 13	4. oligodendrocyte precursors from newborn rodents	2
	5. oligodendrocyte precursors from human embryonic stem cells (ESCs)	2
Bone Marrow Stromal Cells (BMSCs)	1. human BMSCs	9
	2. primate and pig BMSCs (ie. large animal)	3
n = 43	3. rodent BMSCs	31

injured spinal cord but also to form a permissive substrate for regenerating axons, as reported in many of the studies reviewed here.

Of all the cell types examined in the context of this review, SCs have the longest history of transplantation, with the first experiment involving the transplantation of purified SCs occurring in 1981 (Duncan et al., 1981). Much of the early work understanding the basic biology of SC transplantation involved transplanting SCs into the brain and spinal cord in models of demyelination, and is not discussed here (for a review, see Duncan and Milward, 1995). These early transplant studies demonstrated the ability of SCs to myelinate demyelinated CNS axons, as well as the regenerative ability of PNS axons, which made SCs a cell type of interest for SCI injury repair. More recently, it has been recognized that cell transplants (SCs but also OEG and BMSCs) facilitate the invasion of host SCs into the injured spinal cord (Biernaskie et al., 2007; Hill et al., 2006). This invasion of endogenous cells results in a transplant that is a mixture of transplanted cells and host SCs, and suggests that host SCs may contribute to the recovery observed in such transplants.

Most of the studies reviewed were performed with adult rodent (mostly rat) nerve-derived SCs ($n = 35$). Thirty-two of these studies inflicted injuries to the spinal cord at mid to low thoracic levels, and employed blunt contusion/compression type injuries ($n = 11$) or full ($n = 14$) and partial ($n = 8$) transection injuries. Experiments employing full or partial transection injuries have been used in combination with matrix filled channels to examine the ability of SCs to promote CNS axonal regeneration. A number of these studies clearly demonstrated that SCs are very good at enhancing the regeneration of sensory axons from the dorsal root ganglia, as well as propriospinal axons adjacent to the injury site. These studies also highlight the limits of SC transplants, in that SCs alone (at least in complete injury models) are not sufficient to promote regeneration of brainstem spinal axons, nor do they permit axons that enter SCs grafts to exit and reenter the host spinal cord. As a result, there has been demonstrable interest in enhancing the therapeutic utility of SCs by using them in combination with other co-treatments such as neuroprotective agents, with other cell substrates, or after transduction with growth factor expression vectors. All of these studies were carried out in rats.

Clinical translation emphasizes the need for the pre-clinical demonstration of behavioral benefits, and we emphasize this aspect in greater details in this summary below. Of these 35 publications with adult nerve derived SCs, 10 performed behavioral analyses using the Basso, Beattie, Bresnahan (BBB) open field locomotion scale: six after blunt lesions and four after full transection. Of the five studies involving thoracic contusion injury in which a comparison with injury alone can be made, two studies reported significant behavioral benefits in open field locomotion after SC transplantation alone. These included a report by Takami and colleagues (2002) in a subacute setting and a study by Barakat and colleagues (2005) in an 8-week chronic contusion injury (the only chronic study in

Table 2. Schwann Cells

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
1a. Schwann Cells Derived From Human Nerve				
Guest *Exp Neurol* 1997	*Model:* Athymic female nude rats (Hsd:RH-mu/mu) 165–185 g *Injury:* TB Tx 4–5 mm segment of spinal cord removed	■ Human Nerve-Derived SCs (120×10^6/ml) from peripheral nerve and cauda equina of 9 donors (age 2–53 yrs) in Matrigel (MG) @ 0 hr PI □ PAN/PVC channels. □ MP, 30 mg/kg, i.v. to all animals @ 5 min + 2 hr + 4 hr **ACUTE**	SCI + MG cable + 1. SCs with PAN/PVC channel n=18 2. SCs with PAN/PVC channel capped distally n=19 3. SCs n=7 Survival: 6 wk	*Histology:* Grafts without channels had sign. larger cross sectional areas and more myelinated fibers than grafts in channels (1.53 vs. 0.95 mm^2), but the number of myelinated fibers in the middle of the grafts did not sign. differ (channels: 1442 vs. no channels: 2129). ■ Channels likely decrease the invasion of connective tissue. ■ 5-HT+, DBH+ axons, CGRP+ were present within the grafts but did not exit −1% of anterogradely traced propriospinal axons exited the grafts. *Behavior:* Bridging grafts had sig. better BBB scores (8.2) than capped SC channel grafts (6.8) @ 6 wk PI; and a small but sig. improvement on inclined plane
1b. Schwann Cells Derived From Human Nerve, With Co-Treatments				
Guest *J Neurosci Res* 1997	*Model:* Athymic female nude rats (Hsd:RH-mu/mu), 145–165 g *Injury:* TB Tx 4–5 mm segment of spinal cord removed	■ Human Nerve-Derived SCs (120×10^6/ml) from peripheral nerve and cauda equina of 9 donors (age 2–53 yrs) in Matrigel (MG) + PAN/PVC channel @ 0 hr PI □ IN-1 antibody via hybridoma or injection of hybridoma supernatant □ Control HRF antibody □ aFGF-fibrin glue (2.1 μg/ml) in the space between channel rostral/caudal spinal cord stumps (5 μl each) @ 0 hr PI; 2nd injection of 10 μl through window in channel @ 10 d PI □ MP, 30 mg/kg, i.v to all animals @ 5 min + 2 hr + 4 hr **ACUTE**	SCI + MG cable + SCs + 1. IN-1 hybridoma, n=8 2. IN-1 injections 2x/d, n=16 3. HRP control Ab injections 2x/d, n=7 4. aFGF-fibrin glue, n=6 5. No additional treatment, n=3 Survival: 5 wk	*Histology:* CST axons showed little sprouting in response to human SC grafts and the grafts did not prevent die-back of CST (consistant with rat SC studies). • IN-1 delivery from hybridoma had no effect, but IN-1 injections resulted in increased sprouting of CST axons (without preventing die-back) • aFGF treatment significantly decreased CST dieback and some CST fibers were observed to enter the SC grafts in some aFGF treated animals. *Behavior:* Not reported

2a. Schwann Cells Derived from Nerves of Adult Rodents *(Blunt Injury Models)*

Martin. *J Neurosci Res* 1996	*Model:* **Adult** Wistar **rats**, 300 g *Injury:* **T8-T10 balloon inflation**, 50 μl×5 min	■ **Adult rat SCs** from DRGs (syngeneic), 100,000 - 150,000 cells/μl: 5 μl into epicenter + 2.5 μl injected into each of the rostral and caudal margins: **@ 0 hr PI** **@ 3 d PI** **@ 10 d PI** **ACUTE & SUBACUTE**	**SCI+** **1**. SC graft at 0 hr n = 16 **2**. SC graf at 3d n = 10 **3**. SC graft at 10d n = 9 **4**. No graft n = 50 Animals survival: < 1 wk, 1 wk to 1 month; or >1 month	*Histology:* **Best SC survival** seen with transplants at **1 or 10 d PI**; poor with 3 d PI transplant • SC transplants reduced cavitation by 3 fold at 4–27 d and by 4.7 fold at 30 - 365 d post-transplant • NF + **axons invaded SC grafts** with majority CGRP + or substance-P + (85%). Massive increase in number of CGRP/SP + fibers in SC grafts vs. injury alone (by 6–15 fold) • **Brainstem-spinal** (TH + , TPH + or 5HT+) and **CST axons don't penetrate the grafts.** *Behavior:* Not reported
Martin *Brain Res Bull* 1993	*Model:* **Adult** Wistar rats, 300 g *Injury:* **T8-T10 balloon inflation**, 50 μl×5 min	■ **Adult rat SCs,** from DRGs (syngeneic**)**, 100,000–150,000 cells injected; some retrovirally expressed LacZ. **@ 2–4 d PI** **@ 1–2 wk PI** **SUBACUTE**	**SCI+** **1**. SCs unlabeled n = 17 **2**. SCs labeled with LacZ n = 3 **3**. Injury only n = 50 (killed at different time points post-injury) Animal survival mostly to 30 d PT; some survived as short as 2 wk, and some as long as 4 months	*Histology:* Cell **survival greatest** when transplanted either **immediately** after injury **or at least 1 week post-injury**. Cell survival reduced when transplanted 2–4 d PI. • Axons found within SC transplants. **Many fibers of peripheral origin** (CGRP + , bombesin (BOM) + , VIP + , SP+). **Few supraspinal fibers** (TH + , tryptophane hydroxylase (TPH) + , 5HT + or CCK+) **or propriospinal** neurons ENK + ,) • In sections with dorsal rootlets, peptide-containing axons growing within grafts appeared to be continuous with dorsal root afferents. *Behavior:* Not reported
Martin *Neurosci. Lett* 1991	*Model:* **Adult** Wistar **rats**, 200 g *Injury:* **T8-T10 balloon inflation,** 50 μl×5 min	■ **Adult rat SCs**; injection of 18,000 or 90,000 cells/μl **@ 0 hr PI**, survived 3 wk or 4 months PI **@ 1 wk PI**, survived 3 wk PI **@ 2 wk PI,** survived 6 wk PI **ACUTE & SUBACUTE**	**SCI+** **1.** Low density SCs, immediate transplantation n = 2 **2.** High density SCs, delayed transplantation n = 2	*Histology:* • Low density cell transplants: SCs visible at the lesion site. • Higher density cell transplants: Whirls of fusiform cells, most were S100 + . Little cavitation compared to injury only and astrogliosis less pronounced. • Numerous **axons** in SC transplants, **primarily sensory** (SP + , CGRP + and VIP + fibers) and **not supraspinal** (TH + , TPH + , 5HT + and CCK+) **or propriospinal** (ENK+). *Behavior:* Not reported
Hill *Glia* 2006	*Model:* Female Fischer **rats**, 160–180 g *Injury:* **T9/10 contusion NYU/MASCIS** 12.5 mm	**SCs** from: ■ **Adult female Fischer rats** ■ **Transgenic adult female Fischer rat**s expressing human placental alkaline phosphatase (SCs-hPAP) Injection of 2×10^6 **@ 10 min PI** or **@ 7 d PI** • Cyclosporine **(CsA)** 10 mg/kg/day **ACUTE & SUBACUTE**	■ **Acute SCI** **1**. hPAP-SCs, n = 15 **2**. hPAP-SCs + CsA, n = 11 **3**. lysed hPAP-SCs, n = 4 **4**. Wildtype SCs n = 2 **5**. Wildtype SCs + CsA n = 2 ■ **Subacute SCI** **6**. hPAP-SCs, n = 2 **7**. hPAP-SCs + CsA :n = 2 ■ **Uninjured:** **8**. hPAP-SC, n = 4 **9**. Wildtype SCs, n = 2 **10**. Lysed hPAP-SCs: n = 2 **11**. No treatment: n = 6 Animals survived 1d, 8d, 14d post-acute and 14d post-subacute	*Histology:* **SCs die early after transplantation** into the uninjured, acutely- or subacutely- injured spinal cord. Death determined by morphological evidence for apoptosis and necrosis and real time PCR for hPAP DNA. •Two phases of transplanted hPAP-SCs death observed: • **early death** after transplantation**; reduced by delaying transplantation.** • **delayed death** after the hPAP + cells have integrated – **preventable by immunosuppression.** •Endogenous SCs (p75 + hPAP-) invade and by 7 days the majority of p75 + cells are hPAP- and thus host derived. *Behavior:* Not reported

(*Continued*)

Table 2. Continued

Reference	*Model/Injury*	*Intervention Intervention timing*	*Experimental groups*	*Outcomes*
Hill *Eur J Neurosci* 2007	*Model:* Female Fischer **rats,** 160–180 g *Injury:* **T9 contusion NYU/MASCIS** 12.5 mm	■ **Adult female Fischer rat SCs** with lentiviral GFP expression, Injections **@ 7 d PI** **SUBACUTE**	**SCI+** **1.** 250,000 SC n = 25 **2.** 500,000 SC n = 33 **3.** 1,000,000 SC n = 36 **4.** 2,000,000 SC n = 33 **5.** Medium injection, n = 16 Survival: 10 min, 6 hr, 24 hr, 3 d, 7 d or 28 d.	*Histologic/Biochemical/Physiologic:* • 80% of transplanted GFP + SCs cells die between 10 min and 7 days (paralleled GFP + DNA levels), independent of the number of transplanted cells. • **SC death** occurs primarily **within the first 24 hr** after transplantation, 6-times **more** SCs died by **necrosis than apoptosis**. SC survival was minimally affected by the time to transplantation, and passage of cells through the injection pipette (<10% of cells were dead) *Behavior:* Not reported
Schaal *Cell Transplant* 2007	*Model;* Female Fischer **rats,** 180–200 g *Injury:* **C5 Midline OSU contusion**, 1.1 mm displacement	■ **Adult Fischer rat SCs** expressing LV-GFP, (2×10^6) Injection **@ 7 d PI** **SUBACUTE**	**SCI+** **1**. SC transplant n = 18 **2**. No treatment n = 18 No injury: **3**. Control n = 16 Survival: 8 wk	*Histology:* **SC transplants promote tissue sparing** • SC transplants had more NeuN + neurons 1.5 - 2.5 mm rostral and caudal to epicenter, more spared fibers in the lateral white matter, and more anterogradely traced reticulospinal axons (10 vs. 1 axons/section). *Behavior:* **SC transplants significantly improved forelimb hang test** (6.1 sec vs. 1.4 sec), **and forelimb grip test** using Meyer Grip Strength Meter (0.42 N · m vs. 0.11N · m). No significant improvement in BBB scores (8.9 for SCs vs. 8 for control) or incline plane test.
Barakat *Cell Transplant* 2005	*Model:* Female Fischer **rats,** 180–200 g *Injury:* **T8 contusion NYU/MASCIS** 25 mm	Injection of: ■ Dissociated **adult Fischer rat SCs** (2×10^6) □ **OEGs** (2×10^6) Injection **@ 8 wk PI** **CHRONIC**	**SCI+** **1**. SCs n = 12 **2**. OEG n = 12 **3**. No treatment n = 12 Survival: 19 wk	*Histology:* **SCs survive better than OEG** (17.1% vs. 2.3) @ 11 wk after transplantation. NF + axons were present in SC grafts and where OEGs did survive NF + axons were observed. • **SC transplants,** but not OEG transplants**, contained sign. more sensory** (CGRP+) axons (SC: 32; OEG: 9; control: 15 axons/section) and **brainstem-spinal** (5HT+) **axons. Neither SCs nor OEGs promoted CST ingrowth.** *Behavior:* **SCs** but not OEGs resulted in **significant BBB improvement** (at 16 wk: SC: 10.2; OEG: 8.5; SCI: 8.5). On footprint analysis, **SCs** but not OEGs resulted in small but **significant improvements in base of support and hindpaw rotation.**
2b. Schwann Cells Derived from Nerves of Adult Rodents + Drugs/Growth Factors ***(Blunt Injury Models)***				
Golden *Exp Neurol* 2007	*Model:* Female Fischer **rats,** 180–200 g *Injury:* **T8 contusion MASCIS/NYU** 12.5 mm	■ Dissociated **adult Fischer rat SCs** (2×10^6) with or without **GFP** or **D-15A** (a molecule with **BDNF and NT-3** activity). Cell infected using adenoassociated virus (AdV) or transduced using lentivirus (LV) encoding GFP or D-15A **@ 7 d PI** **SUBACUTE**	**SCI+** **1.** D15A/GFP LV SCs n = 27 **2**. D15A/GFP AdV SCs n = 27 **3.** GFP-only SCs n = 27 **4.** GFP-only LV SCs n = 27 **5**. Non-transduced SCs n = 27 Survival: 7 wk	*Histology:* Both **D15A SC** transplants had a **higher myelin ratio** than GFP SC transplants and contained **more myelinated axons** (AdV 18,232; LV 26,624) than GFP (AdV 4,718; LV 4,640) or control (5,158) SC transplants. • LV D15A transplants contained significantly **more axons** (72,108 vs. 32,641 - 49,618). **5HT, DBH and CGRP** axon length was **significantly increased** in AdV-D15A SC grafts, but only DBH was significantly increased in LV-D15A SC grafts. **No brainstem spinal** (reticulospinal) **or CST** axon **regeneration** into SC grafts. *Behavior:* **Similar BBB scores for D15A-SC and GFP-SC transplants** (no significant differences).

Pearse *Nature Med* 2004	*Model:* Female Fischer **rats,** 160 - 180 g *Injury:* **T8 contusion MASCIS/NYU** 12.5 mm	■ Dissociated **adult Fischer rat SCs,** injection, 2×10^6 cells **@ 7 d PI** □ **Rolipram (rol, 0.5 mg/kg/day)** continuously infused subcutaneously, starting either **@ 0 hr PI** or **@ 7 d PI** for 2 wk □ **Dibutyryl cyclic AMP (db-cAMP, 50 mM),** two injections 0.25 μl each, rostrally and caudally to injury site @ 7 d PI **SUBACUTE**	**SCI+** **1.** Acute rol + SCs + cAMP **2.** Delayed rol + SCs + cAMP **3.** Acute rol + SCs **4.** SCs + db-cAMP **5.** SCs **6.** Acute rolipram **7.** Injury only Number per group: • Retrograde tracing: n ~ 6 • Immunohistochemistry: n ~ 6 • EM: n ~ 3 • Behavior: n ~ 12	*Histology:* **Acute rol treatments** but not SCs alone **increased white matter sparing.** • Both SC and Acute rolipram **increased the number of peripherally myelinated axons** within the lesion. Enhanced levels of cAMP further increased this number by decreasing the number of unmyelinated axons. • Rolipram treatment (acute and delayed) **promoted brainstem-spinal (5HT+) but not corticospinal axonal growth** onto SC grafts. *Behavior:* • BBB: Only the triple combination of **acute-rol-SC-cAMP significantly increased BBB score** (15) over injury alone (9.5–10) at 8 wk PT (SCs: 10.4; SCs-cAMP: ~12.5; acute rol-SCs: ~12.5; delayed rol-SCs-cAMP: ~12). • Acute rolipram significantly improved footprints and gridwalk
2c. Schwann Cells Derived from Nerves of Adult Rodents + Other Cells (and drugs) ***(Blunt Injury Models)***				
Takami *J Neurosci* 2002	*Model:* Female Fischer **rats,** 160–180 g *Injury:* **T8 contusion MASCIS/NYU** 12.5 mm	■ **Adult female Fischer rat SCs** (2×10^6) □ **OEGs** (2×10^6) from adult female Fischer rats, nerve fiber layer of the olfactory bulb □ SC 1×10^6 + OEG 1×10^6 Injection in contusion site **@ 7 d PI** **SUBACUTE**	**SCI+** **1.** SCs n = 15 **2.** OEG n = 21 **3.** SCs + OEG n = 11 **4.** DMEM-F12 medium n = 21 Survival: 12 wk	*Histology:* All **cell transplants increase spared tissue** at injury site, with more peripherally myelinated axons within lesions (SC: 5212; SC + OEG: 3,884; OEGs: 2,965; medium: 2125). • **SC and SC + OEG transplants increased** ingrowth and/or sparing of **propriospinal and brainstem-spinal axons** vs. OEGs or medium. • Many sensory (CGRP+) and a few 5HT + brainstem-spinal axons grow into but not beyond the cell grafts. **Brainstem** (DBH+) **and CST axons did not grow onto the grafts.** *Behavior:* **SC transplants significantly improved BBB scores** at 8–11 wk (Final BBBs: SCs: ~11.75 (significant); SC + OEGs: ~11.5; OEG ~ 10.5 SCI: ~10.5).
Pearse *Glia* 2007	*Model:* Female Fischer **rats,** 180–200 g *Injury:* **T8 contusion MASCIS/NYU** 12.5 mm	■ **Adult male Fisher rat SCs, or adult female Fischer rat SC with LV-GFP** □ **Adult male or female Fisher rat OEGs with LV-GFP** □ **Fibroblasts** (FBs) □ SC + OEG Injection **@ 7 d PI** **SUBACUTE**	**SCI+** **1.** SCs (2×10^6) n = 70 **2.** SCs + OEG (1×10^6 each), into epicenter n = 56 **3.** SCs (2×10^6) + OEG (2×10^5), 4 mm rostral + caudal n = 51 **4.** OEG (2×10^6) n = 50 **5.** FBs (1×10^6) n = 12 **6.** No treatment n = 16 Survival: 3 d, 3 wk, 9 wk, 28 wk.	*Histology:* The number of surviving cells was greatly decreased by 3 wk post-transplantation, without further loss at 9 wk as identified by male cells or LV-GFP. **SCs survived better in the lesion than OEGs** (22% vs. 3%). OEGs survived best when transplanted in spared tissue adjacent to the lesion. • SCs increased host p75 + cells invading spinal cord lesions • **Transplants did not increase the in-growth of sensory or supraspinal axons.** *Behavior:* The combination of **SCs/OEGs but not SCs alone significantly improved the BBB scores** at 9 wk PI (SCs:11.5; OEGs: 10.5; SCs/OEGs: 12.3 vs. SCI: 10.7) and subscore (SC: 8; SC/OEG 9.2 vs. SCI: 6.1). • **SC or SC/OEG transplants slightly improved foot rotation**, but not other footprint or gridwalk measures.

(*Continued*)

Table 2. Continued

Reference	*Model/Injury*	*Intervention Intervention timing*	*Experimental groups*	*Outcomes*
Pearse *J Neurotrauma* 2004	*Model:* Female Fischer **rats**, 160–180 g *Injury:* **T8 contusion MASCIS/NYU** 12.5 mm	■ **Adult** female **Fischer rat SCs** (2×10^6) □ **OEGs** (1×10^6) and SCs (1×10^6) from adult Fischer rats. Injection **@ 7 d PI** □ **MP**, 30 mg/kg @ 5 min + 2 hr + 4 hr PI □ **Interleukin-10** (IL-10), 30 mg/kg @ 30 min PI **SUBACUTE**	**SCI+** **1**. MP/IL-10 + SC + OEG n = 14 **2**. SC + OEG n = 13 **3**. MP/IL-10 + SCs n = 13 **4**. SCs n = 14 **5**. MP/IL-10 n = 13 **6.** No treatment n = 15 Survival: 12 wk	*Histology:* **Both acute MP/IL-10 and subacute cell grafts reduced cavity volume**, with a further reduction when used in combination. • MP/IL-10 preserved more tissue when used alone than in with either SCs/OEG or SCs. • **MP/IL-10 alone or in combination with SC/OEG increased the number of retrogradely labeled reticulospinal neurons** from below the injury. • **SCs/OEG** alone or in combination with IL-10, **but not SCs, increased ingrowth of raphe-spinal (5HT+) fibers** into the middle of the grafts. *Behavior:* **MP/IL-10 + SCs/OEG significantly improved and MP/IL10 enhanced BBB scores at 9 wk**. MP/1L10 + SC (12.7) vs. MP/IL-10 (12), MP/IL-10 + SCs (9.5), SCs (10) and control (10.6). **MP/IL-10, SC/OEG and MP/IL-10 + SC/OEG significantly improved BBB subscores** compared to injury alone (MP/IL-10: 5; SCs/OEG: 7; MP/IL-10 + SC/OEG: 7; SCs: 4; SCI: 2). • On footprint, **SCs but not OEGs improved hindpaw rotation**, and SCs + MP/IL-10 improved base of support. • MP/IL-10 alone decreased the number of footfalls on the gridwalk (MP/IL-10: 10; SCI:15)
2d. Schwann Cells Derived from Nerves of Adult Rodents *(Sharp, Complete Transection Injury Models)*				
Xu *J Comp Neurol* 1995	**THORACIC** *Model:* Female Fischer **rats**, 160–180 g *Injury:* **T8 Tx** 4–5 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SCs/ml) within a 10 mm PAN/PVC channel. Spinal cord inserted 1 mm into rostral end, caudal end capped Injection **@ 0 hr PI** **ACUTE**	**SCI+** **1**. MG cable + SCs n = 14 **2**. MG cable n = 8 Survival: 4 wk	*Histology:* **Many axons enter SC/MG cables with capped ends** (2500) compared to MG cables and are myelinated by SCs. Number of myelinated axons decreased along channel, and the ratio of myelinated to unmyelinated axons was 1:4 at graft center. • Axons detected were **primarily propriospinal and sensory**. Brainstem-spinal axons [raphe (5HT+) or coeruleuospinal (DBH+)] did not enter SC/MG cables and **no retrogradely labeled brainstem or CST neurons** were detected. *Behavior:* Not reported
Xu *J Neurocytol* 1997	*Model:* Female Fischer **rats**, 160–180 g *Injury:* **T8 Tx** 4–5 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SCs/ml) within a 7–8 mm PAN/PVC channel both ends open. Rostral and caudal stumps inserted 1 mm Injection **@ 0 hr PI** **ACUTE**	**SCI+** **1.** MG cable + SCs n = 30 **2**. MG cable n = 4 Survival: 4 wk	*Histology:* **Many (>17,000) axons reach the midpoint of SC/MG cables and are myelinated,** with a ratio of myelinated to unmyelinated axons of 1:8. Almost no axons seen in the MG graft. Axons were **primarily propriospinal and sensory axons**. • Retrogradely labeled propriospinal neurons detected as far as C3 and S4. • A few propriospinal axons exited with minimal extension (0.2 mm) into distal host, and a few brainstem-spinal axons. • Only a **few brainstem-spinal axons** seen in the cables **and no CST ingrowth**. 5HT + fibers found within the cable, up to 0.7 mm; DBH fibers stopped at the rostral interface. *Behavior:* Not reported

Plant *Mol Cell Neurosci* 2001	*Model:* Female Fischer **rats**, 160–170 g *Injury:* **T8 Tx** 4 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SCs/ml) within a 6 mm PAN/PVC channel both ends open Injection **@ 0 hr PI** **ACUTE**	**SCI+** **1**. MG cable + SCs n = 34 **2**. MG cable n = 10 **3**. Empty channel n = 8 Survival: 3 wk	*Histology:* Proteoglycan expression as measured by CS-56 and neurocan staining is elevated after transection. Transplantation of SCs resulted in **higher CSPG expression at the caudal interface** relative to the rostral interface. *Behavior:* Not reported
Pinzon *J Neurosci Res* 2001	*Model:* Female Fischer **rats**, 150–180 g *Injury:* **T8 Tx** 4–5 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SCs/ml) within a 7–8 mm PAN/PVC channel both ends open. Injection **@ 0 hr PI** **ACUTE**	**SCI+** **1.** SCs + MG n = 9 **2.** MG only n = 2 No injury: **3.** Control n = 3 Survival: at least 3 months	*Histology and Physiology:* **SCs functionally myelinate axons within the grafts**, as determined by measuring evoked potentials. Electrical stimulation of 2 of the 9 animals receiving SCs + MG grafts produced evoked potentials. Histologically, grafts from animals which responded to electrical stimulation contained the most myelinated axons (mean 1,170 myelinated axons vs. 474 in grafts that did not elicit an evoked potential). *Behavior:* Not reported
2d. Schwann Cells Derived from Nerves of Adult Rodents + Drugs/Factors *(Sharp, Complete Transection Injury Models)*				
Chen *Exp Neurol* 1996	*Model:* Female Fischer **rats**, 160–180 g *Injury:* **T8 Tx** 4–5 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SCs/ml) within a 10 mm PAN/PVC channel, caudal end capped **@ 0 hr PI** □ **MP**, i.v. 30 mg/kg at 5 min, 2 hr & 4 hr PI **ACUTE**	**SCI + MG cable+** **1.** SCs + MP n = 10 **2.** SCs + vehicle n = 8 Survival: 4 - 6 wk	*Histology:* MP promoted tissue sparing and reduced connective tissue at the host-graft interface. • **MP increased SC cable size**, and both the total number of axons (5,447 vs. 1,574 axons) and the number of myelinated axons within the capped SC cables, but not the myelination ratio. • **MP increased sensory and propriospinal ingrowth**. DRG neurons: 359 in SC/MP vs. 84 in SC/vehicle animals. CGRP + sensory axons were present within both groups. • **Brainstem spinal axons** (5HT + and DBH+) **grew on to SC/MP channels** and brainstem nuclei were also retrogradely labeled only with SC/MP transplants. **No CST ingrowth**. *Behavior:* Not reported
Xu *Exp Neurol* 1995	*Model:* Female Fischer **rats**, 160–180 g *Injury:* **T8 Tx** 4–5 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SCs/ml) within a 10 mm PAN/PVC channel, caudal end capped **@ 0 hr PI** □ Recombinant human **BDNF + NT-3** ($168\ \mu$g of each) via mini-osmotic pump ($12\ \mu$g/day) for first 14 days to caudal end of closed channel, started at 0 hr PI **ACUTE**	**SCI + MG cable+** **1.** SCs + BDNF/NT-3 n = 12 **2.** SCs + vehicle n = 9 **3.** BDNF/NT-3 n = 6 Survival: 4 - 6 wk	*Histology:* **BDNF + NT-3 doubled the number of myelinated fibers within the SC cables** and increased the cellularity of the MG only cables. • **BDNF + NT-3 enhanced regeneration of sensory, propriospinal and brainstem-spinal axons into SC cables**. Raphespinal 5HT + fibers extended up to 5 mm into SC/NT cables. Retrogradely labeled brainstem neurons (primarily from vestibular (~67%); red, reticular and raphe nuclei) were present in SC + BDNF + NT-3, but not SC + vehicle animals. **No CST ingrowth**. *Behavior:* Not reported

(*Continued*)

Table 2. Continued

Reference	*Model/Injury*	*Intervention Intervention timing*	*Experimental groups*	*Outcomes*
Menei *Eur J Neurosci* 1998	*Model:* Female Fischer **rats**, 160–180 g *Injury:* **T8 Tx**	■ **Adult Fischer rat SCs** ■ **Adult Fischer rat SCs expressing BDNF** Cells injected directly into injury site and into the caudal stump (5×10^5 SCs in each) **@ 0 hr PI** **ACUTE**	**SCI+** **1**. SCs n = 26 **2**. SCs + BDNF n = 21 **3**. Injury only n = 3 Survival: 4 hr, 24 hr, 7 d, 30 d.	*Histology:* **BDNF increased sensory and brainstem-spinal axon regeneration but not propriospinal axon regeneration**. CGRP + fibers left SC train and extended caudally up to 10 mm. More DRG neurons were retrogradely labeled (1,380 vs. 46 in SC group). 5HT + and DBH + axons sprouted mostly at the level of the Tx but some grew along trail of BDNF-SCs and some brainstem-spinal neurons were retrogradely labeled (primarily reticular and raphe nuclei). *Behavior:* Not reported
Hurtado *Biomaterials* 2006	*Model:* Female Fischer **rats**, 160–180 g *Injury:* **T9/10 Tx** 3 mm segment removed	■ **Adult Fischer rat SCs** ■ **SCs expressing D-15A** (a molecule with **BDNF** and **NT-3** activity) • Cells injected within a poly (D,L-lactic acid) macroporous guidance scaffold **@ 0 hr PI** **ACUTE**	**SCI + Fibrin cable+** **1.** SCs n = 13 **2.** D15A SCs n = 13 **3**. Fibrin cable n = 7 Survival: 1 d, 3 d, 1 wk, 2 wk, 6 wk	*Histology:* **SC survival poor within fibrin grafts** resulting in similar p75 expression in all groups. Few myelinated axons present within the grafts at 6 wk (SC: 27 vs. D-15A-SCs: 69). • D-15A-SC had decreased dieback of NF + axons at 1 week post-injury. NF + axons penetrated pores within SC and D-15A-SC grafts but not fibrin only grafts at 2 and 6 wk. *Behavior:* SC-fibrin cables with or without neurotrophin expression resulted similar BBB scores at 6 wk (SC-Fibrin: 6.3; D15A-SCs-Fibrin: 6.8).
Oudega *Glia* 1997	*Model:* Female: Fischer **rats**, 160–180 g *Injury:* **T8 Tx** 4–5 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SC/ml) within a 10 mm PAN/PVC channel, caudal end capped **@ 0 hr PI** □ **IGF-1** (30 ng/ml) and **PDGF** (15 ng/ml) added to MG during channel preparation **ACUTE**	**SCI + MG cable+** **1.** SCs n = 7 **2.** SC + IGF-I/PDGF n = 7 **3.** IGF-I/PDGF n = 7 Survival: 4 wk	*Histology:* Addition of IGF-1/PDGF to MG may negatively effect some aspects of the SC channels while promoting others. **IGF-I/PDGF promoted brainstem-spinal ingrowth of 5HT + and DBH + up to 2 mm into SC cables**, and promoted myelination as determined by an decrease in the number of unmyelinated axons and the presence of thicker myelin. However, IGF-I/PDGF/SC channels had more cysts rostrally and **fewer axons grew into the cables** (myelinated axons 316 vs. 504). *Behavior:* Not reported
Meijs *J Neurotrauma* 2004	*Model:* Female Fischer **rats**, 140–160 g *Injury:* **T8 Tx** 4 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Fibrin cable** (140×10^6 cells/ml) **@ 0 hr PI** □ **FGF2**,3 μg/100μL **mixed with Fibrin and cells** **ACUTE**	**SCI + Fibrin cable+** **1.** SCs n = 28 **2.** SCs + FGF2 n = 28 Survival: 3 wk, 6 wk, 12 wk	*Histology:* Addition of FGF2 did not alter the number of myelinated axons, the myelination ratio or the number of axons within SC grafts. Similar to SC-fibrin grafts axons did not exit into the caudal spinal cord (no retrogradely labeled neurons were present rostrally). *Behavior:* **SC-fibrin cables with or without FGF2 resulted in similar BBB scores** (SC-Fibrin: 6.8; SC + FGF2-Fibrin 5.8).

2d. Schwann Cells Derived from Nerves of Adult Rodents + Other Cells ***(Sharp, Complete Transection Injury Models)***

Ramon-Cueto *J Neurosci* 1998	*Model:* Female Fischer **rats**, 4 months old *Injury:* **T9 Tx** 4 mm segment of spinal cord removed	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SC/ml) within a 6 mm PAN/PVC channel **@ 0 hr PI** □ **Adult Fischer rat OEGs** (2×10^5) into both rostral and caudal stumps **@ 0 hr PI** Labelled with Hoechst nuclear dye only **ACUTE**	**SCI + MG cable + SCs+** **1**. OEGs n = 9 **2**. Medium n = 7 Survival: 6 wk	*Histology:* **Axons grew into both SC bridges and SC bridges with OEGs** injections, qualitatively more axons within SC + OEG grafts. **SC + OEG bridges contained more CGRP + axons** than SC bridges. **Brainstem-spinal axons** (5HT + or DBH+) **did not enter grafts**. 5HT + axons extended along the connective tissue outside of the channels to the caudal spinal cord up to 1.5 cm. •Labeled propriospinal neurons seen caudally to the graft 0.5–3 mm (1.9 cm in one case). Ascending propriospinal axons observed in the rostral spinal cord, up to 2.5 cm. • Hoechst labeled-OEGs integrated through host and graft tissue up to 1.5 mm from injection sites. *Behavior:* Not reported
Fouad *J Neurosci* 2005	*Model:* Female Fischer **rats**, 165–180 g *Injury:* **T8 Tx** 4 mm segment of spinal cord removed.	■ **Adult Fischer rat SCs + Matrigel (MG)** (120×10^6 SC/ml) within a 6 mm PAN/PVC channel **@ 0 hr PI** □ **Adult Fischer rat OEGs** (2×10^5) into both rostral and caudal stumps **@ 0 hr PI** □ Wound rinsed with **ChABC,** then ChABC infusion (0.02 μg/every other day) into rostral and caudal stumps via catheter for 4 wk. □ mouse **IgG** intrathecally to the caudal stumps via minipump @ 0 hr PI **ACUTE**	**SCI + MG cable+** **1.** OEG + SCs + ChABC + IgG (ChABC) n = 5 **2.** OEG + SCs + galactosidase + IgG n = 7 **3.** MG only n = 8 (control) Survival 12 wk	*Histology:* Axons grew into all grafts. **Significantly more myelinated axons were observed following ChABC treatment** (MG alone: 1,009; SCs + OEG: 1,949; SCs + OEG + ChABC: 3,058). •. **Brainstem spinal axons (5HT+) entered all grafts**, but more 5HT axons found in and caudal to grafts with ChABC. No correlation between 5HT staining and Behavior. *Behavior:* **ChABC significantly improved BBB scores at 9 wk** as compared to control or SC + OEC (MG: 2.1; SC + OEG: 4; SC + OEG + ChABC: 6.6), and **significantly improved forelimb-hindlimb coupling** (without weight supported stepping. Both SC + OEG and SC + OEG + ChABC **reduced tactile sensitivity** at tail base (von Frey force): control 0.6 g, SCs: 0.31 g, ChABC 0.33 g, sign vs. control
Moon *Restor Neurol Neurosci* 2006	*Model:* Female Fischer **rats**, 160–192 g *Injury:* **T10 Tx** 2 mm segment of spinal cord removed	■ **Adult Fischer rat SCs** (1.8×10^6 cells) in a 3 mm **fibrin** cable □ **OEG**, injection of 2×10^5 into each of the rostral and caudal stumps □ Basic housing (BH) or motor enriched housing (**MEH**), started at 1 wk PI Cells injected **@ 0 hr PI** **ACUTE**	**SCI+** **1**. SCs + Fibin cable/OEG + MEH n = 11 **2**. SCs + Fibrin cable/OEG + BH n = 8 **3**. OEG + MEH n = 9 **4**. OEG + BH n = 10 Survival: 22 wk	*Histology:* **Many axons entered SC + OEG grafts** (up to 13,279 SC-myelinated axons). CTB fibers did not penetrate any of the grafts. **No 5HT axon regeneration** into or beyond the grafts nor on the connective tissue bridges surrounding the grafts. Longest survival of all SC studies, however, mortality was a problem (9/37 rats). *Behavior:* **No significant differences in BBB scores between the groups**, however, 100% of OEG + MEH group maintained a score of at least 7 and 4 MEH rats obtained scores of ≥ 9 while no BH rat achieved a BBB score > 8. Final BBBs at 22 wk: SC/OEG/MEH: ~ 6; SC/OEG/BH: ~5; OEG/MEH: ~ 8 OEG/BH: ~ 5.5.

(*Continued*)

Table 2. Continued

Reference	*Model/Injury*	*Intervention Intervention timing*	*Experimental groups*	*Outcomes*
Vavrek *J Neurotrauma* 2007	*Model:* **Adult** female Fischer **rats** *Injury:* **T8 Tx** 4 mm region encompassing T8 removed.	■ **Adult Fischer rat SCs** + Matrigel (5×10^6) in guidance channels **@ 0 hr PI** □ **OEG,** injections of 2×10^5 rostral and caudal **@ 0 hr PI** □ Wound rinsed with **ChABC** + infusions of 0.02 μg/every other day; rostrally and caudally for 4 wk □ **IgG,** infusions into caudal spinal cord. **ACUTE**	**SCI+** **1.** OEG + SCs in MG bridge + ChABC n = 5 **2.** No treatment n = 4 Animals survived 12 wk.	*Histology:* **Both propriospinal and brainstem-spinal axons enter the caudal spinal cord in SC + OEG + ChABC group** but not in injury alone group (fluorogold injected into T13). Most labeled propriospinal neurons were thoracic (238 ± 123) with a few located cervically (12 ± 3.6). • Most labeled brainstem neurons arose from the vestibular (70%), reticular (18%), and raphe (12%) nuclei. *Behavior:* Not reported
2e. Schwann Cells Derived from Adult Rodent Nerve *(Sharp, Partial Transection Injury Models)*				
Xu *Eur J Neurosci* 1999	*Model:* Female Fischer **rats**, 155–165 g *Injury:* **T8 lateral hemi-section** with removal of 2.5–2.8 mm of spinal cord.	■ **Adult Fischer SCs** (120×10^6/ml) + Matrigel within a 3 mm PAN/PVC channel open at both ends, **@ 0 hr PI** **ACUTE**	**SCI** **1.** SCs + MG cable, n = 54 **2.** MG cable, n = 21 Survival: • 5 wk PI. n = 65 • 55–70 d PI, n = 7 • 100 d PI, n = 3	*Histology:* More myelinated axons in SC vs. MG only grafts (1,004 vs. 185). Many axons (~10,000) including some brainstem spinal (5HT + and DBH+) axons enter SC hemi-grafts. In 4/10 cases axons re-entered caudal stump 3.2–3.5 mm. **Sensory, propriospinal and brainstem neurons reach the midpoint of SC hemi-grafts** and originate further from the graft. Propriospinal axons from as far as C3 - S4 grow in (550 in SCs; 126 in MG grafts), as well as axons from 19 brainstem nuclei (primarily: raphe, reticular, vestibular, locus coeruleus and red). *Behavior:* Not reported
Hsu *J Neurosci Res* 2005	*Model:* Female Fisher **rats**, 145–160 g *Injury:* **T8 lateral hemi-section** with removal of 2.8 mm of spinal cord.	■ **Adult Fischer SCs** (120×10^6/ml) + Matrigel within a 3 mm PAN/PVC channel open at both ends, **@ 0 hr PI** **ACUTE**	**1.** SCI + MG cable + SCs n = 26 Survival: 2 d, 4 d & 7d: n = 6/time point; 14 d: n = 8	*Histology:* **The number of axons within the guidance channel increased with time**. **Propriospinal axons entered SC grafts within 2 days** but did not exit by 14 days. Axons rapidly increased the length in which they extend into channels within one week (2d: 0.90 ± 0.21 mm; 4d: 1.60 ± 0.15 mm; 1w: 2.70 ± 0.26 mm; 2w: 3.10 ± 0.12 mm). Scarring increased within the first week. At the host-graft interface, strong GFAP reactivity was present at 7 days with a further increase at 14 days, and CSPG staining was observed first at 3 days and was increased at 7 and 14 days. *Behavior:* Not reported
Imaizumi *Brain Res* 2000	*Model:* **Adult** Wistar **rats** *Injury:* **T11 dorsal column transection** with sparing of the central vein	■ Freshly dissociated **adult female Wistar rat SCs** (6×10^4) **@ 0 hr PI** □ Freshly isolated **neonatal Wistar rat OEG** (6×10^4) • Injection of cells in DMEM into rostral and caudal injury stumps (3×10^4/site) **ACUTE**	**SCI+** **1.** SCs n = 12 **2.** OEG n = 12 **3.** Medium (DMEM) injection n = 10 **No SCI:** **4.** Control n = 11 Survival for 5–6 wk	*Physiology:* **Detectable compound action potentials (CAPs)** in more animals **with SCs or OEGs transplants** (SCs: 11/12; OEGs: 10/12; Medium: 1/10) with CAP amplitudes significantly higher with both cell transplants (SCs: 19.9 m/s; OEG: 18.9 m/s; Medium: 13.8 m/s). *Histology:* More peripherally myelinated axons traversing the lesion in SC or OEG transplants (SC: 421 ± 96; OEG: 536 ± 117; vehicle; 168 ± 60) immediately rostral to lesion. Larger axons with peripheral myelin in both the ascending dorsal column fibers and descending CST fibers after SC or OEG transplantation *Behavior:* Not reported

2g. Schwann Cells Derived from Adult Rodent Nerve, With Other Drugs *(Partial Transection Injury Models)*

Bamber *Eur J Neurosci* 2001	*Model:* "**Rats**" *most likely Fischer as per previous publications* *Injury:* **T8 lateral hemi-section** with removal of 2.5–2.8 mm of spinal cord.	■ **Adult Fischer SCs** (120×10^6/ml) + Matrigel within a 3 mm PAN/PVC channel open at both ends, **@ 0 hr PI.** □ **Neurotrophins** (BDNF: 0.83 μg/μl; NT-3: 0.83 μg/μl or BDNF and NT-3: 0.83 μg/μl each) delivered 2.5 - 3 mm caudally for length of study **ACUTE**	**SCI + MG cable**+ **1.** SCs + vehicle n = 4 **2.** SCs + BDNF n = 4 **3.** SCs + NT-3 n = 4 **4.** SCs + BDNF + NT-3, n = 8 **5.** BDNF + NT-3 n = 4 Survival for 30d PI + tracer time	*Histology:* Many axons were observed within all SC-seeded MG cables; neurotrophins did not alter the number of myelinated axons within channels. **Neurotrophins** did, however**, increased the exiting of axons from the SC hemi-graft** - labeled axons seen to exit SC-neurotrophin grafts and extend 6 mm in some cases (2/4 of NT-3; 2/4 of BDNF; 4/5 of BDNF + NT-3 animals). In SC grafts without neurotrophins, there was limited exiting of axons from the grafts. *Behavior:* Not reported
Vroemen *Cell Tissue Res* 2007	*Model:* **Adult** female Fischer **rats**, 160–180 g *Injury:* **C3 dorsal column Tx** with Tungsten wire knife	■ **Adult Fischer SCs (**3×10^5 cells/μl alone or with FB or 1.25×10^5 with NPC) **@ 0 hr PI** □ **Neural precursor cells** (NPC) (2.5×10^5 cells/μl) from cervical spinal cord □ **Skin fibroblasts** (FBs). 1.5×10^5/μl alone, 1.5×10^4 cells/μl with SCs, 6.3×10^3 cells/μl with SCs and NPC Total volume 2.5 μl of cells injected **ACUTE**	**SCI** **1.** SCs n = 8 **2.** SC + NPC + FB n = 6 **3** SCs + NPC n = 7 **4.** SC + FB n = 6 **5.** FB n = 10 Survival 3 wk	*Histology:* SCs alone or with NPCs did not reduce cyst formation, but the addition of FB prevented cyst formation. • SCs (GFP+) and NPCs (BrDU+) survived transplantation. SCs were restricted to the injury site. NPC were mostly in the spinal cord adjacent to the graft with few cells within the graft. • **More axons were found in SC grafts** (NPC/SC/FB: 6,366 pixels/mm^2; SC/FB 4,998 pixels/mm^2) **than FB grafts** (3,632 pixels/mm^2). Few anterogradely labeled CST axons were present within grafts. CST labeling was reduced within SC/FB grafts compared with FB grafts (1,601 vs 6,879 pixels/graft). More CST axons were able to enter SC/FB grafts when NPCs were present (NPC/SC/FB grafts: 5,105 pixels/graft). *Behavior:* Not reported
Weidner *J Comp Neurol* 1999	*Model:* **Adult** Fischer **rats**, 160–200 g *Injury:* **T7 dorsal hemisection,** (including the CST and RST)	■ **Adult Fischer SCs** (2.1×10^6/ml) with or without enhanced **NGF** expression **@ 0 hr PI** □ **Primary fibroblasts** (FBs) (5:1 ratio SCs/FBs) **ACUTE**	**SCI + collagen + FB**+ **1.** SCs n = 22 **2.** NGF-SCs n = 23 Survival: • 3d, 6d, 10d, 14d, 21d (n = 2/group) • 1 month: n = 4/group • 3 months: n = 3–4 • 6 months: n = 5/group	*Histology:* Modest growth of axons into SC grafts, primarily of sensory origin (CGRP+). • **NGF-SC increased graft size** over time and **increased fiber ingrowth, primarily of sensory** (CGRP+) **fibers** (6 months: NGF-SC ~35,000 vs. SC <3,000 pixels/field). Also **some supraspinal** growth of coerulospinal (TH+) axons (9,000 pixels per field). *Behavior:* Not reported
Iannotti *Exp Neurol* 2003	*Model:* Female Fischer **rats**, 155–165 g *Injury:* **T8 lateral hemi-section** with removal of 2.5 mm of spinal cord.	■ **Adult Fischer SCs** (120×10^6 /ml) + Matrigel within a 3 mm PAN/PVC channel open at both ends, **@ 0 hr PI.** □ Neurotrophin (**rhGDNF**: 3μg/μl) added to MG at the time of channel preparation. **ACUTE**	**SCI + MG cable**+ **1**. SC n = 8 **2**. GDNF + SCs n = 8 **3**. GDNF n = 4 **4**. MG cable only n = 4 Survival for 5 wk	*Histology:* **GDNF increased myelinated axons** within the SC grafts (6,000 vs. 1,000), and myelination ratio but not the total number of axons. • GDNF (with and without SCs) reduced GFAP expression, macrophage infiltration and the number of cavities at the graft interface. • GDNF/SC grafts **had more retrogradely labeled propriospinal neurons over a greater distance** (as far as C2 and S2). Most propriospinal neurons arose ipsilateral to the lesion (75%) at T8 and T10, the remaining arose contralateral (25%). *Behavior:* Not reported

(*Continued*)

TABLE 2. CONTINUED

Reference	*Model/Injury*	*Intervention Intervention timing*	*Experimental groups*	*Outcomes*
Chau *FASEB J* 2004	*Model:* **Adult** female Fischer **rats** *Injury:* **T8 lateral hemi-section** with removal of 2.5–2.8 mm of spinal cord.	■ **Adult** female **Fischer SCs** (120×10^6 /ml) + Matrigel within a 3 mm PAN/PVC channel open at both ends, **@ 0 hr PI.** □ Infusion of **ChABC** 0.5 μl/hr from minipump with implantaion of infusion tip @ T9 **@ 0 hr PI** **ACUTE**	**SCI + MG cable+** **1.** SCs + ChABC **2.** SCs + vehicle Survival for 2 wk (n = 4/group) or 4 wk (n = 12/group)	*Histology:* **ChABC decreased CSPG expression in the caudal spinal cord, but not within SC cables**. CS56 deposits observed at the caudal interface in SC only grafts. ChABC treatment **increased the number of myelinated axons** in SC hemi-cables (1,500 vs. 400). **Propriospinal axons** entered all cables, and **exited some cables treated with ChABC** (7 of 12 animals) but not SC only hemi-cables and extended as far as 5 mm into grey matter (3.18 ± 0.98). *Behavior:* Not reported
3a. Schwann Cells Derived from Newborn Rodents *(Blunt Injury Models)*				
Firouzi *Neurosci Lett* 2006	*Model:* Female Wistar **rats**, 100–140 g (immature <60d old) *Injury:* **T10 clip compression** 1.16 N closing force for 10 sec.	■ **Neonatal SCs,** 50,000 injected into the subarachnoid space above injury **@ 7 d PI** **SUBACUTE**	**SCI+** **1.** SCs prelabeled with Hoechst (HO+) n = 5 **2.** SCs n = 8 **3.** Medium n = 8 **4.** No treatment n = 12 Animals survived • 7 d post-transplant and • 60 d PI	*Histology:* 7 days post-transplant into young rats: a few HO + cells found in subarachnoid space, attached to pia mater or migrated into the middle of the spinal cord. • Counts of Marsland silver-stained axons through lesion center at 60 d PI indicate more axons (presumably primarily in spared white matter) following SC transplantation versus medium injection or injury alone (39,606 vs. 27,865 vs. 23,916 axons/mm^2). *Behavior:* **Implantation of neonatal SCs into the subarachnoid space of immature rats resulted in significant BBB recovery (**Injury alone: 9.4; Medium injection: 11.4; Subarachnoid SCs:13.5)
Azanchi *J Neurotrauma* 2004	*Model:* Female SD **rats**, 200 g *Injury:* **T10 contusion MASCIS,** 25 mm plus □ **Demyelination** of dorsal columns with gal-C and complement @ 3 d PI.	■ **Neonatal P2 male SCs,** 2.4×10^5 injected at the site of injury **@ 4 d PI** **SUBACUTE**	**SCI+** **1.** SCs (n = 12) **2.** Demyelination + SCs 12 **3.** Demyelination n = 12 **4.** SCI control n = 12 **NO SCI+** **5.** Demyelination control n = 8 **6.** SC control (intact spinal cord) n = 8 **7.** Uninjured control n = 12	*Histology:* Transplanted SCs spread from transplant site to demyelination area. Some host SCs found within the region of demyelination in controls. • **Demyelination + SCs increased the number of sensory axons in** dorsal columns 4 mm rostrally **within the region of demyelination** (128 labeled axons), **but not beyond demyelination zone.** *Behavior:* **No significant differences in BBB** (SCI + demyelination + SCs: 11.5 vs. SCI alone: 10.5)
3b. Schwann Cells Derived from Newborn Rodents - combination treatment *(Blunt Injury Models)*				
Papastefanaki *Brain* 2007 *See Caitlin's comment*	*Model:* C57BL/J6 **mice**, 3 month old *Injury:* **T7-9** Laminectomy, **Crush with forceps** 1 min	■ **Transgenic GFP-C57BL/J6 mouse SCs (postnatal day 5)** transduced with either **STX** (SC-STX) or alkaline phosphatase (SC-AP). *(STX increases expression of PSA form of neural cell adhesion molecule)* Injection of 1×10^5 cells **@ 0 hr PI** **ACUTE**	**SCI+** **1.** SC-STX **2.** SC-AP **3.** Medium Survival: 1d, 3d, 1 wk, 2 wk, 4 wk	*Histology:* Both transplants spread 2–3 mm at 2 wk PI, but no proliferation of SCs seen. • More myelinated fibers in SC-STX > SC-AP > medium controls. Most P0 + profiles were GFP negative, indicating that most of the myelination was by host SCs. • SC-STX increased 5HT + fiber growth by 6% vs. 1% in SC-AP *Behavior:* **Significant improvement in BMS in SC-STX group: 5.5 vs. 2.1 in SC-AP** & 0.3 in controls at 3 wk and 4 wk

3c. Schwann Cells Derived from Newborn Rodents *(Sharp Injury Models)*

Keirstead *Exp Neurol* 1999	*Model:* Female SD **rats**, 12 wk old *Injury:* **L2 dorsal column transection**	■ **Neonatal P2 SCs**, 6×10^4 cells injected at the site of injury @ **24 hr PI** □ **Demyelination** at L1 with gal-C and complement **ACUTE 24 hr**	**SCI+** **1.** Demyelination + SCs (n = 10) **2.** SCs n = 3 **3.** Vehicle n = 3 **4.** SCI only n = 3 **No SCI:** **5.** SCs only n = 4 **6.** Demyelination only n = 3	*Histology:* Demyelination enhances the spread of SC transplants. • SCs **myelinate axons within the demyelinated region, and promote axonal growth,** as determined by the number of growth cones when demyelination is combined with axotomy. *Behavior:* Not reported.

4. Schwann Cells derived from Other Sources

Biernaskie *J Neurosci* 2007	*Model:* Male SD **rats**, 286 ± 7g *Injury:* **T9 ESCID (OSU) contusion**, 1.5 mm displacement, 286 ± 5 kdyn	**Cells derived from skin of neonatal mice,** P0-P3, eYFP-expressing transgenic mice ■ SKP-derived **SCs** (**SKP-SCs**) □ Skin precursors (**SKPs**) from dorsal skin □ **Neonatal** neurospheres from forbrain subventricular zone (NPCs). 8×10^5 cells injected into lesion epicenter **@ 7 d PI** • Cyclosporine A **(CsA)** 15 mg/kg daily starting on day 5 PI **SUBACUTE**	**SCI+** **1.** SKP-SCs, 1–2 wk survival n = 10 **2.** SKP-SCs, 12 wk survival n = 16 **3**. SKPs, 12 wk survival n = 13 **4.** NPCs, 12 wk survival n = 11 **5.** Medium, 1–2 wk survival n = 8 **6**. Medium, 12 wk survival n = 4 Survival: see above	*Histology:* SKPs & SKP SCs survive better than NPCs (180,000 & 140,000 vs. 15,000). • SKP-SCs promoted tissue sparing, reduced scarring, and extended through the length of the injury. • **SKP-SCs promoted axonal ingrowth of both sensory** (CGRP + and CTB traced) **and brainstem-spinal axons** (TH + and 5HT+). SKP-SCs improved axonal myelination within the lesion and attracted endogenous myelinating Schwann cells. *Behavior:* **BBB** at 12 wk, **SKP-SCs were significantly better than SKPs but not NPC** (SKP-SC: 12; SKP: 10.5; NPC: 11). No significant difference in horizontal ladder test. • Hindlimb withdrawal was more sensitive in SKP and NPC treated animals at 5 wk, compared to SKP-SC
Kamada *J Neuropath Exp Neurol* 2005	*Model:* Male Wistar **rats**, 200g *Injury:* **T7 Tx** and removal of **T7** segment	■ **Bone marrow stromal cell-derived SCs** (BMSC-SCs) from **adult** male Wistar rats in Matrigel (**MG**) within 5 mm length of ultra-filtration membrane 2×10^6 cells **@ 0 hr PI** **ACUTE**	**SCI+** **1**. BMSC-SCs n = 9 **2.** MG only n = 7 Survival: 6 wk 2 animals were re-transected at 6 wk	*Histology:* BMSC-SC grafts contained twice as many NF + axons within the graft and 0.5 mm rostral and caudal as MG only. Few sensory fibers (CGRP+) grew into BMSC-SC or MG only grafts. • More brainstem-spinal axons (TH + and 5HT+) were present within BMSC-SC grafts than in MG grafts *Behavior:* **BBB: BMSC-SCs showed significant improvement at wk 4, 5 & 6** (6 wk: MG alone: 3.6; BMSC-SC: 7). Re-transection of BMSC-SC animals at 6 wk abolished improvement in BBB (BBB = 1), with no further improvement over 4 wk

d: day, days; hr: hour, hours; i.v.: intravenous; PI: post-injury; PT: post-transplant; SCI: spinal cord injury; SD – Sprague Dawley; Tx: transection; wk: week, weeks

5HT: serotonin; AdV: adenoviral; aFGF: acidic fibroblast growth factor; BBB: Basso, Beattie and Bresnahan locomotor test; BDNF – brain-derived neurotrophic factor; bFGF (FGF2): basic fibroblast growth factor; CCK: cholecystokinin; CGRP - calcitonin gene-related peptide; ChABC: chondroitinase ABC; CsA: Cyclosporine; CSF: cerebrospinal fluids; CSPG - chondroitin sulfate proteoglycan; CST - cortico-spinal tract; CTB: cholera toxin beta; DBH: dopamine-β-hydroxylase; DMEM: Dulbecco's modified Eagle's medium; DRG - dorsal root ganglion; EM: electron microscopy; ENK: enkephalin; FB: fibroblast; GDNF: glial cell line-derived neurotrophic factor; GFAP: glial fibrillary acidic proteins; GFP – green fluorescent protein; FBs: fibroblasts; IGF-1: insulinlike growth factor; LV: lentivirus; LacZ – beta-galactosidase; MG: matrigel; MRI-magnetic resonsnce imaging; MP: methylprednisolone; NF: neurofilament; NGF: nerve growth factor; NPC: neural progenitor/precursor cells; NT: neurotrophin; OEC: olfactory ensheahting cell; OEG: olfactory ensheathing glia; PAN/PVC: poly(acrilonitrile-vinyl chloride); PDGF: platelet-derived growth factor; PSA: polysialic acid; SCs:- Schwann cells; SKPs: skin precursors; SP: substance P; STX: sialyl-transferase; TH: tyrosine hydroxylase; TPH: tryptophan hydroxylase; VIP: vasoactive intestinal peptide.

the SC literature). The three other studies only saw benefits when SCs were used in combinations with either rolipram plus cAMP (Pearse et al., 2004a), olfactory ensheathing cells (OECs) (Pearse et al., 2007), or the combination of methylprednisolone, IL-10, and OECs (Pearse et al., 2004b). Despite the lack of effect of SCs alone in these studies, SCs score almost one point above injury controls in two of these studies with BBB scores ranging from 10 to 11.5 versus 9.5 to 10.7 for SCI controls. It is noteworthy that the two studies in which SCs were efficacious involve pre- and post-transplantation behavioral assessment, whereas the three studies in which SCs alone had no effect did not score behavior prior to one week post-transplantation. To date, the BBB score comparing SCs with matrix (Fibrin or Matrigel) only has not been reported, making it difficult to ascertain the functional benefits of SCs in either the complete or incomplete transection model. Only one of the four studies that performed behavioral analysis after full transections yielded clear behavioral benefits. In this study, the SCs were used in combination with a Matrigel-filled PAN/PVC channel plus OECs injections into the stumps plus ChABC and mouse IgG (Fouad et al., 2005), and it did not include a SC-only control. Given that the BBB score reported for the combination treatment (6.6) was similar to that of human SCs suspended in the same matrix (8.2) (Guest et al., 1997b) and rat SCs suspended in fibrin (6.3) (Hurtado et al., 2006), it remains unclear if the combination treatment is truly better than SCs alone. These behavioral findings suggest that additional, well-designed, and properly controlled studies are needed to assess the functional benefits of SCs. While the focus of interest is in the development of SCs as a transplantation therapy, as multiple centers move toward or have begun human clinical trails (Saberi et al., 2008), there is strong preclinical interest in the use of additional experimental co-treatments. However, translating such a combination of two experimental treatments into clinical trials has significant implications from a regulatory standpoint, particularly if each treatment in the "co-treatment" is experimental.

Despite the clinical observation that about 60% of human SCIs occur at the cervical level, experiments involving cervical injuries are rare. Only two studies that evaluated adult nerve-derived rodent SCs were performed at the cervical level of the spinal cord. One study that used a clinically relevant contusion injury without co-treatments reported improvements in forelimb grip strength and forelimb hang tests (Schaal et al., 2007).

SCs from nerves of newborn rodents were used in four studies, three of these employing a thoracic contusion injury and the other a lumbar partial transection. While all three contusion studies employed the BBB, only two showed behavioral benefits. It should be pointed out that in one of these two positive studies, efficacy was not observed with wildtype SCs, but rather in SCs transduced to express the cell adhesion molecule PSA. In the other positive study, an astonishing BBB improvement from 9 to 13.5 was reported using the mere injection of 50,000 neonatal SCs into the subarachnoid space after clip compression injury (Firouzi et al., 2006). It should be noted that the rats in this study were very young (100–140 grams), which corresponds with 45–60 days of age. Recently, it has been suggested that SC precursors may be more beneficial than SCs from newborn rodents. However, even with enhanced CST regeneration (something that SCs from adults typically do not promote), these SCs from newborns did not result in functional improvements in a cervical crush model (Agudo et al., 2008). Further studies are warranted to compare the effects of SCs derived from young versus adult animals. The ethical and logistical considerations of acquiring human SCs from embryonic or early postnatal sources makes them less attractive as a therapeutic approach than human SCs derived from the nerves of adults, particularly if an autologous transplantation can be performed.

Considering the fact that a clinical trial of SCs requires the transplantation of human cells, it is remarkable that human SCs have been reported in only two pre-clinical rodent studies. Both of these studies by Guest and colleagues were in thoracic full transection SCI models (Guest et al., 1997a, 1997b). In one study, in which behavior was assessed, the authors report a small but significant behavioral benefit in the BBB and the inclined plane test. The SCs were used in conjunction with a guidance channel and Matrigel, for which there is presently no FDA-approved formulation available. Pre-clinical experiments examining the survival and efficacy of human SC in contusion models of SCI are clearly needed. Despite the paucity of human SC experience in traumatic SCI, it is noted that a number of studies have looked at the effects of cultured human SCs in rodent models of demyelination (Kohama et al., 2001) and peripheral nerve injury (Hood et al., 2009). Additionally, autologous human SCs have been implanted into humans with multiple sclerosis (Brierly et al., 2001; Halfpenny et al., 2002).

An autologous transplantation approach is appealing as it eliminates concerns regarding immune rejection and avoids the controversy over embryonic or neonatal sources. However, an autologous approach necessitates sacrificing a peripheral nerve (the significance of which may be negligible in a completely paralyzed individual), and despite improvements in amplification techniques, a number of weeks are still needed before enough cells can be generated for transplantation. To obviate the need for harvesting a peripheral nerve from a patient, alternative sources of SCs from postnatal skin or adult bone marrow have recently been pursued and tested after thoracic transection (Kamada et al., 2005) or contusion (Biernaskie et al., 2007) injuries. Both studies reported modest (but significant) improvements on the BBB scale and subscale, suggesting that other sources, which may potentially be less invasive that peripheral nerve biopsies, may be an alternative source for autologous SCs. Intriguingly, the SCs from skin-derived progenitors formed bridges across the injury site, migrated into the host parenchyma, and formed myelin with minimal astrocyte hypertrophy (Biernaskie et al., 2007).

Despite the lack of pre-clinical data using human SCs, clinical trials involving the use of human SCs are moving ahead. Saberi and colleagues (2008) in Iran recently published the first results from 4 of 33 patients with chronic thoracic SCI (2.0–6.5 years post injury) that underwent autologous SC transplantation. No detrimental (or beneficial) effect was reported in the first four patients. While it has been possible to identify SC cables using MRI in rats (Iannotti et al., 2002) and fetal tissue transplants in the human spinal cord (Wirth et al., 2001), MRI failed to identify the SC transplants in the study by Saberi and colleagues. This trial, following ICCP guidelines, is a promising first step in the move toward human translation of SCs in SCI. A summary of the pros and cons and knowledge gaps for SC transplantation is depicted in Table 3.

Table 3. Summary Statement for Schwann Cell Transplantation

Pros	*Cons*
Arguably the most extensively studied cell type, with beneficial effects after transplantation into thoracic SCI demonstrated by numerous investigators.	Schwann cells provoke a more robust astrocytic reaction, resulting in less effective integration into the host spinal cord compared to neural precursors such as oligodendrocyte precursors or neural precursor/stem cells.
Can be harvested from patients for an autologous transplantation approach	An autologous transplantation approach requires *in vitro* amplification which may take several weeks and imposes a delay on the intervention.
Behavioral efficacy in "chronic" rodent contusion models has been reported.	In many cases, appears to require adjuvant treatment to increase efficacy (e.g. Matrigel, rolipram, cAMP, neurotrophic factors)

Knowledge Gaps

Long axon regeneration through Schwann cell bridges and reentry into the host spinal cord still poses a challenge, and some fiber tracts like CST axons are not stimulated by SCs.

More preclinical data using clinically relevant (blunt) cervical injury models are desirable (only one study).

It is still necessary to address different injury severities – only one study with behavioral assessment used severe contusions, representing the most likely clinical treatment group.

The optimal source of Schwann cells, i.e. isolating them from nerves versus other tissues progenitors is yet to be determined.

A confirmation that human Schwann cells behave in the same way as rodent Schwann cells is implied, but direct demonstrations are still highly desirable prior to translation.

Olfactory ensheathing cells (also known as olfactory ensheathing glia; Table 4)

OECs are found in the nerve fiber layer of the olfactory bulb, as well as in the nasal olfactory mucosa. These cells have garnered considerable interest because of their ability to facilitate the lifelong repeated regeneration of olfactory axons from the PNS environment of the nasal olfactory mucosa to the CNS environment of the olfactory bulb (Doucette, 1991). Significant differences have been revealed between OECs of various origins (Richter et al., 2005), and therefore these are considered separately in the tables. In addition, the properties of OECs can change considerably depending on the culture conditions (e.g., the number of passages *in vitro*) (Au et al., 2007). Hence, optimal cell source and treatment for transplantation into the injured spinal cord is subject to an ongoing debate.

OECs derived from the olfactory bulbs of adult rodents are the most commonly studied OECs, compared to OECs derived from the lamina propria of the olfactory mucosa. Thirteen such studies were reviewed, which employed both blunt and sharp injury models of the thoracic and cervical spinal cord (thoracic contusion in three, thoracic transection in four, and partial transection in six, five of which were performed in the cervical spinal cord). None of the three thoracic contusion studies reported that OECs alone conferred a behavioral benefit (as per BBB scores) when injected into the cord in either a subacute time frame (Pearse et al., 2007; Takami et al., 2002) or a chronic setting 8 weeks after injury (Barakat et al., 2005). The combination of OECs with SCs, however, appears to promote significant behavioral benefits (Pearse et al., 2007). Takami and colleagues (2002) and Barakat and colleagues (2005) have promoted SC transplantation for SCI, and in direct comparisons between OECs and SCs, these authors have reported that the latter yielded superior behavioral outcomes.

The evaluation of OECs in complete thoracic transection injury models has been based on the rationale that OECs may promote axonal regeneration across a spinal-cord lesion site and facilitate the reentry of axons into the host at the distal host/graft interface. The study by Ramon-Cueto and colleagues (2000) gained considerable international attention due to the apparent regeneration of corticospinal axons and improvements of motor behavior in a non-standardized climbing test after 3 and 7 months post injury. When combining this OEC treatment after full transection injury with treadmill step training, the ability of rats to perform plantar stepping was further improved (Kubasak et al., 2008). Similarly, in an independent study, Cao and colleagues (2004) studied both OECs, as well as OECs modified to overexpress glial cell line-derived neurotrophic factor, which were injected into the stumps of the fully transected spinal cord. They reported significant improvements on the BBB (by six to eight points), as well as on the inclined plane test. While both the Ramon-Cueto and Cao studies reported extensive axonal regeneration of various systems (CST, RST, raphespinal, coeruleospinal), there were no differences in serotonergic fibers below the injury site in the study by Kubasak and colleagues (2008). This is only one of many studies in which the claims of robust axonal regeneration after OEC transplantation were not confirmed, and possible differences in the types of OECs utilized may be one of many potential explanations. For example, a similar study to that of Ramon-Cueto and colleagues (2000), but using primate OECs implanted into nude rats, failed to reveal any CST regeneration but a modest regeneration of 5-HT fibers (Guest et al., 2008).

In partial lesion models (seven studies to date), the transplantation of adult bulb-derived OECs appeared to improve directed forepaw reaching after dorsal column transection, as well as electrolytic lesions of the dorsal columns (Li et al., 1997; Nash et al., 2002). Whether this is due to the claimed corticospinal axon regeneration facilitated by OECs or the enhancement of plasticity and sparing in the host spinal cord is subject of ongoing debate. There is heightened awareness within the field about the potential for sparing in partial lesion models and how this may influence both the interpretation of the histological and behavioral outcomes. No significant regeneration of ascending fibers after dorsal column transection was reported by Toft et al. (2007) although there was electorphysiological evidence for preserved function in rats transplanted with olfactory cells. Regeneration of rubrospinal

Table 4. Olfactory Ensheathing Glial Cells

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
1. OECs (or OEC-like cells) from Olfactory Bulb of Humans				
Deng *Cytotherapy* 2008	*Model:* **Adult** female SD **rats**, 240–270 g *Injury:* **T10 weight drop contusion,** 10g×50 mm	■ **Human OECs** (from outer layers of olfactory bulb from aborted fetuses; 5–7 months gest'n; 3–4 passages) @ 30 min PI *Comment: only p75 used as marker of cell identity* □ **HUMAN BMSCs** (5–7 passages) 250,000 cells/in 5 μl injected into the spinal cord "at 3 spots on average": injury center, 2 mm rostral, and 2 mm caudal to lesion @ 30 min PI • **CsA** (10mg/kg/day, s.c.) for 2 weeks following transplant. ACUTE	**SCI+** **1.** 5 μl of OECs **2.** 5 μl of BMSCs **3.** 3.4 μl of BMSCs & 1.6 μl of OECs **4.** 5 μl of DMEM-F12 (control) n = 15 per group Survival: 5 wk	*Histology/Physiology:* All cell groups had significantly more axons in the lesion than the control group, and the BMSC & OEC co-grafted group had significantly more axons in the lesion site than all other groups. The authors claim neuronal and glial differention of the transplanted BMCs • transcranial motor evoked potentials (in ms) at 5 wk PI revealed BMSC & OEC group (7.3 ± 0.7); OECs (8.0 ± 1.6); BMSCs alone (9.6 ± 2.6); all were significantly better than control (13.4 ± 3.7). *Behavioral:* BBB Scores – At 5 wk PI, the BMSC & OEC group achieved coordinated weight-supported stepping (BBB ~ 15), the BMSC group demonstrated frequent coordination (BBB ~ 13), the **OEC group had weight support and occasional coordination (BBB ~ 11)** and the **control group** failed to gain weight support at all **(BBB ~ 6)**.
2a. OECs from Olfactory Bulb of Primates into Rats				
Guest *Exp Neurol* 2008	*Model:* **Nude rats (immunotolerant)** female 140–155 g *Injury:* **Full transection T9/10** *no immunsuppression required*	■ **OECs from olfactory bulb (nerve fiber layer) of PRIMATES *Macaca fascicularis*** cells were cultured in the presence of DMEM and fetal calf serum with Heregulin and forskolin and enriched by p75 immunopanning (3rd passage) • Cells were labelled with Hoechst and a subgroup of OECs was also labelled with GFP expressing Lentivirus 400,000 cells total injected into the midline of the stumps: 50,000 in 0.5ul each at 4 different depth positions, rostral as well as caudal **immediately** ACUTE	**SCI+** **1**. OECs n = 38 rats **2**. L-15 culture medium n = 19 Survival: 6 wk, 8 wk, 14 wk and 24 wk CST labeling in n = 13 rats with OECs and n = 8 controls at 17–20 weeks after transplantation	*Histology:* **Discrepancies between widespread Hoechst labelled cells and GFP-expressing OECs, which were rather confined. This is a strong indication that Hoechst prelabelling is not reliable Some 5HT fiber regeneration** in 6 of 11 transplanted animals for up to 6 mm distal - but none in control animals **No CST regeneration in any rat** but more collateral CST branches in the OEC transplanted rats above the lesion. *Behavior:* 5 rats showed autotomy – of these 4 had OECs transplanted **BBB:** the **OEC transplanted rats had significantly higher BBB scored from 8 to 18 weeks after injury** – but not thereafter: peak group averages were reached around 12 weeks with a scores around **BBB 9 after OEC transplant ation versus scores around 4 in controls**. After retransection one of 5 rats showed a delcine on the BBB, the other 4 not. The decline in BBB after 12 weeks correlated with the renal pathology seen in these rats.
2b. OECs from Olfactory Bulb of Pigs into Rats				
Imaizumi *Nat Biotechnol* 2000	*Model:* **Adult** Wistar **rats** *Injury:* **T11 dorsal column transection**	■ **OECs** from anterior tip of Olfactory bulb of hCD59 transgenic pigs □ **Schwann cells** from sciatic nerves of hCD59 transgenic pigs 60,000 cells in total of each injected into the dorsal column at 0.5 mm rostral and caudal to the lesion **@ 0 hr PI** **ACUTE**	**SCI+** **1.** Pig OECs n = 10 **2.** Pig Schwann cell n = 5 **3.** DMEM n = 9 **No SCI**: n = 11 Survival: 4–5 wk	*Histology, Physiology:* Cell labeling indicated that the donor cells migrated into the denervated host tract. The **axons** seemed **thickly myelinated** with a peripheral pattern of myelin expected from the donor cell type. • In animals receiving pig **OECs or** Schwann cells, **impulse conduction** was restored across the lesion site for **more than 1 cm**, whereas non-transplanted rats (lesion only) exhibited no impulse conduction across the transection site. *Behavior:* Not reported

3a. OECs from Olfactory Bulb (OB) of Adult Rodents *(Blunt Contusion/ Compression Injury Models)*

Takami *J Neurosci* 2002	*Model:* **Adult** female Fischer **rats**, 160–180 g *Injury:* **T9 contusion** **NYU** Impactor, 10g×12.5 mm	■ **OEGs** (2×10^6) from adult female Fischer rats, nerve fiber layer □ **Adult female Fischer rat SCs** (2×10^6) Injection in contusion site **@ 7 d PI** **SUBACUTE**	**SCI+** **1. OECs n = 21** **2.** Schwann cells n = 15 **3.** SCs + OECs n = 11 **4.** Medium n = 21 Survival: 12 wk	*Histology:* Less cavitation and more sparing in all grafted groups @ 12 wk PI • All cell grafts filled with axons, primarily of spinal origin. SC grafts contained more myelinated axons than OEC or OEC + SC grafts. • **Less intense GFAP and CSPG staining in OEC-only grafts** versus grafts containing SCs. • Significantly higher number of retrograde traced propriospinal and brainstem axons reached 5–6 mm beyond the SC and SC/OEG grafts vs. controls, but not the OEG grafts. • Corticospinal fibers terminated closer to the lesion epicenter in all grafts. *Behavior:* **No improvements of BBB in OEC group.** BBB improvement at 8–11 wk PI in SC group only (from 10.8 to 11.8)
Pearse *Glia* 2007	*Model:* Female Fischer **rats**, 180–200 g *Injury:* **T8 contusion** **MASCIS/NYU,** 10g×12.5 mm	■ **OEGs** (2×10^6) from adult female Fischer rats, nerve fiber layer of the olfactory bulb □ **Adult Fisher rat SCs** (2×10^6) from adult Fischer rats □ **Fibroblasts** (FBs) (1×10^6) Injection into injury site or 4 mm on either side **@ 7 d PI** **SUBACUTE**	SCI + **1.** SCs n = 70 **2.** SCs + OEG, into epicenter, n = 56 **3.** SCs + OEG, 4 mm rostral + caudal n = 51 **4.** OEG n = 50 **5.** FBs n = 12 **6.** No treatment, n = 16 Survival: 3 d, or 3, 9, or 28 wk	*Histology:* Cell survival decreased to a low level by 3 wk post-transplantation, without further loss at 9 wk. OEC-only survived poorly within the lesion (<5%) and did not migrate. • At later times, p75ρ + /EGFP- cells in the lesion outnumbered EGFP + cells in all paradigms, evidence of host SC infiltration. • No sensory or supraspinal axon ingrowth into transplants • Numerous myelinated axons were found within regions of grafted SCs but not OECs. *Behavior:* At 9 wk, **only the SCs + OEG into the injury site group significantly improved BBB scores** (12.3) vs. FB transplant (9.8) and injury controls (10.7). BBB subscores also improved. • No transplant decreased gridwalk errors or reduced deficits in base of support and stride length
Barakat *Cell Transplant* 2005	*Model:* Female Fischer **rats**, 180–200 g *Injury:* **T9 contusion** **NYU/MASCIS,** 10g×25 mm	■ **OEGs** (2×10^6) from olfactory bulb of adult Fisher rats □ Dissociated **adult Fischer rat SCs** (2×10^6) Injection **@ 8 wk PI** **CHRONIC**	**SCI+** **1.** OEG n = 12 **2.** SCs n = 12 **3.** No treatment n = 12 Survival: 19 weeks	*Histology:* SCs survive better than OEG (17.1% vs. 2.3%) @ 8–11 wk after transplantation. Where OEGs did survive, NF + axons were observed. • SC transplants, but not OEG transplants, contained significantly more sensory (CGRP+) axons: SC: 32; OEG 9; control: 15 axons/section and brainstem-spinal (5HT+) axons. Sensory fibers arise primarily from DRGs adjacent to the lesion. SCs did not promote CST ingrowth. *Behavior:* **SCs but not OEGs** resulted in significant BBB improvement (at 16 wk: **SC: 10.2; OEG: 8.5; SCI: 8.5)**. On footprint analysis, SCs but not OEGs resulted in small but significant improvements in base of support and hindpaw rotation

(*Continued*)

Table 4. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
3b. OECs from Olfactory Bulb (OB) of Adult Rodents (*Full Transection (Tx) Injury Models*)				
Ramon-Cueto *Neuron* 2000	*Model:* **Adult** female Wistar **rats**, 200–230 g, 2.5 month old *Injury:* **T8/T9 complete Tx**	■ **OECs** from 2.5 month old Wistar rats injected @ **0 hr PI** into 4 sites (ventral funiculus, grey commissure, dorsal CST, and gracile fasciculus) of the midline of both cord stumps at 1 mm from lesion site 50,000 cells in 0.5 μl per injection (400,000 cells total) **ACUTE**	**SCI+** **1.** OECs n = 9 **2.** DMEM n = 12 **No SCI:** laminectomy alone n = 11 Survival: 7 months	*Histology:* Authors claim long distance regeneration of relevant motor axons (CST, raphespinal and coerulospinal) within caudal cord stumps in OEC transplanted animals, but not in control. *Comment: a similar study with primateOECs fails to find this (Guest et al 2008).* *Behavior:* From 3 to 7 months PI, all OECs-transplanted animals recovered locomotor function and sensorimotor reflexes (climbing test). They regained voluntary hindlimb movements, supported body weight, and the hindlimbs responded to light skin contact and proprioceptive stimuli
Cao *Brain* 2004	*Model:* **Adult** female SD **rats**, 2.5 month old *Injury:* **T8 Complete Tx**	■ **OECs from adult** Sprague Dawley rat olfactory bulb (ONL) injected rostrally and caudally at 1 mm from the transection site, 2×25,000 cells in each stump @ **0 hr PI.** ■ **OECs** modified with retrovirus to secrete **GDNF**, 2×25,000 cells in each stump @ **0 hr PI.** **ACUTE**	**SCI+** **1.** Normal OECs n = 18 **2.** GDNF-OECs n = 15 **3.** DMEM n = 8 Survival: 8 wk	*Histology:* GDNF mRNA expression was detected at least 8 wk after implantation. ■ Compared to controls, **increased** amounts of **NF-positive fibers** were growing through lesion site of GDNF-OEC group ■ BDA-traced **CST fibers** appeared to have **grown through the lesion**, reaching distal segments *Behavior:* GDNF-OEC group regained more functional recovery than normal OEC group 8 wk PI on both BBB **(10 for GDNF-OEC, 8 for OEC, 2 for DMEM) and** Inclined plane: (above 70 for GDNF-OEC, above 60 for OEC, below 50 for DMEM)
Kubasak *Brain* 2008	*Model:* **Adult** female Wistar Hannover **rats**, 10–12 weeks old *Injury:* **T9 complete Tx**	■ **OECs** from Wistar Hannover rats (**8–10 wk old**), injections at 1 mm from the transection site, 4 injections per rat, 400,000 cells in total @ **0 hr PI** Additionally, rats were trained daily for 5–20 min during recovery over 4–7 months, receiving more than 50 hr of manual step training per rat. **ACUTE**	**SCI+** **1.** OEC-trained n = 10 **2.** OEC-untrained n = 10 **3.** Media-trained n = 9 **4.** Media-untrained n = 9 **No SCI: sham operation** n = 8 For morphologic analysis, n = 3 Survival: 7 months	*Histology:* OECs promoted tissue sparing at the transection site, regeneration of noradrenergic axons and serotonergic axons spanning the injury site. However, there was no difference in the 5-HT + fibre density 250 mm below the transection site in media- and OEC-injected rats. *Behavior:* **OECs** (group 2), but not training (group 3), significantly **increased the number of plantar steps performed by the 7-month** endpoint of the study. **OECs with training (group 1) resulted in significant improvement of stepping abilities between the 4- and 7-month evaluations**. Task-specific training enhances OEC effect.

Ramon-Cueto *J Neurosci* 1998	*Model:* Female Fischer **rats**, 4 month old *Injury:* **T9 Tx** 4 mm segment of spinal cord removed	■ **OECs** from **adult Fischer rats** (2×10^5) injected into both rostral and caudal stumps **@ 0 hr PI** □ **Schwann cells** from adult Fischer rats within a PAN/PVC **channel and Matrigel (MG)**; proximal and distal spinal cord stumps inserted 1 mm into channel. **ACUTE**	**SCI + MG cable + SCs+** **1.** OEGs n = 9 **2.** Medium n = 7 Survival: 6 wk	*Histology:* Numerous NF +, GAP-43 +, CGRP +, and 5HT fibers traversed both interfaces of the cord-SC channel with the OECs added. 5HT + axons extended along the connective tissue outside of the channels to the caudal spinal cord up to 1.5 cm. • Labeled spinal neurons seen caudally to the graft 0.5 - 3 mm (1.9 cm in one case). Ascending propriospinal axons observed in the rostral spinal cord, up to 2.5 cm. • OEGs integrated through host and graft tissue up to 1.5 mm from injection sites. *Behavior:* Not reported
3c. OECs from Olfactory Bulb (OB) of Adult Rodents ***(Partial Transection and Electrolytic Injury Models)***				
Ruitenberg *J Neurosci* 2003	*Model:* **Adult** female Fischer F344 **rats**, 175–200 g *Injury:* **C4 Tx** of left **dorsolateral funiculus** as deep as 1 mm ventral.	■ **OECs** from adult female Fischer rats **10,000 cells, injected 1 mm proximal and distal to lesion @ 0 hr PI** • OECs were transfected with **BDNF, NT-3** or B-galactosidase (LacZ controls). **ACUTE**	**SCI+** **1. OEC** transplant n = 10 **2.** OEC - BDNF n = 12 **3.** OEC - NT-3 n = 12 **4.** OEC - BDNF + NT-3 (1:1) n = 12 **5.** OEC - LacZ n = 12 **6.** No transplant n = 10 Survival: 4 months	*Histology:* BDNF-expressing OECs stimulated extensive sprouting of RST axons, 1–1.5 mm into the lesion area – but not across and beyond. NT-3 expressing OECs did not stimulate RST axons. *Behavior:* **OECs with BDNF/NT-3 adenoviral vector enhanced recovery of hindlimb function** in horizontal rope walking vs. controls. ■ BBB was not sensitive nor appropriate in this model
Ruitenberg *Brain* 2005	*Model:* **Adult** female Fischer **rats**, 120 – 150 g *Injury:* **C4 dorsolateral Tx,** 1.5 mm deep (intending to cut the CST also)	■ **OECs** from **adult** female Fischer 344 rats injected 0.5 mm lateral, 1 mm proximal and distal to lesion **@ 0 hr PI**; total of 200,000 cells in 1 μl • OECs were transfected with **NT-3** or B-galactosidase (LacZ controls) **ACUTE**	**SCI+** **1.** Non-modified OECs n = 7 **2.** OEC-NT-3 n = 7 **3.** OEC-LacZ n = 7 **4.** Vehicle OECs n = 6 Survival: 12 wk	*Histology:* OEC groups had greater % of spared spinal tissue and decreased area of degenerative tissue • Only in OEC-NT-3 rats was there a significant increase in anterogradely labelled CST axons distal to the lesion. This was interpreted as either regeneration and/or maintenance of spared CST axons due to OEC-NT-3. *Behavior:* **No significant difference in recovery** between experimental groups.
Deumens *J Neurosci Res* 2006	*Model:* **Adult** male Lewis **rats**, 9 weeks *Injury:* **T11–12 dorsal hemisection**	■ Mixed **OEC**s and **fibroblasts** from the outer two layers of **adult** rat **OB,** implanted **@ 4 wk PI** into the lesion site and into the rostral and caudal cord stumps, 1 mm from lesion site **CHRONIC**	**SCI+** **1.** OEC/ONF complexes + OEC/ONF suspension n = 8 **2.** Culture medium injections 1 mm rostral/ caudal to lesion sites n = 8 Survival: 16 wk	*Histology:* 12 wk after transplantation: enhanced presence of injured CST axons rostral to the lesion, but the OEC/ONF did not stimulate regrowth of injured CST axons across the lesion gaps *Behavior:* No Behavioral recovery (neither BBB (~11) nor CatWalk gait analyses)
Nash *J Neurosci* 2002	*Model:* **Adult** SD **rats**, 300–400 g *Injury:* **C3 Tx of dorsal column**. (15 of 61 animals died)	■ **OECs** from the olfactory **nerve rootlets** and **OB** of adult rats, 100,000 injected into rostral and caudal cut surfaces **@ 0 hr PI.** □ Methylprednisolone (**MP**) IV, 30 mg/kg×4 injections every 6 hr. **ACUTE**	**SCI+** **1.** OEC injections n = 9 **2.** OEC/MP injections n = 10 **3.** MP injections n = 10 **4.** Vehicle injection n = 5 **5.** Lesion, no injection **No SCI** (Sham) n = 5 Survival: 7.5 wk	*Histology:* MP-treated rats had significantly more anterogradely traced CST axons vs. controls, up to 7 mm caudal to the lesion. • OECs-MP group was claimed to have **significantly more axons** than all other lesioned rats, up to 13 mm caudal to the lesion *Behavior:* Functional recovery was assessed in directed forepaw reaching (DFR) test. The percent age of **success was significantly higher** in injured rats **with OEC** transplants, both with (78%) and without MP (72%), as well as in MP group (57%). Vehicle control animals achieved 42%.

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Table 4. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Toft *Brain* 2007	*Model:* Adult male Fischer 344 rats *Injury:* L3/L4 dorsal column Tx	■ OECs from adult or P7 olfactory bulbs of Fischer 344 rats, either purified (98%) or as mixtures of otfactory cells (10-50% p75 + cells mixed with fibroblasts, endothelial, meningeal cells) injection of up to 4 μl (50,000-100,000 cells/μl) into lesion either as suspension or with matrix from cultures dish @ 0 hr PI **ACUTE**	SCI+ 1. adult OECs suspension n=8 2. adult OECs in matrix n=7 3. P7 OECs in suspension n=8 3–4 animals in each group were injected with BDA into the L4 DRG or CTB into the nerve Survival 2 weeks or 2–3 months;	*Histology:* extensive growth of axons (of undefined origin) into the lesion sites filled with OECs (both cell suspensions or cells with their matrix); new blood vessel formations. No evidence for significant regeneration of sensory ascending axons across the lesion sites (neither with BDA nor with CTB tracing) despite good filling of axons caudal to injury and some sprouting into the lesion sites – with any of the transplant types. *Electrophysiology:* dorsal column recordings demonstrated that only animals with transplants (but not controls) had preserved potentials cranial to injury (i.e. beyond the site of injury). Similar findings for sensory evoked cortical responses. The presense of electrophysiological function in the absence of axonal regeneration indicates either neuroprotective or neuroplasticity promoting effects of the transplants. *Behavior:* Not reported
Li *Science* 1997	*Model:* **Adult rats** *Injury:* **C1-C2 focal electrolytic lesion** unilaterally in medioventral part of dorsal columns.	■ **OECs** obtained from the olfactory nerve and glomerular layers of syngeneic adult rat OBs • OECs were cultured 14–17 d before transplantation, then injected @ **0 hr PI** **ACUTE**	**SCI+** **1.** OEC implantations n = 7 **2.** No transplantation n = 21 No SCI: **3.** Unoperated rats used in Behavioral testing n = 5 Survival: up to 3 months	*Histology:* Axons extended through the transplant and continued into the caudal host cord. Authors claim regeneration of corticospinal axons (see discussion). *Behavior:* Rats with more extensive lesions and no transplanted cells did not use the forepaw on the lesioned side for directed reaching @ 2–3 months PI. **Rats in which the transplanted cells had formed a continuous bridge across the lesion exhibited directed forepaw reaching on the lesioned side** like unoperated control animals.
Li *J Neurosci* 1998	*Model:* **Adult** female **rats** of a locally bred AS strain, 200–240 g *Injury:* **C1-C2 focal electrolytic lesion** to attempt destroying the corticospinal tract.	■ **OEC**s from the outer nerve fiber and glomerular layers of adult female AS rat OBs. 100,000 cultured OECs were injected into the lesion site @ **0 hr PI** • Immunosuppression not mentioned **ACUTE**	**SCI+** **1. OEC** transplantation n = 86 **2.** No transplantation n = 22 No injury: **3.** Intact n = 5 Survival: between 6 d to 3 months; n = 10 at 6d; n = 19 at 10d; n = 7 at 2 wk; n = 21 at 3 wk; n = 51 at 4 wk; n = 4 at 6 wk; n = 3 at 7 wk; n = 11 at 9 wk n = 3 at 3 months.	*Histology:* Within the first week after transplantation, the cut corticospinal axons (identified by anterograde transport of biotin dextran) extended caudally along the axis of the corticospinal tract as single, fine, minimally branched sprouts that ended in a simple tip, often preceded by a small varicosity. ■ By 3 wk PI the traced axons, ensheathed by P0-positive peripheral myelin had accumulated into parallel bundles, which extended across the full length of the lesion into the caudal part of the host corticospinal tract. *Behavior:* Not reported

4. OECs from Olfactory Bulb or Lamina Propria of Prenatal or Newborn/ Juvenile Rodents

Boyd *PNAS* 2004	*Model:* **Adult** female Wistar **rats**, 225–250 g *Injury:* **T10 Clip compression,** 22g	■ **OECs** from E18 Wistar rat (ONL), transfected with B-galactosidase (LacZ), injected into the cystic cavity @ 1 wk PI • 4×10^5 OECs in a volume of 4 μl of grafting solution at a rate of 1 μl/min • Immunosupression not mentioned **SUBACUTE**	**SCI+** **1.** OEC with LacZ n = 10 **2.** OEC without LacZ n = 22. **3.** Grafting media n = 13 Survival: 3 wk post transplantation	*Histology:* 3 weeks post transplantation: GAP-43-positive axons (most likely of CNS origin) and Schwann cells were seen invading the cystic cavity. • LacZ-expressing OECs were visualized in the injury site and did not migrate from the site of implantations; LacZ OECs were not observed directly associating with myelinated or unmyelinated axons *Behavior:* Not reported
Richter *J Neurosci* 2005	*Model:* **Adult** male SD **rats,** 150–200 g *Injury:* **C3-C4 dorsolateral funiculus crush** (1 mm deep×18–20s)	■ **OECs** from **OB or Lamina Propria** of P5 mice expressing eGFP, injected **@ 0 hr PI** either into the lesion site; or 1 mm rostral/caudal (R/C) to lesion site Each rat received a total of 75,000–90,000 cells in a volume of 1.5 μl • **CsA** 10 mg/kg/d, i.p **ACUTE**	**SCI+** **1.** LP OECs into lesion site $n = 5$; n = 3 **2.** LP OECs R/C $n = 4$; n = 5 **3.** OB-OECs into lesion site $n = 4$ **4.** OB-OECs R/C $n = 4$; n = 4 **5.** DMEM control into lesion site $n = 4$ **6.** DMEM R/C $n = 4$; n = 5 Survival: 28d (1^{st} n) or 24 hr (2^{nd} n)	*Histology:* At 28 days: • OECs survival was better when transplanted R/C than into lesion site. • 45% of OB-OECs were still found in caudal injection point, compared to only 6% of LP-OECs • significantly greater number of LP OECs migrated than OB OECs, in vivo and in vitro. • LP-OECs stimulated outgrowth of axonal subpopulations but also significantly enhanced autotomy *Behavior:* Not reported **Autotomy was reduced by OB-OECs but increased by LP-OECs**
Imaizumi *Brain Res* 2000	*Model:* **Adult** Wistar **rats** *Injury:* **T11 Tx of dorsal column.**	■ **OECs** from P2–4 Wistar rat pup **Obs** □ **Schwann cells** from the sciatic nerves of adult female Wistar Rats. Injections into the dorsal column at depths of 0.5 and 0.7 mm rostral and caudal (about 0.5 mm) to the lesion, 60,000 cells **@ 0 hr PI** • Immunosupression not mentioned **ACUTE**	**SCI+** **1.** Neonatal OECs **2.** Schwann cells **3.** control DMEM n = 7–12 per group/ per measurement Survival: 5–6 wk	*Histology, Physiology:* Donor cells migrated into the denervated host tract. Limited number of regenerated axons was observed. • Following cell transplantation, **impulse conduction was restored for over a centimeter beyond the lesion.** • More peripherally myelinated axons traversing the lesion in SC or OEG transplants (SC: 421; OEG: 536; vehicle: 168) and detected immediately rostral to lesion. Regenerated axons conducted faster and had larger axon areas than normal axons. *Behavior:* Not reported
Chuah *Exp Neurol* 2004	*Model:* **Adult "rats"**, 270–380 g *Injury:* **T8-T9 Tx of dorsal columns.** T8–9 laminectomy.	■ **OECs** from P3 Wistar rat pup **OB-ONL and mucosa** • OECs injected 1 μl at each of 4 stereotaxic sites rostral and caudal to the lesion + 4 μl in the lesion, 80,000 cells in total **@ 0 hr PI.** • OECS encapsulated in culture medium (CM) capsules inserted into the dorsal median sulcus of the spinal cord, centred anteroposteriorly on the injury site **@ 0 hr PI.** **ACUTE**	**SCI+** **1.** Encapsulated OECs n = 5 **2.** Injected + Encapsulated OECs, n = 6 **3.** CM-filled capsules n = 6 **4.** Injected + Encapsulated CM n = 5 **No SCI** n = 4 Survival: 5 wk PI.	*Histology:* • No significant difference in the number of anterogradely traced CST fibers in all treatment groups; few regenerating fibers from the sectioned CST of rats which had received OECs. • More axonal branching was seen in encapsulated OEC group compared to control groups. *Behavior:* Not reported

(*Continued*)

Table 4. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Lopez-Vales Neurobiol Dis 2006	*Model:* **Adult** female SD **rats**, 250–300 g *Injury:* **T8 Complete Tx**	■ **OECs from P22–P23** SD rat OBs • Two injections 1 mm rostral, two injections 1 mm caudal to the lesion epicenter 375,000 cells in 3 μl in total @ **0 hr, 30 min, or 7 d PI** **ACUTE & SUBACUTE**	**SCI+** **1.** OEC, 0 hr PI (immediate) **2.** OEC, 30 min PI (acute). **3.** OEC 7 days PI (subacute). **4.** Control: DMEM/F12 30 min PI **5.** Control: DMEM/F12 7 d PI. n = 8 per group Survival: 9 months	*Histology, Physiology:* • Claim of long axonal regeneration of raphespinal, coerulospinal, and corticospinal axons within the caudal cord stump. • Expression of GFAP and NG2 was down-regulated in perilesional cord segments in transplanted animals, • **OEC transplants recover motor evoked potentials (MEP).** *Behavior:* **OEC reduced hindlimb hyperreflexia, and significantly recovered some movements of hindlimb joints** (BBB: control group: 0–2; acute transplant: 4.4; delayed transplant: 3.7). The BBB scores significantly correlated with the MEP amplitudes
Lopez-Vales Glia 2007	*Model:* **Adult** female SD **rats**, 250–300 g *Injury:* Complete **T8 Tx**	■ **OECs** from **P22-P23** SD rat **OBs;** p75 192 immunopanning. 4 injections: rostral, caudal and two lateral injections 375,000 cells in 3 μl in total @ **45 d PI** **CHRONIC**	**SCI+** **1.** OEC n = 12 **2.** DMEM Control: n = 8 Survival: 5 months	*Histology, Physiology:* • **No** significant amounts of CST (BDA labeled) and serotonergic (5HT+) axons regrowth through the lesion site and into the caudal spinal cord in OEC animals. • Some serotonergic axons elongated long distances through the gray matter. • Delayed transplantation of OECs failed to reduce posttraumatic astrogliosis. • The electrophysiological tests (**MEP**) - **limited recovery in OEC** vs. no recovery in control. *Behavior:* **BBB score of 2.50 in OEC group vs. 0.92 in control** ($P < 0.05$)
5. OECs from Pieces of Lamina Propria (Olfactory Nasal Mucosa) of Adult Rats				
Lu *Brain Res* 2001	*Model:* **Adult** female SD **rats,** 250–300 g *Injury:* **T10** Complete **Tx** (1–2 mm gap).	■ Pieces of lamina propria (**LP**) or **OEC**s from **LP** of **adult** SD rats, transplanted **@ 0 hr PI**. Suspensions of 100,000 cells in 1 μl, injected into dorsal columns, ventral funiculus, and ventral grey commissure of each rostral and caudal spinal cord stump • Three animals with transplants of LP were re-transected through the scar tissue at T10 @ 10 wk PI **ACUTE**	**SCI+** **1.** 4–6 pieces of olfactory LP n = 10 **2.** OEC suspensions n = 9 **3.** OEC suspensions + rat collagen matrix filled with OECs n = 9 **4.** Medium + rat collagen matrix n = 4 **5.** 6–8 pieces of respiratory LP n = 6 **6.** Rat collagen matrix n = 4 Survival: 10 wk.	*Histology and Physiology:* In the olfactory tissue groups: • Nerve fibres passed through the transection site. • Serotonegic fibres found distal to the transection site, and retrograde labelling of brainstem raphe and gigantocellularis neurons were observed, indicating regeneration of descending pathways. • The recovery of spinal reflex circuitry was observed, assessed using the rate-sensitive depression of the H-reflex from an interosseous muscle. *Behavior:* **10 wk PI both LP and OEC animals partially recovered movement of their hind limbs reaching 6–8 BBB points vs. 0–2 point in controls**); this was abolished by a second spinal cord transection.

Lu *Brain* 2002	*Model:* **Adult** female SD **rats**, 250 g *Injury:* **T10 Complete Tx**	■ Pieces of **lamina propria** containing OECs from **adult** rats, placed into the spinal cord gaps □ **Respiratory lamina propria** pieces □ **Collagen** matrix All interventions @ **4 wk PI.** **CHRONIC**	**SCI+** **1.** OEC implantation (pieces of OECs) n = 6 **2.** Transplants of respiratory lamina propria pieces n = 7 **3.** Implants of a collagen matrix n = 3 Survival: 10 wk PI for histology, 14 wk for BBB	*Histology:* • Serontonergic axons were observed caudal to the transaction site in 2/3 OEC animals. Longest distance being 4 mm • No recovery in respiratory LP transplant animals *Behavior:* **BBB significantly improved: 4.3 ± 0.8 vs. 1.0 ± 0.2 in control**
Steward *Exp Neurol* 2006	*Model:* **Adult** female SD **rats**, 250 g, 4–5 month old *Injury:* **Complete T10 Tx** .	■ **Olfactory lamina propria** (LP) □ **Respiratory lamina propria** (controls) • both from adult female SD rats (250 g), inserted to fill the gap after dissection of scar tissue. • 7–8 pieces of OLP or RLP @ **4 wk PI** **CHRONIC**	**SCI+** **1.** Olfactory LP n = 3; n = 5 **2.** Respiratory LP n = 6; n = 3 Survival: 10 wk post-transplantation	*Histology:* Fluorogold injections caudally did not reveal evidence for regeneration of descending axons across transaction site • A few serotonergic axons extended in both respiratory and olfactory LP transplants and a few serotonergic-axons were found caudal to the injury in 2 animals that received olfactory LP transplants and 1 animal that received respiratory LP transplant. *Behavior:* **No significant difference in BBB scores (0–1) between groups at any time point. No difference in bladder retention of urine.** *NOTE: This was an attempt to replicate the study of Lu et al, Brain 2002.*
6. OECs from Immortalized Cell Lines				
Moreno-Flores *Mol Ther* 2006	*Model:* **Adult** male Wistar **rats** *Injury:* **C3 bilateral dorsal** columns lesion with **forceps**	■ **Immortalized rat OECs** (TEG1–20), clonal cell line TEG3, which is a SV40 large T antigen stable transfectant of OEG primary cultures prepared from adult rat **olfactory bulbs**. • 300,000 cells injected into the dorsal half of the cord (depth 2 mm) at 3 rostrocaudal levels: at lesion site and 1 mm caudal and rostral to the lesion @ **0 hr PI** **ACUTE**	**SCI+** **1**. OEC-TEG3 n = 20 **2**. DMEM n = 12 No SCI: **3**. Sham-operated n = 6 **4**. Intact animals n = 6 Survival: 2, 4, 10 wk PI	*Histology:* In the injured spinal cord, prelabeled OEC-TEG3 cells survived for at least 10 wk after grafting and they integrated into the spinal cord, adopting Schwann cell-like, astrocyte-like, or intermediate morphologies. • In OEC-TEG3-transplanted animals, sensory projection axons grow into the lesion site and there was robust sprouting/axonal growth of the corticospinal tract, both into and beyond the lesion site, after crushing of the spinal cord–dorsal columns. *Behavior:* OEC-TEG3-transplanted animals at 8 wk **recovered sensory** and **motor function** in tape removal and beam walking Behavioral tests.

d: day, days; hr: hour, hours; PI: post-injury; PT: post-transplant; SCI: spinal cord injury; s.c.: subcutaneous; SD: Sprague–Dawley; Tx: transection; wk: week, weeks

5HT: serotonin; BBB: Basso, Beattie and Bresnahan locomotor test; BDA: biotinylated dextran amine; BMSCs: bone marrow stromal cells; CGRP - calcitonin gene-related peptide; CsA: Cyclosporine; CSPG - chondroitin sulfate proteoglycan; CST - cortico-spinal tract; DMEM: Dulbecco's modified Eagle's medium; EGFP: enhanced green fluorescent protein; FBs: fibroblasts; GDNF: glial cell line-derived neurotrophic factor; GFAP: glial fibrillary acidic proteins; GFP – green fluorescent protein; LacZ – beta-galactosidase; LP: Lamina Propria; MEP: motor-evoked potentials; MG: matrigel; MP: methylprednisolone; NF = neurofilament; OEC: olfactory ensheathing cell; OEG: olfactory ensheathing glia; OB: olfactory bulb; ONL: olfactory nerve layer; SCs: Schwann cells.

or corticospinal axons across and beyond the lesion site was also not found in the studies using olfactory bulb derived OECs from adult rats (Deumens et al., 2006; Ruitenberg et al., 2003) or with OECs from the mucosa of newborn mice (Bretzner et al., 2008; Lu et al., 2006). Behavioral benefits were seen when the adult bulb-derived OECs were transfected to express BDNF/NT-3 (Ruitenberg et al., 2003). This might have been due to the enhanced plasticity and neuroprotection by these trophic factors in the absence of significant regeneration.

Four studies evaluated OECs derived from the olfactory bulb of prenatal or newborn rodents (one and four studies using a blunt or sharp injury model respectively) but none reported behavioral outcomes. There is consistent evidence for axonal growth into the OEC-filled lesion sites, yet only one study demonstrated axonal growth and myelination across and beyond the dorsal column lesions that was confirmed by electrophysiology (Imaizumi et al., 2000a,b). These same authors reported similar restorations of conduction in the dorsal columns of rats when transplanting OECs from the olfactory bulbs of pigs.

The approach of transplanting pieces of olfactory nasal mucosa into a T10 full transection injury model has been championed by Lu and colleagues (2001, 2002). When transplanted acutely after injury, the authors reported that OECs improve open field locomotor scores from values of around 0–2 in controls to 6–8 in treated animals. Intriguingly, significant improvements were still observed when these transplantations were performed 4 weeks after injury (Lu et al., 2002); however, these results were not seen in a formally conducted replication study that was performed in the laboratory of Steward and colleagues (2006). It is worth mentioning that a similar protocol is already used in chronically injured humans using an autotransplantation paradigm (Lima et al., 2006). While this systematic review of cell transplantation was in review for publication, an unblinded, non-randomized study by Lima and colleagues (2010) reported improvements in 11 of 20 individuals with chronic (>18 months) SCI, including six individuals who improved from an AIS score of A to C (motor/sensory complete to motor and sensory incomplete). It must not be overlooked that this intervention was combined with a very aggressive rehabilitation regimen.

Despite the description of culture conditions for human OECs (Barnett et al., 2000) only one animal spinal cord injury study to date has used human OECs (p75-positive "OEC-like" spinal cord injury cells) harvested from the outer layers of the olfactory bulbs from human fetuses 5 to 7 months gestation (Deng et al., 2008). After a severe contusion injury, the transplantation of these cells alone was reported to improve open field locomotor scores from 6 to 11, and from 6 to 15 when combined with human bone-marrow stromal cells. These data clearly require independent replication.

Taken together, the literature on OECs contains many claims of axonal regeneration that cannot be confirmed independently by others. The reasons for these discrepancies are not fully understood, although experimental bias, variability of the cell sources and culture conditions, and animal or injury model systems are all likely contributing factors. Importantly, about two thirds (13 of 18) of the studies reported improved behavioral outcomes, yet increased autotomy was seen in some studies (Guest et al., 2008; Richter et al., 2005), which raises the question of neuropathic pain and cautions against the indiscriminative application of OECs. Further studies with human OECs are clearly warranted, but these will require a better understanding of OEC biology with which to strengthen the rationale for human translation. A summary of the pros and cons and knowledge gaps for olfactory ensheathing cell transplantation is depicted in Table 5.

Neural stem/progenitor cells (Table 6)

Adult neural stem/progenitor cells (aNPCs) are typically harvested from the subventricular zone of the brain or the spinal cord of rodents, and amplified as neurospheres in EGF and/or bFGF for several rounds of passages. They contain precursors for neurons, astroglia, and oligodendrocytes – and likely some stem-like cells with capacity for self-renewal.

Adult rodent NPCs were applied to thoracic contusion or compression injuries in eight rodents studies (six rat, two mice) and to cervical dorsal column transections in four rat studies. A subacute regimen was chosen in most of these studies. While some authors reported mainly astrocytic differentiation of the transplanted aNPCs (e.g., Cao et al.,

Table 5. Summary Statement for Olfactory Ensheathing Cell Transplantation

Pros	*Cons*
Demonstrates good integration into host spinal cord; claims of axonal sprouting/regeneration reported.	No robust behavioral benefits after transplantation into moderate or severe thoracic contusion injuries.
Behavioral improvements have been frequently reported, albeit after partial or full transection SCI models. Improvements may be due to some axonal regeneration, although trophic effects on host spinal cord likely play a role.	Efficacy of olfactory lamina propria pieces in chronic thoracic transection models could not be formally replicated.
Offer the possibility of autologous transplantation, although human protocols for such procedure still need refinement (see below)	In many cases, appears to require adjuvent treatment to increase efficacy (e.g. Schwann cells, Matrigel, rolipram, cAMP, neurotrophic factors)
Knowledge Gaps	
The body of literature focused mainly on partial or full laceration models, which are clinically rare. More data on the performance of these cells in contusion injuries is needed.	
Efficacy in a chronic injury model is currently lacking, and is particularly important given that this cell transplantation strategy has been forwarded into human patients with chronic SCI.	
Only one study published on human olfactory bulb derived cells in the injured spinal cord.	
A better understanding of the possible effects on sensory perception and provocation of pain is needed.	
Optimization of biomaterials to support survival and OECs bridging lacerations is still desirable.	

TABLE 6. NEURAL STEM/PROGENITOR CELLS

Generally, NSPCs are taken from the CNS of rodent (or human aborted) embryos and expanded as neuropheres (in the presence of EGF and/or bFGF or other trophic factors) before they are dissociated and injected. These neuropheres contain precursor cells for Neurons, Astrocytes, and Oligodendrocytes plus stem cells capable of self-renewal. Heterogeneities likely exist due to some variations in the donor species, ages, passages, anatomical origins and trophic factors/culture conditions used.

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
1. Neural Stem/Precursor Cells from Prenatal/Embryonic Humans (huNSPCs)				
Iwanami *J Neurosci Res* 2005	**PRIMATE** *Model:* **Adult** female common **marmosets,** 280–350 g *Injury:* **C5 contusion** with modified **NYU** Impactor (tip 3.5 mm, 17g×50 mm onto exposed dura)	■ **Human eNSPC** from Human fetuses (8 weeks, abortions), >10 passages, BrDU labeled, • 1 million cells in 5 μl of medium into the lesion epicenter **@ 9 d PI.** • **CsA** daily for 8 wk 10 mg/kg s.c. **SUBACUTE**	**SCI+** **1**. NSPC n = 5 these cells are 52% nestin, 12% βIII tubulin, 34% GFAP positive **2**. Media injection n = 5 Survival: 8 wk	*Histology:* • **Smaller lesions** in MRIs (T2 rated) • Differentiation in vivo into 25% nestin, **21% βIII tubulin**, 46% GFAP, **5% Olig2 ve + cells.** • Neurofilament positive bundles (axons) in lesion site filled by transplanted NSPCs. *Behavior:* **Significant improvement of bar grip power and spontaneous motor activity in the NSPC transplanted group**
2. Neural Stem/Progenitor Cells from Human Immortalized Cell Lines These cells are initially isolated like the NSPC cells but subsequently selected and immortalized by e.g. c-myc transduction. Hence their characteristics are less hetereogenous than the NSPCs and at the same time they may be significantly different.				
Lee *Neurol Res* 2009	***LARGER MAMMAL*** *Model:* 18-month-old mongrel **DOGS**, 25–32 kg *Injury:* **L2 Lateral** (left-sided) **hemisection**	■ **Human neural stem cell line** (hNSCs**, HB1.F3 clone**) immortalized from human embryos (15 wk of gestation) Transplantation in canine spinal cord hemisection **@ 0 hr PI** No immunosuppresion **ACUTE**	**SCI+** **1**. Matrigel seeded with hNSCs (1×10^7 cells/200 μl) n = 5 **2**. MG (200 μl) alone as a growth-promoting matrix n = 7 Survival: 12 wk.	*Histology:* Human nuclei-positive cells were found mainly near hemisected areas in dogs treated with Matrigel + NSCs. • Colocalization of human nuclei and neuronal nuclei or myelin basic protein was observed • NSCs were found to express neuronal and/or oligodendrocyte markers • The MG + NSC group showed more ascending sensory axon regeneration. *Behavior:* Using a **canine** hind limb locomotor scale, assessed over 12 weeks. **Significantly better functional recovery in hNSC group (15.6 ± 0.7 versus 10.3 ± 0.7**, respectively, at 12 weeks; $p < 0.05$).
Takeuchi *Neurosci Lett* 2007	***RODENT*** *Model:* Female KSN **mice**, 8 wk old *Injury:* **T8 Weight compression** 10 g, 2 mm×1 mm for 5 min	■ **Human neural stem cell line** (hNSCs**, HB1.F3 clone**) established from human embryos 14 wk of gestation • Labeled with LacZ vector or CellTracker (CM-DiI); 10 million cells in 100 μl **i.v. in tail vein** **@ 3 d PI** or **@ 7 d PI** or **@ 10 d PI** **SUBACUTE**	**1**. SCI + hNSCs n = 7; n = 8; **2**. SCI + PBS n = 7 **3**. Uninjured + hNSCs n = 7 1st number: injected at 7 d; 2nd number: injected on either day 3, 7, 10 PI **4**. Additional groups like **1** & **3** for BBB n = 8.	*Histology:* Labelled NPCs were found at the spinal cord lesion site; more cells found when injected on day 7 than on day 3 or 10. This correlated with a peak in expression of hepatocyte growth factor, stromal derived factor 1. Authors present CellTracker positive cells that also stain for GFAP and βIII tubulin – no confocal confirmation *Behavior:* **A trend of a better BBB score at 21 days (p = 0.06.)**

(*Continued*)

TABLE 6. CONTINUED

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Tarasenko *J Neurosci Res* 2007	*Model:* Male SD **rats,** 230–240 g *Injury:* **T10 contusion,** 130 kDyn force, **IH** Impactor, no dwell time	■ **Human fetal NSPC (line K048)** isolated from 8 week forebrain expanded in EGF/FGF/LIF; □ **Primed** with laminin • **Unprimed** • AAVegfp transduced 200,000 in 2 μl at epicenter injected **@ 0 hr PI** or **@ 3 d PI** or **@ 9 d PI** Oral **CsA** in drinking water 100μg/ml **ACUTE-SUBACUTE**	**SCI+** **1**. Primed hNSC n = 28 **2**. Unprimed hNSC n = 25 **3**. Vehicle n = 25 **4**. Sham n = 13 7–10 animals for each of the injury-transplant intervals Survival: 3 months	*Histology:* Only 0.75% of primed and 0.47% of unprimed cells survived at 3 months when they had been transplanted on day 9 after injury; • Differentiation of primed hNSC towards oligodendroctes (39% OSP+) and neurons (38% MAP-2+) – somewhat less in the unprimed group *Behavior:* **No significant differences** in transplanted vs. controls at 3 months in BBB, activity box, **only the duration of rearing in the 9th day graft group was normalized** (interpreted as improved trunk stability)
Cummings *PNAS* 2005	*Model:* NOD-*scid* **mice** (immunodeficient) *Injury:* **T9 contusion** 50 or 60 kdyn **IH** Impactor	■ **HUMAN Neural stem/ progenitor cells;** referred to as hCNS-SCs from **long term** neurospheres cultures • originially from "human fetal spinal cord and brain tissues" □ Human fetal liver **fibroblasts (FBs)** 75,000 cells per μl; 250 nl at T8 and T10 **@ 9 d PI** **SUBACUTE**	50 kdyn **SCI+** **1**. hCNS-SCs n = 11 **2**. Vehicle n = 15 60 kdyn **SCI+** **3**. hCNS-SCs n = 8 **4**. hFBs n = 8 **5**. Vehicle n = 8 Survival: 24 hr, 48 hr, 4 & 17 wk post transplantation	*Histology:* Extensive migration from the injections sites >1 cm from lesion epicenter; Wrapping of spared axons by hCNS-SC using light microscopy and electron microscopy – indicative of **myelination**; • Immunohistochemistry with human specific cytoplamic AB (SC-121) **Neuronal differentiation** (labeled synapses) by immuno-electron-microscopy *Behavior:* BBB scores in mice: 50kdyn exp: **More hCNS-SC animals reached some FL-HL co-ordination** (scores >12); **scores ~13 vs. ~11**. 60kdyn exp.: **higher frequency of stepping** in the hCNS-SC group than both control groups 4 and 5. BBB ANOVA not significant, hCNS-SC not sig different from fibroblast group; scores ~10.7 vs. 10. Linear horizontal ladder beam task: hCNS-SC made fewer errors than controls; effects lost post diphtheria toxin killing of the human cells in both experiments
3a. Neural Stem/Progenitor Cells from Prenatal/Embryonic Rodents (reNSPCs) ***(Blunt Injury Models)***				
Ogawa *J Neurosci Res* 2002	*CERVICAL* *Model:* **Adult** female SD **rats**, 200–230 g *Injury:* **C4-C5 weight compression** 35g placed onto dura for 15 min (3.0 by 2.2 mm)	■ **NSPCs embryonic rat spinal cord** (E14.5 SD or Ta1-EYFP transgenic **rats**) as neurospheres **bFGF**, 2–5 passages, BrdU labeled; 100,000–400,000 cells injected into cavity in a volume of 20–40 μl **@ 9 d PI** **SUBACUTE**	**SCI+** **1**. NSPCs (no FGF) n = 15 **2**. Media (no FGF) n = 17 **3**. Injury alone n = 13 **4**. Naïve unoperated n = 10 **5**. Another group received Tα1-EYFP NSPCs plus BrdU i.p. *("n" unknown)*	*Histology:* 5 wk after transplantation transplanted BrdU positive cells express neuronal (5.9%, Hu) astroglial (GFAP 32.6%) and oligodendrocyte (CNP, 4.4%) markers. • Immunostaing for neuronal marker Hu and BrdU (given to host) suggested that transplanted cells divided and expressed neuronal markers. Immuno electron microscopy revealed EYFP + ve synaptic structures - integration *Behavior:* **Skilled reaching task** (Bregman & Diener): On day 44**, 13 of 15 transplanted rats could reach and eat more than 5 food pellets versus 9 of 17 control rats**. Testing session after a 48 hr fast – (only day 44 reported)

Okada *FASEB J* 2005	***THORACIC*** *Model:* **Adult** female C57BL/6J **mice,** 20–22 g *Injury:* **T10 contusion**, 60 kdyn **IH** Impactor	■ **NSPCs** from striatum of E14 **mice** C57BL/6J; as neurospheres **EGF/ bFGF** 2 passages; • Transduced with lentivirus luciferase and GFP 500,000 injected either **@ 0 h PI** or **@ 9 d PI** presumably into lesion epicenter (missing detail) **ACUTE & SUBACUTE**	**SCI+** **1**. Acute NSPCs @ time of injury n = 8 **2**. Delayed NSPCs @ 9 d PI n = 8 **3**. Medium @ 9 d PI n = 8 Plus calibration group	*In vivo Bioluminescence study:* Calibration of cell number to signal was linear. Drastic reductions in signal by 80% within the first 4 days after injury; stable after that for 6 wk around 20%; no difference in signal between immediate vs. delayed transplantation (TP); in the delayed paradigm, light signal spread further caudally indicative of migration. *Histology:* Mainly astrocyte differentiation 80%; higher % of differentiated neurons and oligodendrocytes ~8–10% in the delayed group vs. ~1% the acute group. Some caudal migration of what appears to be neurons; *Behavior:* **BBB** score (in mice): locomotor scores after 6 wk in acute (~10.0) and delayed transplantation **(~10.6) versus ~8** in controls, **reached significance in only is subacute = delayed transplantation group.**
Ikegami *Eur J Neurosci* 2005	*Model: Female SD rats, 230–250 g* *Injury: T10 contusion, NYU/ MASCIS 10g×25 mm*	■ **NSPC** grown as **EGF/bFGF** neurospheres from E14 striata of GFP expressing SD **rats** • 5 μl (2.0×105 cells/μl) were injected into the lesion epicenter **@ 2 wk PI** □ 0.2 ml of 200 U/ml **ChABC** or inactivated control ChABC in saline intrathecally via osmotic minipump @ 1 wk PI for 1 wk. **SUBACUTE**	**SCI+** **1**. ChABC, n = 12 **2**. Inactivated ChABC n = 11 **3**. ChABC + NPC n = 14 **4**. Inactivated ChABC + NPC n = 7 **5**. Inactivated ChABC + conditioned media n = 5	*Histology:* CSPG decreased the migration of NPC *in vitro* • ChABC significantly reduced the amount of CSPG to a level comparable to uninjured controls • ChABC treatment increased the migration and integration of transplanted NPCs • Significant differences between groups with regards to GAP-43 IR axons in and around lesion site. *Behavior:* Not reported
Meng *Cell Biol Int* 2008	*Model:* Female Wistar **rats,** 280–350 g *Injury:* **T9-T11 contusion**, modified **NYU** with diameter of 3.5 mm, 20g×30 mm	■ **NSPCs from forebrain** of fetal Wistar **rats** grown as neurospheres in **EGF/bFGF**, Iabeled with Hoechst □ **Rat amniotic epithelial cells (AEC) from E-14-E16** Wistar rats Transfected with eGFP-h**bFGF** or control eGFP-C1 control construct • 2.5 μl at a concentration of 1.0×10^5 cells/μl were transplanted into the injured spinal cord**@ 7 d PI** **SUBACUTE**	**SCI+** **1**. NSPC n = 12 **2**. NSPC + AEC with eGFP-hbFGF n = 12 **3**. NSPC + AEC with eGFP-C1 n = 12 **4**. Saline control n = 12 **5**. Sham surgery n = 6	*Histology, Physiology:* Expression of transfected mRNA was detected by RT-PCR • **Transplanted cells reported to express MAP-2** *(based on Hoechst label)* • More NPC expressing MAP-2 in group 2 > group 3 > group 1 • **Amplitude (μV) of CSEP and CMEP** in group 2 (0.29/ 0.24) > group 3 (0.24/ 0.18) > group 1 (0.19/ 0.13) > group 4 (0.19/ 0.13) • **Latency (ms) of CSEP and CMEP** group 2 (2.86/ 2.89) < group 3 (3.09/ 3.06) < group 1(3.42/ 3.28) < control (3.66/ 3.7) *Behavior:* Animals were tested with **BBB** scale and on their ability to climb stairs. • Authors claim that group 2 **NSPC + AEC + bFGF (~14)** > group3 (~12) > group 1 (~9.5) > **control (~7)**

(*Continued*)

Table 6. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Wu *Neurosci Lett* 2002	*Model:* SD **rats**, 4 wk old, 70–90 g *Injury:* **T8/9 compression** 25 g glass rod placed on T8/9 dura mater for 90 sec.	■ **NSPCs** from E16 embryonic hippocampus GFP transgenic SD rats cultured as neurospheres **bFGF** • 1×10^6 cells were injected **intrathecally** (through the fourth ventricle) **@ 0 hr PI** **ACUTE**	**SCI** + embryonic neurosphere cells: **1**. Fourth ventricle (1×10^6 cells) n = 18 **2**. Cisterna magna (1×10^6 cells) n = 18	*Histology:* Animals were sacrificed at 1, 3, and 6 weeks after SCI (n = 6 for each time point). Immunohistochemistry: • Transplanted cells were transported by CSF within the subarachnoidal space, and survived as clusters on the pial surface of the spinal cord. Some cells migrated into the lesion site and became integrated with the host tissue. • No quantification. *Behavior:* Not reported
Setoguchi *Exp Neurol* 2004	*Model:* Male outbred ICR strain, 15 wk old *Injury:* **T9 compression** 30 g of extradural static weight for 2 min	■ **NPSCs** from E14 ICR **mice** (as neurospheres **bFGF**), transfected with a vector that expressed **noggin** or a control vector, 1μl (1×10^5cells/μl) was transplanted into lesion site **@ 8 d PI** • Labeled with DiI or GFP • **CsA** daily 10 mg/kg and gentamicine 8 mg/kg **SUBACUTE**	**SCI+** **1**. NPSCs n = 13 **2**. NPSCs transfected with noggin n = 13 **3**. Media control n = 13	*Histology:* Authors claim 100% of NPSCs express GFAP, while those with noggin have ~5 % expressing βIII tubulin and ~6% expressing GSt π with the rest expressing GFAP. • Cells survived @ 4 wk post transplantation *Behavior:* **NPC ~7 and NPC with noggin ~10 reported to improve on the BBB compared to media control ~3**.
Kimura *Stem Cells* 2007	*Model:* **Adult** female Lewis rats, 220 ± 25 g *Injury:* **T8 contusion IH** 200kdyn	■ **NSPC** from E 14.5 **rat** forebrains as neurospheres in **EGF/bFGF** (5×10^5) transplanted 8 mm caudal and 0.8 mm deep relative to the epicenter **@ 7 d PI** shRNAi inhibition of S1p1 (sphingosine phosphate pathway) • **Tacrolimus** (FK506) 0.64 mg/kg intramuscularly daily **SUBACUTE**	**SCI+** **1**.NSPC transplant + random shRNAi sequence **2**. NSPC transplant + shRNAi interference of $S1p_1$ n = 3–4 per group/per measurement	*Histology:* All tissue was assessed 3 days after transplantation. • Transplanted NSPCs migrated to the injury site. • Inhibiting the sphingosine 1-phosphate (Sph-1-P)/$S1P_1$ pathway (via RNA interference of the receptor on the NSPCs cells) inhibited migration of the NSPCs. *Behavior:* Not reported
Fujiwara *Neurosci Lett* 2004	*Model:* Male SD **rats** (230 g avg.) *Injury:* **compression** 25-g glass rod put on **T7** exposed dura mater for 90 sec.	■ **NSPCs** from E15 fetal hippocampus GFP transgenic **rats** cultured as neurospheres in **bFGF** only. • 1×10^5 cells or PBS media control were transplanted intravenously via penile vein **@ 24 hr PI** • **FK506** 1 mg/kg 30 min before transplantation and then daily **ACUTE 24 hr**	**SCI+** **1**. NPCs n = 34 **2**. Media (PBS) control n = 16	*Histology:* The intravenously transplanted NPC (labeled with GFP) migrated to the lesion site. The migrating NPCs were claimed to differentiate into neurons, astrocytes, and oligodendrocytes, and some survived up to 56 days post injury. *Behavior:* Not reported.

3b. Neural Stem/Progenitor Cells from Prenatal/ Embryonic Rodents (reNSPCs) *(Sharp Injury Models)*

Pan *J Clin Neurosci* 2008	*Model:* SD **rats,** 250–300 g *Injury:* **T8-T9 complete transection** with 2 mm gap	■ **NSPCs** from E14/15 SD **rats**; cortex, striatum and mesencephalon (as **neurospheres, EGF**) • 10μl (5×10^5cells/μl) or saline was transplanted into lesion site which was subsequently filled with fibrin glue and covered in gel foam **@ 0 hr PI** □ **G-CSF injections** for 5 days (50μg/kg/day) • Cells labeled with BrDu • 5 d before sacrifice all animals received injections of BrdU (50 mg/kg/day) for 5 d **ACUTE**	**SCI+** **1**. NPC n = 10 **2**. NPC with G-CSF n = 10 **3**. Saline control with G-CSF n = 10 **4**. Saline control n = 10	*Physiology, Histology:* With regards to MEP (mV) the NPC + G-CSF group (47.7 ± 3.17) > NPC group(38.97 ± 2.3) > G-CSF group(31.6 ± 3.1 and Control group (24.7 ± 3.5) • **Conduction latency smaller in** the NPC + G-CSF group (1.29 ± 0.02 msec) < NPC group(1.39 ± 0.04) and G-CSF group(1.39 ± 0.03) < Control group(1.54 ± 0.04) • **BrdU density at the lesion site** NPC + G-CSF group > NPC group > G-CSF group > Control group • **Neu-N expression 5 mm distal** to lesion site NPC + G-CSF group > NPC group > G-CSF group > Control group • **MAP-2 expression 5 mm distal** to lesion site NPC + G-CSF group > NPC group/ G-CSF group/ Control group • GFAP expression 5 mm distal to lesion site NPC + G-CSF group/ NPC group/ G-CSF group > Control group *Behavior:* **The NPC + G-CSF group (11.67 ± 0.17) > NPC group (9.63 ± 0.26) > G-CSF group (7.88 ± 0.22) > Control group (3.86 ± 0.26) on the BBB.**
Ishii *J Neurosci Res* 2006	*Model:* **Adult** female SD **rats,** 250–300g *Injury:* **T6** right **overhemisection** including both dorsal columns	■ **NSPCs from spinal cords** of E14 SD **rats**, as neurospheres **EGF& bFGF**) 2–3 passages, BrdU label, 10 million cells in a piece of gelfoam into lesion site **@ 0 hr PI** □ Infusion of **anti-CNTF** antibody intrathecally into vicinity of transplants for 14 d **ACUTE**	**SCI+** **1**.NSPCs anti-CNTF n = 8 **2**.NSPCs control n = 5 for 2 weeks survival **3**.NSPCs anti-CNTF n = 3 **4**.NSPCs control n = 3 for 6 weeks survival another group 8 wk survival	*Histology:* CNTF reached the target evidenced by less phosphor STAT-3 around the lesion; **significantly less GFAP immunoreactivity around the lesion site**; More extensive rostro-caudal migration of the transplanted cells; Fewer cells differentiated into astrocytes (about half the %) and more into oligodendroctyes (from 8% in controls to 18% in anti-CNTF treated rats by 4 wk). By 8 wk, significantly more CST axons in the transplant and spinal cord below the lesion indicative of SCT regeneration – but growth of spared CST axons from caudal into transplant not entirely ruled out. *Behavior:* Not reported

(*Continued*)

Table 6. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Lowry *Exp Neurol* 2008	*Model:* Female Swiss Webster **mice**, 10–12 wk old *Injury:* **T8 Dorsal over-hemisection** (1.0 mm depth)	■ **SPINAL CORD STEM CELLS** (SCSCs) **from early embryonic spinal cord of mice** (E8–9 ! rare!) using LIF and bFGF □ Co-culture with **Endothelial cells** from the bovine pulmonary artery (**BPAE**) were obtained commercially • NSPCs cultured with BPAE with or without **1μM Shh** and **1μM RA** were transplanted to injury site and 1 mm rostral (0.5μl of 10^5 cells/μl each) **@ 0 hr PI** • Labelled with GFP • **No CsA** **ACUTE**	**SCI+** **1**. SCSCs without Shh or RA treatment n = 7 (two groups) **2**. SCSCs with Shh and RA n = 7 (two groups) **3**. Saline injection n = 5	*Histology:* • In vitro, both co-culture with BPAE and addition of Shh + RA increase the ability of SCSC to produce neurons • In vivo, authors report that the Shh + RA treated cells were more likely to differentiate into oligodendrocytes where those not treated with Shh + RA were more likely to become astrocytes and migrated more away from the injury zone than with Shh + RA grown cells. *Behavior:* **Mice receiving cells with Shh + RA had decreased errors on horizontal ladder (2 vs. 0.5 errors per run) and time to sense tape on tape removal test (~0.1 vs. ~0.8 min)** compared to mice receiving cells without Shh and RA. *There were no baselines for these animals.*
4. Neural Stem/Progenitor Cells from Neonatal Rodents				
Guo *Spinal Cord* 2007	*Model:* Female SD **rats**, 200–220 g *Injury:* **T9–10 Tx,** a 2 mm segment was resected	■ **NSPC** from the hippocampus of **neonatal** SD rats were cultured. □ **Schwann cells** (SC) from **neonatal SD rats** were modified by AdvNT-3 (NT-3-SC) and AdvLacZ (LacZ-SCs). • 4×10^6 cells were transplanted into the type 1 collagen scaffold ($2\times2\times2$ mm^3) that was put into the injured cavity **@ 0 hr PI** **ACUTE**	**SCI+** **1**. Scaffold + NSPCs n = 10 **2**. Scaffold + SCs + NSPCs n = 10 **3**. Scaffold + LacZ-SCs + NSPCs n = 10 **4**. Scaffold + NT-3-SCs + NSPCs n = 10 **5**. Scaffold + medium n = 10 **6**. No SCI, no transplant n = 10 Survival: 60 d	*Histology:* Some NSPCs in the injury site. In the transplanted groups 5-HT, CGRP, and SP positive fibers were found within the lesion site. Some evidence for axonal regeneration based on retrograde tracing. *Physiology:* The latency and amplitude of cortical motor (and somatosensory) evoked potential (CMEP, CSEP) improved in all transplanted groups shorter latency and higher amplitude than the SCI control group. The best group was the NT-3-SCs + NSPCs. *Behavior:* At 60 days after transplantation (the only time point tested) BBB scores: SCI **control = 0.54** ± 0.32, NSPCs = 3.63 ± 1.71, SCs + NSPCs 7.03 ± 2.35, LacZ-SCs + NSPCs = 6.87 ± 3.06, **NT-3-SCs + NSPCs = 10.76** ± 3.43. Inclined grid results discussed (better) – but no numbers given.

These cells are typically harvested from the subventricular zone of the brain and amplified as neuropheres in bFGF and EGF for several rounds of passages. They contain precursors for neurons, astroglia and oligodendrocytes – plus some stem-like cells with capacity for some self-renewal. They can be obtained from adult animals –here referred to as adult NPCs – or from embryos – here referred to as embryonic NSPCs.

5a. Neural Stem/ Progenitor Cells from Adult Rodents *(Blunt models)*

Alexanian *J Neurosci Methods* 2006	*Model:* **CATS** ***XENOTRANSP LANT.*** *Injury:* **T8–9 Contusion** weight drop (300g×cm = 25g×12 cm) T8–9 laminectomy	■ Adult **NPCs** from **SVZ** adult mice; (lineage restricted neural precursors) ~100,000 cells in 25μl **@ 14 d PI** • **Tacrolimus**, 0.3 mg/kg.day from the day of transplantation **SUBACUTE**	Sacrificed after **SCI** at: 2 d n = 1 2 wk n = 1 4 weeks n = 2	*Histology:* • In vitro characterization: Nestin positive, neuronal (NCAM) and glial (A2B5) precursor cells were identified • Quantitative Immunohistochemical analysis of cell differentiation: Majority of the cells expressed βIII tubulin-(neuronal marker), GFAP (astrocyte marker) and immature oligodendroglial marker, NG2 in a ratio of 2.1 to 1.3 to 1. *Behavior:* Not reported
Cao *Expl Neurol* 2001	***RODENTS*** *Model:* **Adult** female Fischer 344 **rats**, 170–200 g *Imjury:* **T8 contusion** **NYU** Impactor (12.5-g/cm)	■ Adult **NPCs** from brain SVZ versus ■ **Embryonic NSPCs** (ES), cerebral cortices of E14 Rat Fischer 344 rats. • Cells (passage 2) labeled with BrdU, transplanted into epicenter (500,000 in 10 μl) or one segment rostral/caudal to the epicenter (150,000 cells) **@ 10 d PI** **SUBACUTE**	**SCI +** Either adult NPCs or embryonic NSPCs cells: **1./2.** Into epicenter n = 12 **3./4.** Rostro/Caudal n = 12 No SCI: **3.** Cells in to uninjured cord (1.5×10^5 cells) n = 12	*Histology:* Animals were sacrificed at 1, 2, 4, 8 weeks after SCI. • Adult NPCs in injured spinal cord mainly differentiated into GFAP-astrocytes, no oligodendrocytes or neuronal differentiation. • The percentage of nestin-positive cells decreased significantly along postgraft time in both normal and injured spinal cord • **ES** cells showed similar trend *Behavior:* Not reported
Ziv *PNAS* 2006	*Model:* C57BL/6J **mice** (Size or age not mentioned) *Injury:* **T12 contusion, IH** (force of 200 kdyn, for 1 s)	■ Adult **NPCs** from subventricular zone (**SVZ**) or mice, GFP labeled, via **intracerebro-ventricular (i.c.v.)** delivery **@ 7 d PI** number of cells not given □ T-cell-based vaccination with a **myelin-derived peptide** (MOG) @ 7 d Pre-Injury (1st number in the Groups) or @ 0 hr PI (2nd number in the Groups) **SUBACUTE**	**SCI+** **1.** Vaccination + aNPC (MOG/aNPCs) n = 7; n = 9 **2.** Vaccination and no NPCs (MOG/PBS) n = 7; n = 9 **3.** no Vaccination + aNPC (aNPCs/PBS) n = 6 **4.** No vaccination, no aNPC (PBS/PBS) n = 7; n = 7	*Histology:* • 1 wk after transplantation, smaller lesion site in the MOG/aNPC group • aNPCs migrated to the injury site and survived up to 60 d. Did not express neuronal or glial cells markers. • Less activated macrophages and microglia and more T-cell in MOG/aNPC • More BDNF and Noggin in the MOG/aNPC; • Increased endogenous neuronal differentiation! (BrdU and DCX) around the lesion site in the MOG/aNPC group *Behavior:* **BMS, Significant improvement in MOG/aNPCs group (4.2), vs. MOG/PBS s (2.7) vs. aNPCs/PBS (1.5) or PBS/PBS only (1.5) groups**

(*Continued*)

Table 6. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Bottai *Mol Med* 2008	*Model:* **Adult** male CD1 **mice** 28 - 30 g *Injury:* **T8 contusion IH** 50 kdyn (applied for 1s)	■ **aNPCs adult mice neural stem cells (NPCs)** were obtained from the subventricular zone of CD-1 albino GFP transgenic mice and cultured as neurospheres in **EGF/FGF** for 5–15 passages Within **30 min** after SCI 330,000 cells were injected **intravenously** the tail vein, followed by the same number of cells at 6hr and 18 hr. For **intraspinal** injections 50,000 cells were transplanted rostrally and caudally to the injury site. □ Murine **Fibroblasts** from skin i.v **ACUTE**	**Experiment 1:** **1**. SCI + i.v. NPCs n = 9 **2**. SCI + i.v. Fibroblasts n = 9 **3**. SCI + i.v. PBS n = 9 **4**. Laminectomized n = 9 **5**. Not laminectomized, and not lesioned n = 9 **Experiment 2: SCI+** **1**. NPCs i.v. **2**. Intraspinal injected NPCs **3**. intraspinal injected PBS (*"n" is not mentioned*)	*Histology:* 2% of the i.v. injected cells home in to lesion site but remained undifferentiated Spared tissue was increased in i.v. NPC treated animals (3 months post injury). At 2 weeks post injury, the NPC treated group had less TUNEL positive cells than vehicle treated *Behavior:* Experiment 1: **NSC i.v. injected animals performed better on the BMS (5.14 ± 0.06)** than i.v. fibroblast group (~ 3.7) and i.v. PBS control (3.20 ± 0.08), starting at 4 days post-injury and continuing all the way to 90 days post-injury. Experiment 2: **The NSC i.v. transplant group performed better on the BMS (~ 5.6)** than both the PBS intraspinal group (~ 3.8) and the NSPC intraspinal group (~ 4.9) (from 25 days post-injury to 60 days). The NPC intraspinal group performed better than the PBS group. The SCI mice did not indicate any signs for forepaw allodynia (hotplate, cold stimulation)
Parr *J Neurotrauma* 2007	*Model:* **Adult** female SD **rats,** 250–300 g *Imjury:* **T8–9 Clip compression** 35 g	■ **aNPCs** isolated from the **spinal cord of adult male Wistar rats** expressing enhanced green fluorescent protein (eGFP), 140,000–520,000 cells in 5 μl in neurospheres either into the injury site, or 1 mm rostral and caudal to the injury site (**R/C**). **@ 0 hr PI** **@ 9 d PI** **@ 28 d PI** • **CsA** 10 mg/kg/d s.c. **ACUTE, SUBACUTE & CHRONIC**	**SCI+** **1.** Acutely NPCs into the injury site **2.** Acutely NPCs R/C **3.** Subacute NPCs R/C **4.** Chronic NPCs R/C **5.** SCI only • For morphology, animals from each group sacrificed @ 7, 14, 28 d (n = 3/time point) For Behavior: n = 9 for group 1 and n = 8 for group 5	*Histology:* • Better survival of NPCs when transplanted R/C than the epicenter. 1% survival at 7 d after transplantation, and further decline • Better survival of NPCs in subacute model (7% survival at 7 d) • Cell differentiation**: 31.2% astrocytes (GFAP), 50.3% oligodendrocytes** (APC), less than 1% neurons (MAP2) 37.0% undifferentiated (nestin), the relative percentages did not change with time. • Low proliferation rate (<1%, Ki-67) *Behavior: (group 1 and 5)* **No difference at 16 wk between the two groups**

Parr *Neuroscience* 2008	*Model:* **Adult** female SD **rats,** 250–300g *Injury:* **T8 Clip compression** 27 g for 1 min.	■ **aNPCs from adult rats' spinal cords** (eGFP-Wistar) grown as neurosperes in **bFGF/EGF** transplanted **@ 9 d PI** □ bone marrow stromal cells **(BMSCs)** from eGFP-transgenic Wistar rats; (adherence to plastic) 4–6 passages @ 0 hr PI 100,000 NPCs or BMSCs were injected each 1 mm rostral and 1 zmm caudal to the epicenter • **CsA** 15 mg/kg/d s.c. **SUBACUTE**	**SCI +** **1**. Medium for BMCs on day 0 and **NPC**s on d 9 **2. BMSCs** on d 0 and medium for NSPC on d 9 **3. BMSCs** on day 0 and **NPCs** on d 9 **4**. Medium for BMSCs on day 0 and medium for NPCs on day 9. All groups: n = 10, 12 wk survival, plus a small cohort of NPCs or medium for a 7 d survival n = 3	*Histology:* Only 1.2% of the NPCs survived 12 wk, and 2% in group 4; After 7 days only 4.6% survived; differentiation (at day 7) mainly into astrocytes (18%) oligodendrocytes (63%) and neurons (1.1%); these numbers are 11%, 41% and 4.2% in the weeks group. BMSCs have no effect on NPC differentiation and do not differentiate neurally themselves. NPCs wrap around axons but did not ensheath them (by EM). More sparing of long tracts in the NPC group. *Behavior:* **Significant Improvements of NPC only group #3 on BBB (∼16)**, BBB subscore (∼4.5) and horizontal ladder ∼2 – better than all other groups (#1,2,4) **BBB ∼12**, subsc. ∼1; footsli[s ∼7. The effect is early – already within 7 days PI Not seen with BMSCs.
Hofstetter *Nat Neurosci* 2005	*Model:* **Adult** female SD **rats**, 250 g *Injury:* **T8–9 contusion**; weight drop (12.5 mm height, *weight unknown)*	**COMBINATORTIAL TREATMENTS:** ■ **aNPCs** (EGF-FGF resp.) from female SD **rat spinal cord** (BrdU labeled) into the injury site (naïve NPCs) ■ **Adult NPCs** transduced to express **neurogenin-2** (Ngn2- NPCs) • 4 injections arounf the lesion, 100,000 cells/animal in total **@ 7 d PI** • Immunosuppression not mentioned **SUBACUTE**	**SCI+** **1**. Naïve NPC transplantation n = 29 **2**. Ngn2-NPC transplantation n = 38 3. Vehicle n = 37	*Histology:* • 80% survival @ 2 wk and 40% @ 9 wk • **Increased myelination** and white matter sparing in the Ngn-2. • Increased axonal sprouting of CST (BDA) but not axonal regeneration beyond the injury site. • Increased sprouting of CGRP + afferents rostral to the injury site in NPC group vs. Ngn2-NPC and controls. • Recovered fMRI (blood flow) after stimulation of hind paws in Ngn2-NPC. *Behavior:* • **Enhanced pain sensation in naïve NPCs**, not in Ngn2 group • BBB (improvement with NPC-Ngn2 to ∼16; compared to ∼12 vehicle control, and NPCs around ∼14). • @ 9 wk, Ngn2-NPC group better in Grid Walk followed by NPC. • Lower threshold for Hot-plate testing (sensory) in Ngn2 group

(*Continued*)

Table 6. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Karimi-Abdolrezaee *J Neurosci* 2006	*XENOTRANSPL.* *Model:* **Adult** female Wistar **Rats** 250 g *Injury:* **T7 clip compression** (23 g)	■ Dissociated **NPCs** from **SVZ** of transgenic **adult mice** expressing YFP • 3×10^5 in total of NPCs injected into 4 locations bilaterally rostrally and caudally to the injury site **@ 14 d PI @ 56 d PI** □ Cocktail of **growth factors** (**GF:** EGF, bFGF and PDGF-AA) infused intrathecally @ the time of transplantation for 1 wk □ **Minocycline** for 10 d (starting 3 d before transplantation) • **CsA** entire experiment **SUBACUTE & CHRONIC**	**SCI+** **1.** GF cocktail + minocycline + CsA + NPCs n = 26 **2.** No cells: GF cocktail + minocycline + CsA n = 26 **3.** SCI control n = 5 "n" for Behavior tasks: Plain injured n = 5, Control injured n = 8, NPC transplanted n = 10 Survival: 10 wk PT	*Histology:* • Subacute: 37% of cells survived @ 6 wk after transplantation (n = 3), migrated ~ 5 mm rostro-caudally. • **51% differentiated into oligodendroglia**, very few astrocytes (5%). No neurons, no p75 + SCs. • Axonal ensheathment, expression of myelin basic protein (MBP) by NPC-derived oligodendrocytes, greater myelin index in NPC-transplanted rats. • Chronic: Low survival rate, no NPCs after 1–2 wk in the cord. *Behavior:* • BBB significant improvement from @ 3 wk after transplantation. **@ 6 wk: transplant − 12.3 vs. "no cells" − 10.3, plain injury − 11.0.** • Significant decrease in foot falls in Grid walk, improved coordination and angle of rotation @ 5–6 wk after transplantation
5b. Neural Stem/Progenitor Cells from Adult Rodents (sharp models)				
Vroemen *Eur J Neurosci* 2003	CERVICAL *Model:* **Adult** female Fischer 344 **rats**, 160–180 g *Injury:* Spinal cord dorsal column **Tx** at **C3**	■ **Adult NPCs from rat spinal cord** (syngenic) labeled with GFP-retrovirus or BrdU 1.6–1.8$\times10^5$ cells/μl into the lesion site **@ 0 hr PI** • No indication of immunosuppression **ACUTE**	**SCI+** **1.** NPC-GFP/BrdU n = 4 **2.** NPC-BrdU n = 8 **3.** NPC-GFP n = 8 **4.** No transplant n = 6 Survival: 3 wk	*Histology:* • Transplantation did not affect lesion size. • Transplanted NPCs resided predominantly in the rime around the cystic lesion and central canal. • Low proliferation rate with Ki-67 labeling. • **No effects of NPC transplantation on the CST re-growth** (3 wk PI). • At 3 wk PI, the majority of cells differentiated to astrocytes (GFAP) and radial glial markers (BLBP), fewer cells for oligodendroglial (APC) or glial precursors (NG2). **No neuronal differentiation** was observed (βIII tubulin). *Behavior:* Not reported

Pfeifer *Eur J Neurosci* 2004	*Model:* **Adult** female Fischer 344 **rats**, 160–180 g *Injury:* **C3** dorsal column **transection**	■ **Adult NPC from rat spinal cord** and □ **Fibroblasts** (FBs), from Fisher 344 rats. • Into the injury site @ **0 hr PI** **ACUTE**	**SCI+** **1.** aNPC + FBs ($4\times10^5 + 0.6\times10^5$) n = 8 **2.** FBs ($1.2\times10^5$) only n = 8 **3.** Lesion only n = 6 Survival: 3 wk	*Histology:* •FBs are required for differentiation of aNPCs in vitro to astrocytes (30%) and Oligodendrocytes (5%). • 3 wk PI, FBs alone are sufficient to fill the lesion cavity. aNPCs required FBs to fill the cavity. NPCs were found within the grafts. • aNPCs-derived glial cells **enhanced axonal regrowth and sprouting into the graft from the severed CST tract** (BDA anterograde tracing, 1 wk PI). • The majority of surviving cells were **astrocytes** (radial glial or regular GFAP+) and **oligodendrocytes (<20%). No neuronal differentiation**. *Behavior:* Not reported
Vroemen *Cell Tissue Res* 2007	*Model:* **Adult** female Fischer 344 **rats,** 160 - 180 g *Injury:* **C3** dorsal column t**ransection** (wire knife)	■ SVZ-**NPCs/Schwann cells (SCs)** or NPCs/Schwann cell/fibroblast (FB) co-grafts • NPC labeled with GFP □ **Fibroblasts** and **Schwann cells** labeled with BrDU • into the lesion site @ **0 hr PI** **ACUTE**	**SCI+** **1.** NPC/SCs/FBs co-grafts n = 6 **2.** NPC/SCs n = 7 **3.** SCs/FBs n = 6 **4.** SCs n = 8 **5.** FB n = 10	*Histology:* • FBs but not SCs filled the cyst, no NPCs in the graft @ 3rd w PI • **NPCs mainly differentiated into GFAP + astrocytes**, a few APC + oligodendrocytes. No neuronal differentiation. • Neurofilament + ve profiles (axon) density in gaft: NPC/SC/FB > SC/FB > FB • **CST axons sig. more sprouting** into NPC/SC/FB or FB grafts vs. SC/FB • Only peripheral type remyelination of axons was observed in all groups *Behavior:* Not reported
Pfeifer *Regen Med* 2006	*Model:* **Adult** female Fischer 344 **rats**, 160–180 g *Injury:* **C3** dorsal column **transection** (wire knife)	■ **Autologous SVZ aNPCs** labeled with BrdU • Autograft or allograft aNPC (2.4×10^5 cells in 3 μl) □ Autograft **fibroblasts** (FBs, 0.6 10^5 in combination with aNPC or 1.5 10^5 of FBs alone) • Injection into the lesion site @ **8 wk PI** **CHRONIC**	**SCI+** **1.** Allogenic NPCs combined with FBs n = 8 **2.** Autologous NPCs combined with FBs n = 8 **3.** Autologous FBs alone n = 8 Survival: 4 wk post transplantation	*Histology:* • 4 wk post-transplant: exclusive glial fate (astroglia and radial glia – 90%, no neuronal specific reactivity). • Autologous cells (aNPC/FBs or FBs) fill lesion site and survive better than allogenic cells. • **Miminal sprouting of corticospinal axons into aNPCs containg grafts** (n.s.) • Axonal sprouting (neurofilament + ve profiles) in auto and allo-grafted aNPC *Behavior:* Not reported

(*Continued*)

Table 6. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
6. Neural Stem/ Progenitor Cells from Rodent Immortalized Cell Lines: (c17.2, RN46A-B14; RADIAL GLIA (RG3.6)				
Macias *Exp Neurol* 2006	*Model:* Female SD **rats,** 200–250 g *Injury:* **T8 contusion, NYU** Impactor 10 g from 25 mm	■ **Mouse NSC (line c17.2)** with or without transfection of GDNF • 4×5μl injections 1 mm rostral/caudal to the lesion site with a total 1.0×10^5 cells **@ 8 d PI** • Immunosuppressant **Prograf** (50 mg/kg) **SUBACUTE**	**SCI** + **1**. Cells n = 10 **2**. Cells with GDNF n = 10 **3**. Saline n = 3 **4**. Sham n = 6	*Histology:* • Majority of cells (with or without GDNF) display astrocytic markers. • Enhanced density and area of GAP-43 and CGRP in the dorsal horn of animals transplanted with cells or cells with GDNF. *Behavior:* **No significant difference between groups on BBB** • **Significant decrease in withdrawal latency in response to thermal stimulus** for forelimbs, but not hindlimbs, in groups 1 and 2 compared to saline injected or sham controls • Significant **increase in the number of forepaw, but not hindpaw, withdrawals** in response to von frey hairs in groups 1 and 2. **"PAIN WITH NO GAIN"** title by the authors
Teng *PNAS* 2002	*Model:* **Adult** female SD **rats** *Injury:* **T9-T10 hemisection**, 4 mm aspirated from lateral hemisection	■ **Mouse NSC (line c17.2)** were injected alone (10μl of 1.0×10^7 cells/ml) or seeded into **scaffold** composed of PLGA and polylysine block (50:50) into lesion site **@ 0 hr PI** • **No CsA** **ACUTE**	**SCI**+ **1**. Scaffold plus cells n = 13 **2**. Scaffold without cells n = 11 **3**. Cells in media without scaffold n = 12 **4**. Injury control n = 12	*Histology:* Group 1 and 2 showed anterogradely traced CST axons rostral and caudal to the injury site as well as GAP-43 axons rostral to the injury site. • Cells inside of scaffold were mostly co-labeled with nestin, a progenitor marker *Behavior:* **BBB** at 70 d: **group 1 (∼11** injured side; ∼12 uninjured side**) vs. group 2** (∼**8** injured side; ∼9 uninjured side)**, group 3** (∼**6** injured side; ∼6 uninjured side) and **control** (∼**5** injured side; ∼**6** uninjured side)**.** *Comment: Unusually low scores for hemisections* • Group 1 improved on angled plane test in downward orientation (not upward) at most time points as well as improved righting reflex at 56 d PI only. • Group 1 had fewer animals exhibiting a spastic response to toe pinching stimuli
Lu *Exp Neurol* 2003	**CERVICAL** *Model:* **Adult** female Fisher **rats,** 160–200 g *Injury:* **C3 Dorsal column transection** – wire knife	■ **Mouse NSC line C17.2** • **C17.2 cells** transduced to produce **hNT-3** □ **Rat (Fisher) Fibroblasts (FF227,** from skin). All transduced with GFP 2.5 μl of 40,000 cells/μl into epicenter **@ 0 hr PI** • **CsA** 10mg/kg daily started one day prior injury **ACUTE**	**SCI**+ **1**. C17.2 NSCs n = 16 **2**. C17.2-NT-3 NSCs n = 16 **3**. Fibroblasts n = 8 **4**. Lesion only n = 4	*Histology:* Lesion size similar among all grafted groups • Found neither neuronal nor glial markers in the C17.2 NPCs 2 wk PI. • Occasional C17,2-NT-3 NPCs expressed the neuronal marker MAP2. • **4-fold greater number axons infiltrating the C17,2-NT-3 NPCs versus unmanipulated C17.2 NPCs.** More CGRP + ve axons in the NPC groups versus fibroblast groups; More ChAT positive axons in the C17.2 than C17.2-NT-3 group, none in fibroblasts; More dorsal column sensory axons in 17.s-NT-3 group. • The C17.2-NT-3 cells express less BDNF and GDNF than controls *Behavior:* Not reported

Hains *Neuroscience* 2003	*Model:* Male SD **rats**, mean 175 - 200 g *Injury:* **T13** Unilateral spinal **hemisection**	■ RN46A-**B14 immortalized serotonergic precursor cells** (from E12.5 medullarly raphe nucleus **rat** neurons), which secrete serotonin and BDNF. • RN46A-V1 vector as control • 1×10^6 cells were trans-planted intrathecally L2-L3 **@ 28 d PI** • **CsA** 40 mg/kg/day for 7 d **CHRONIC**	**SCI+** **1**. RN46A-V1 n = 15 **2**. RN46A-B14 n = 15 **3**. Sham transplant n = 15 10 animals in each group were used for e-Phys.	*Histology /Physiology:* E-Phys of dorsal horn neurons in animals with RN46A-B14 transplantation were less responsive to sensory stimuli compared to the 2control groups, and were near the normal baseline l *Behavior:* The group receiving B-14 cells showed improvement of mechanical and thermal allodynia, while the other groups did not change.
Hasegawa *Exp Neurol* 2005	*Model:* Female SD **rats**, 200–250 g *Injury:* **T9/10 contusion NYU/ MASCIS** Impactor 10.0 g×12.5 mm	■ **Immortalized RADIAL GLIA (RG3.6) clone originally from (**E 13.5) neurospheres isolated and grown from GFP SD **rat** forebrain – (v-myc) • 4×10^5 RG3.6 cells were injected into the center of the contusion site and 2×10^5 2 mm rostral and caudal to the injury site **@ 0 hr PI** □ GFP rat skin fibroblasts $0.5\times10^5/\mu l\times4$ in the same sites • **CsA** 10 mg/kg.day s.c. **ACUTE**	Experiment 1: **SCI+** **1**. RG3.6 n = 8 **2**. Media injected n = 4 Experiment 2: **SCI+** **1**. RG3.6 n = 10 **2**. Fibroblast transplant n = 10	*Histology:* Transplanted fibroblasts remained in the lesion site while the RG3.6 cells migrated into the spared white matter and displayed polarized morphology. Both fibroblast and RG3.6 resulted in less CSPG and NG2 immunostaining. The RG3.6 transplanted group had highest density of neurofilament staining in the spared host rim compared to both controls groups. *Behavior:* Experiment 1 – The **RG3.6 transplanted group performed better on the BBB** throughout the observation period (1 wk PI: control = 5.123 ± 1.0008, RG3.6 = 8.0 ± 0.590) and 6 wk PI (media control ~ 10.7, RG3.6 ~12.5). Experiment 2: RG3.6 cells by 2 days PI (fibroblasts ~0.2, RG3.6 ~ 1.6), and still better at 6 weeks PI (fibroblast ~9.0, RG3.6 ~10.1).

EMBRYONIC STEM CELL-DERIVED NEURAL PRECURSORS

Embryonic stem cells are the pluripotent cells of the inner cell mass of embryos that can generate all mesodermal ectodermal and endodermal derivatives (except germ cells). Hence they can and have been show to produce teratomas after transplantation. Some groups developed protocols to direct their differentiation into neural cells or glials that could be used for transplantation. The possible contamination of ESCs in these cultures is raising serious safety concerns in some author's mind.

7. Neural Stem/Progenitor Cells from Embryonic Stem Cells

McDonald *Nat Med* 1999	*Model:* Long Evans **rats** 275 ± 25 g *Injury:***T9–10 NYU,** 10g×25 mm	■ Neural differentiated in culture **mouse D3 ESCs or** ROSA26 ES cells with LacZ transgene and expressing β-galactosidase **(mESCs)** (labeled with Brdu) **@ 9 d PI** □ **Mouse neocortical cell** control ~1×10^6 cells directly into the lesion site @ 9 d PI • **CsA** 10 mg/kg.day **SUBACUTE**	Group I (Behavior, 5 wk survival): **SCI+** **1**. D3 mESC n = 11 **2**. Media control n = 11 **3**. Sham operated n = 11 Groups II (histology, 2wk vs. 5 wk) and III (Behavior, 5 wk survival): **SCI+** **1**. ROSA26 mESC n = 11; n = 6 **2**. Neocortical cells n = 6 **3**. Media control n = 11; n = 6	*Histology:* 2 wk PT, ESCs-d-OPCs were found to fill the lesion. They colocalized with oligodendrocyte (CC1), astrocyte (GFAP) and neuronal (NeuN) markers – based on BrdU label in the ESCs-d-OPCs • By 5 wk, ES cell-derived cells in part replaced with an extracellular matrix. *Behavior:* In both group 1 and group 2 the mESC cell transplanted showed significant improvement on the BBB over the neocortical cells and the media control and the neocortical cells (appr. **10 vs. 8 @ 5 wk** post-transplantation)

(*Continued*)

Table 6. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Chen *J Neurotrauma* 2005	*Model:* Female C57BL/6J **mice**, 3 month old *Injury:* **T7-T10**, 100% **compression** with an electromagnetic mouse compression device	■ **NPC derived from C57BL/6J ESC line** (95% are nestin+) • Labeled with GFP • Expressing cell adhesion molecule L1 under the control of the promoter of the isoform 1 of the 3-phosphoglycerokinase • Injected 1.0×10^6 cells in 10 μl into both 0.5 mm rostral and caudal to the lesion **@ 7 d PI** **SUBACUTE**	**SCI+** 1. GFP-ESC-NPC-L1 n = 26 2. GFP-ESC-NPC n = 28	*Histology:* L1-cells survived and appeared to express NF200 as well as astrocyte and oligodendrocyte markers. • L1-cells more closely associated with axons than non-transected ESC-NPC. • Both L1 non-transected ESC-NPC cells fill the lesion cavity. • In L1-cell transplanted animals anterogradely traced CST is significantly closer to the lesion site than the non-transected ESC-NPC cell group • Survival is poor following transplantation of non-transected ESC-NPC cell group • L1- ESC-NPC graft site appears to be compressing the host spinal cord *Behavior:* Not reported

d: day, days; hr: hour, hours; IH – Infinite Horizon Impactor; i.v.: intravenous; n.s.: not significant; PI: post-injury; PT: post-transplant; s.c.: subcutaneous; SCI: spinal cord injury; SD: Sprague–Dawley; sig: significant; Tx: transection; wk: week, weeks; + ve: positive; -ve: negative

5HT: serotonin; AdV: adenoviral; APC: adenomatous polyposis coli gene protein; BBB: Basso, Beattie and Bresnahan locomotor test; BDA: biotinylated dextran amine; bFGF (FGF2): basic fibroblast growth factor; BLBP: brain lipid binding protein; BMSC: bone marro stromal cell; CGRP - calcitonin gene-related peptide; ChABC: chondroitinase ABC; ChAT: choline acetyltransferase; CMEP: cortical motor evoked potential; CNP: ciliary neurotrophic factor; CNTF: Ciliary neurotrophic factor; CNP: 2′3′-cyclic nucleotide 3′- phosphodiesterase/phosphohydrolase; CMEP: cortical motor evoked potential; CsA: Cyclosporine; CSEP: cortical somatosensory evoked potentials; CSF: cerebrospinal fluid; CSPG - chondroitin sulfate proteoglycan; CST - cortico-spinal tract; DMEM: Dulbecco's modified Eagle's medium; EGF: epidermal growth factor; EM: electron microscopy; EYFP: enhanced yellow fluorescent protein; FBs: fibroblasts; G-CSF: granulocyte colony stimulating factor;GDNF: glial cell line-derived neurotrophic factor; GFAP: glial fibrillary acidic proteins; GFP – green fluorescent protein; LacZ – beta-galactosidase; MAP-2: microtubule-associated protein-2; MG: matrigel; MRI: magnetic resonance imaging; NCAM: neural cell adhesion molecule; NSPCs: Neural Stem/Precursor Cells; NPCs: neuronal progenitor cells; OPC: oligodendrocyte precursor cell; OSP: oligodendrocyte-specific protein; PDGF: platelet derived growth factor; PLGA: poly(lactic-co-glycolic) acid; RA: retinoic acid; SCs: Schwann cells; Shh: sonic hedgehog; SVZ: sub-ventricular zone.

2001), many authors also observed the expression of up to 60% oligodendroglial markers (Karimi-Abdolrazaee et al,. 2006; Parr et al., 2007, 2008; Pfeifer et al., 2004; Vroemen et al., 2007); expression of neuronal markers was generally rare (0–1%). The extent to which these oligodendrocytes can mature in the injured spinal cord and generate compact myelin is inconsistently reported (Karimi-Abdolrezaee et al., 2006; Parr et al., 2008).

Six of the eight contusion studies evaluated behavioral recovery with open field BBB locomotor scores. Five of these six studies reported significant improvement with the transplantation of aNPCs, three in rats (Hofstetter et al., 2005; Karimi-Abdolrezaee et al., 2006; Parr et al., 2008) and two in mice (Bottai et al., 2008; Ziv et al., 2006). However, it needs to be pointed out that in some of these studies, co-treatments were also applied, such as myelin vaccination (Ziv et al., 2006) or a cocktail of trophic factors infused intrathecally for 1 week (Karimi-Abdolrezaee et al., 2006). Of note, Hofstetter and colleagues (2005) reported an alarming lowering of sensory thresholds to non-noxious stimuli (i.e., allodynia) in the naïve aNPCs transplanted animals, illustrating the very real potential that such cells may promote neuropathic pain. Interestingly, Bottai and colleagues (2008) observed even better behavioral outcomes with intravenous compared to intraspinal delivery of mouse aNPCs into mice – while all other studies employed a direct transplantation approach with the cells injected rostrally and caudally.

Embryonic neural stem/progenitor cells (NSPCs) are taken from the CNS of rodent embryos and expanded as neurospheres before they are dissociated and injected into the injured spinal cord. These neurospheres contain precursor cells for neurons, astrocytes, and oligodendrocytes, plus stem cells capable of self-renewal. The group is somewhat heterogeneous, as they may be taken between embryonic day 13 and 16 from various parts of the CNS (forebrain to spinal cord) and expanded in EGF or bFGF or a combination thereof, plus other potential growth factors. In addition, the number of passages varies significantly between labs, which may favor different subpopulations within the neurospheres.

Embryonic NSPCs were applied in eight studies of compression/contusion injuries in rodents at the thoracic level, in one study with weight compression at the cervical level, and in two full transection and three different partial transection models at the thoracic level of rodents. Expression of astrocyte, oligodendrocyte, and neuronal markers was observed to a variable degree in several studies. Behaviorally, the cervical weight-compression model revealed improvements on a skilled reaching task (Ogawa et al., 2002). In the seven thoracic contusion studies, behavior was reported in only three, and all observed significant improvements on the BBB locomotor scale (Meng et al., 2008; Okada et al., 2005; Setoguchi et al., 2004). In two of these studies, the effects were further enhanced with adjuvent treatments of noggin (Setoguchi et al., 2004) or bFGF expressing rat amniotic epithelial cells (Meng et al., 2008). All three studies used a subacute time frame for transplantation (7–9 days), while one direct comparison with the cells transplanted acutely demonstrated the failure of this approach. This again underlines the general notion in the field that the acutely injured spinal cord is a hostile environment for many transplanted cells, and in this regard points to an important distinction from the neuroprotection field.

Four of the five studies using sharp models of SCI reported on behavioral outcomes. Pan and colleagues (2008) claimed BBB scores of 9.6 versus 3.8 (control) after filling a complete spinal cord transection site immediately after injury (i.e., acute intervention) with eNPSC and fibrin glue from embryonic rats. Administering five injections of G-CSF over 5 days further improved the scores to 11.7. Using the same model, Guo and colleagues (2007) reported BBB scores of 3.6 after transplantation of NSPCs from neonatal rats plus type1 collagen compared to 0.54 in controls. In the latter study, the benefit was greatly enhanced by co-transplantation with SCs from neonatal rats, especially when these were transduced to express NT-3 (BBB $\sim$ 10.7).

The transplantation of eNSPCs from human fetuses at 8 weeks of gestation into cervical contusion sites of marmoset monkeys (Iwanami et al., 2005) is interesting from a translational perspective for both the human source of cells and the primate model of cervical injury. Expression of astrocytic, neuronal, and a small percentage of oligodendrocyte markers was observed. Behaviorally, bar grip power and spontaneous motor activity was improved, which is promising, although validation of these test models is still pending. Given the ethical controversy around the use of human abortion material, as well as the technical variability and logistical problems involved, several authors have pursued human immortalized neural stem-cell lines (HB1.F3 clone; line K048) or long-term human neurosphere cultures (Cummings et al., 2005) and transplanted them to dogs, mice, and rats. However, only two of the four studies listed were met with behavioral success (Cummings et al., 2005), including the transplantation into dogs (motor scores of 15 vs. 10) (Lee et al., 2009). It is conceivable that eventually these approaches will yield viable sources of human cells for clinical translation. A general summary of the pros and cons and knowledge gaps for neural stem-cell transplantation is depicted in Table 7.

Neural and glial restricted precursors (Table 8)

The nine studies of glial restricted precursors (GRPs) and/or neural restricted precursors (NRPs) included here were performed with rodent cells transplanted into the injured rodent spinal cord – six of these employed blunt contusion models. While transplantation of NRPs alone into uninjured spinal cords resulted in neural differentiation, such neuronal differentiation is far less complete in the environment of the SCI site, underlining the fact that the environment of the injured spinal cord inhibits neuronal differentiation (Cao et al., 2002). Similarly, GRPs differentiate mainly into astroglial cells in the lesion centre, while only some express oliogdendrocyte markers, usually after they migrate into the spared host spinal cord (Enzmann et al., 2005; Han et al., 2004; Hill et al., 2004). Still, the degree to which GRPs form myelinating oligodendrocytes in the contused spinal cord is somewhat limited (Enzmann et al., 2005). Furthermore, it appears that behavioral recovery requires the transduction with the neurotrophin D15A (Cao et al., 2005), which has BDNF and NT-3 activities and also enhances oligodendrocyte differentiation. The extent to which the observed benefits are related to increased myelination, neuroprotection, or neural plasticity can only be speculated on.

Four studies transplanted a mixture of GRPs and NRPs from rodent embryos, and in two experiments this was performed in the context of severe thoracic contusion injuries. Both cases reported moderate but significant improvements

Table 7. Summary Statement for Neural Stem/Progenitor Cells (NSPCs)

Pros	*Cons*
Appear to integrate well into the host spinal cord.	Differentiate primarily into astroglial cells, with some oligodendrocytes seen; neurons are rare.
The majority of studies (17 of 20) with behavioral assessments reported improved outcomes (in both blunt and sharp models).	NSPCs do not provide optimal bridges for axonal regeneration – hence less likely suited for axonal repair strategies.
Behavioral improvements have been demonstrated in large animal models of SCI: acute primate cervical contusion model (human embryonic NSPCs), and acute canine lumbar hemisection model (human neural stem cell line)	Harvesting NSPCs from fetal material is met with ethical and safety issues and will likely result in variable quality. Allo-transplantation of cells from human brain material is logistically difficult and would likely require immunosuppression.
Knowledge Gaps	
Importantly, a plethora of different cells have been described, and the optimal source for NSPCs for transplantation purposes has yet to be determined.	
While the "body of literature" is large, the extent to which a specific cell from a single laboratory has been studied is modest. Whatever specific cell is proposed for human translation will require much more detailed characterization, including application in different injury models, severities, and treatment intervals.	

on the BBB scale from ~7 to ~9; in addition, bladder control was improved and the hypersensitivity to thermal stimuli ameliorated (Mitsui et al., 2005; Neuhuber et al., 2008). The cells were neuroprotective, and many differentiated into astrocytes, some into oligodendrocytes.

The isolation and transplantation of oligodendrocyte precursors from newborn rodents (as opposed to embryos) using antibodies to A2B5 or O-2A resulted in improved BBB scores after mild as well as after moderate contusion injury (Bambakidis and Miller, 2004; Lee et al., 2005). Both studies reported reduced latencies of motor evoked potentials, consistent with either remyelination and/or neuroprotection.

From a translational perspective, harvesting human GRPs and NRPs from abortion materials is met with logistic and ethical concerns in many countries. Hence, alternative sources for oligodendrocyte precursors have been pursued. Most prominent is the differentiation of oligodendrocyte precursors (OPC) from a human ES-cell line, an approach that received FDA approval to proceed with a Phase 1 clinical trial in January 2009, but was subsequently put on hold (Geron Corp, Menlo Park, CA). In essence, these ESC-derived OPCs enhance myelination, are neuroprotective, and they appear to mediate moderate improvement of locomotor function when transplanted after subacute but not after chronic SCI (Keirstead et al., 2005). This study has not been independently replicated by other laboratories, although Geron performed extensive "in-house" safety and efficacy studies prior to obtaining FDA approval. Such studies further characterizing the efficacy of this technology have yet to be released to the academic community. Efficacy in blunt cervical models would be desirable if that will be a major human target for translation. Similarly, no larger animal models with OPC transplants exist so far. Concerns regarding the risk of teratoma formation have been voiced. A summary of the pros and cons and knowledge gaps of GFP/NRP transplantation is given in Table 9.

Bone-marrow-derived stromal cells – mesenchymal stem cells (Table 10)

The stromal cells from bone marrow are isolated and separated from the hematopoietic cell fraction of the bone marrow by their property to adhere to plastic. Some authors go further by using FACS to purify hematopoietic cells (which are CD34 positive). Bone-marrow-derived stromal cells (BMSCs) are hence typically a crude mixture of stromal cells that support the growth of hematopoietic stem cells and mesenchymal stem cells, and some authors do provide additional (albeit somewhat unspecific) markers to characterize these mesenchymal stem cells. This heterogeneity and uncertainty of origin likely explains the highly variable results among different laboratories regarding the ability of these cells to survive, integrate, and differentiate as neural cells in the injured spinal cord. In addition, there is evidence that rather non-specific treatments can induce the expression of a neuronal marker without truly specifying these cells as neuron or glial cells (Lu et al., 2004).

A narrative review of BMSC transplantation in TBI, stroke, and SCI has recently been published by Parr and colleagues (2007). Nine SCI studies were performed with human BMSC, of which six used a blunt contusion or compression model. Three of these report beneficial behavioral effects, while three groups observed no transplant-related improvements. Deng and colleagues (2008) claimed impressive BBB scores of 13 (weight-supported stepping with frequent coordination) versus a BBB of 6 in their controls. However, Kim and colleagues (2006) found less dramatic benefits (13 vs. 10) in a milder contusion model when combining these cells with FGF. Similarly, Cizkova and colleagues (2006) reported benefits after balloon compression. The behavioral benefits in the sharp models are questionable, since in the studies by Mansilla and colleagues (2005), as well as the study by Zhao and colleagues (2004), a large percentage (80% and 35% respectively) of control animals died (but not in the treated groups), which suggests suboptimal animal care standards (and leading to exclusion of the former study from this review). Neuhuber and colleagues (2005) tested human BMSC from four different donors and found highly variable outcomes in a rat hemisection model using various tests, which illustrates the heterogeneity of these cells. Hence, it appears that we need a better understanding of the types of cells in the BMSC fraction that might mediate these benefits.

Table 8. Neural and Glial Restricted Precusors - NRPs and GRPs

These cells are isolated from embryos and rather than propagating them as neurospheres they are immunoselected (immunopanned) with antibodies to select glial precursors (A2B5 antibody for a tripotential glial precursor or O-2A for oligodendrocyte precursors) or neuronal precursors (PS-N-CAM). The main rationale behind the use of the GRPs is to replace lost oliogdendrocytes in order to remyelinate demyelinated axons in the spared host spinal cord. In addition, these cells may provide neuronal replacement and deliver trophic factors for neuroprotection and enhancement of plasticity.

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
1. Neural Restricted Precursors				
Cao *Exp Neurol* 2002	*Model:* **Adult** Female Fisher-344 **rats**, 170–200 g *Injury:* **T8 contusion NYU** Impactor 12.5 g-cm	■ **NRPs from E14 Fischer-344 rat** spinal cord (immunopanned with PS-NCAM). Cells were labeled with either BrdU or by retrovirus expressing **EFGP**. • 1.5×10^5 cells were transplanted into 3 sites unilaterally into the gray matter one segment rostral (T7), one segment caudal (T9) and into the lesion epicenter (T8) @ **10 d PI** **SUBACUTE**	**SCI+** **1**. NRP transplant n = 15 3 groups with 1 wk; 1 and 2 months survival Non-injured: **2**. NRP transplant n = 15 Survival: 1 wk; 1 and 2 months	*Histologic/Biochemical/Physiologic:* The NRPs transplanted into the **non-injured** spinal cord had good survival and the majority differentiated into βIII tubulin-positive neurons. Some of these cells expressed GABA, and a smaller number were positive for glutamate and ChAT. • Of those **NRPs** transplanted into the epicenter of the contused spinal cord some **remained undifferentiated** (2 weeks to 2 months post injury), a few were MAP2- or βIII tubulin-positive "neurons". The expression of GABA, glutamate, or ChAT were NOT detected. *Behavior:* Not reported.
2a. Glial Restricted Precursors (GRPs)				
Hill *Exp Neurol* 2004	*Model:* Female Long Evans **rats**, 83–95 days old *Injury:* **T9–10 contusion NYU/MASCIS** 10 g×12.5 mm	■ **GRPs** isolated by dissociating embryonic rat spinal cord and the immunopanning with A2B5. • Labeled with PLAP or GFP • 5×10^5 GRPs injected into the impact site @ **0 hr PI** □ Some animals received 30 mg/kg **MP** @ 5 min, 2 hr, and 4 hr PI • **CsA** 10 mg/kg.day i.p. for 10 days and then from drinking water **ACUTE**	4 groups (2 times points 8 day and 6 wk hence 2 n values given) SCI n = 6; n = 8 SCI + MP/CsA n = 6; n = 8 SCI + MP/CsA + conditioned media n = 6; n = 8 SCI + MP/CsA + conditioned media + GRP cells n = 9; n = 14 1st number – animals sacrificed at 8 d; 2nd number – animals sacrificed at 6 wk.	*Histology:* Transplanted cells survived in spared tissue around the lesion site (8 day and 6 week), with trend to lowering macrophage infiltration. Cells become astrocytes and oligodendrocytes. • GRP transplantation lowered CSPG expression in the lesion and at the lesion boundary as well as decreased GFAP expression at the lesion boundary. • No affect on CST axonal dieback. No increase in 5-HT sprouting. PLAP were used to detect transplanted glial-restricted precursor (GRP) cells. *Behavior:* No Significant differences, but injury models not sensitive to behavioral improvements.
Enzmann *Exp Neurol* 2005	*Model:* Female Fischer 344 **rats**, 170–200 g *Injury:* **T9 contusion IH** Impactor150kdyn	■ **GRPs** from E14 Fischer rats, dissociated and immunopanned for A2B5. • Cells were infected with either an **EGFP** or a **noggin**-EGFP retrovirus • 5×10^5 GRPs injected into 5 injection sites: the epicenter and distal aspects of the lesion area @ **7 d PI** **SUBACUTE**	**SCI+** **1**. GRP-noggin n = 3 **2**. GRP-EGFP transplanted animal n = 3	*Histology:* The transplanted GRPs expressing noggin, showed no more oligodendroglial differentiation than GRP-EGFP. Transplantation of noggin-GRPs increased the lesion area compared to EGFP-GRP and extension correlated to the number of injected cells. *Behavior:* Not reported

(*Continued*)

Table 8. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Han *Glia* 2004	**CERVICAL** *Model:* Female SD **rats**, mean 225 g; *Injury:* **C3/4 Tx**, partial, Lateral Funiculus **cut**	■ **GRPs** were isolated by dissociating from PLAP transgenic embryonic (E13.5) rat spinal cord. Once dissociated the cells were FACS sorting with A2B5. • 1×10^5 cells in 2μl injected either directly into the lesion, rostral and caudal (on segment) or just caudal **@ 1 wk PI** • **CsA** 10 mg/kg.day s.c. **SUBACUTE**	**SCI+** **1**. Lesion injection, 1 wk survival n = 2 **2**. Rostral/caudal injection, 1 wk survival n = 2 **3**. Lesion site + caudal injections, 5 wk survival n = 5 **4**. No transplant, but lesion filled with gelfoam at time of injury, BrdU 1 d prior to sacrifice n = 2 **5**. No SCI + cells n = 17	*Histology:* Transplanted cells were found in in spared white matter and along lesion border interface and expressed astrocytes and oligodendrocytes markers at 5 wk post transplantation. • Endogenous glial precursors proliferate after spinal cord Injury *Behavior:* Not reported
2b. Glial Restricted Precursors (GRPs), in combinatorial treatments				
Cao *J Neurosci* 2005	*Model:* Female Fischer 344 **rats**, 170–200 g *Injury:* **T9 contusion** **IH** Impactor 150 kdyn	■ **D15A-GRPs** with EGFP (GRPs immunopanned with A2B5 from embryonic rat spinal cord) □ **D15A-NIH3T3** cells as control • **D15A** is a multi-neurotrophin • 4×10^5 cells in total into 4 sites 1 mm rostral/ caudal on left and right sides of the midline **@ 9 d PI** • No mention of immunosupression **SUBACUTE**	**SCI+** **1**. D15A-GRP n = 13; 11 for BBB **2**. EGFP-GRP n = 14; 11 for BBB **3**. D15A-NIH3T3 cells n = 12; 11 for BBB **4**. Adenovirus injection of D15A n = 4, no BBB **5**. Media n = 13; 11 for BBB **6**. Laminectomy n = 4	*Histology Physiology:* D15A displayed oligodendrocyte and astrocyte markers. Roughly 1/3 of the D15A-GRP become oligodendrocytes, twice that of the GFP-GRP. • The D15A-GRP showed a recovery in latency of the tcMMEP response as well as an increase in amplitude. *Behavior:* Transplantation of D15A-GRP significantly improved recovery over GFP-GRP, D14A-NIH3T3 cells, and media controls (BBB: 15.2 vs. 12.6 for next best group). No improvement with either GFP-GRP or D15A-NIH3t3. This suggests that GRPs have to be modified to show improvement.
3. Neural and Glial Restricted Precursors in Combination				
Mitsui *J Neurosci* 2005	*Model:* Female SD **rats**, 225–250 g *Injury:* **T8/9 contusion** **NYU/ MASCIS** Impactor dropped from 25 mm and allowed to rest on spinal cord for 5 sec	■ **Neuronal** and **glial** restricted precursors (**NRP/GRP**) mixture, 25:75%. NRP and GRP from embryonic rat spinal cord; FACS for eNCAM and A2B5, respectively. ■ 5×10^5 cells in liquid collagen injected into the injury site + 2.5 $x10^5$ cells 3 mm rostral/ caudal to the injury center **@ 9 d PI** □ **MP** 30 mg/kg 10 min PI ■ **CsA** 10 mg/kg for day 2 wk, then oral (50 μg/ml). **SUBACUTE**	**SCI+** **1**. NRP/GRP n = 8 **2**. Media injected n = 10 **3**. Unoperated controls n = 6	*Histology:* The NRP/GRP group had a greater amount of spared tissue. ■ The grafted cells differentiated into astrocytes and oligodendrocytes. ■ Increase in 5-HT in the DLF, DH, and LF in the NRP/GRP group compared to the media control. ■ Increase in CRF in the SPN and DH of the NRP/GRP group compared to the media control *Behavior:* Cell transplantation increase voided volume in 2 & 3 wk vs. media control. NRP/GRP group had a decreased micturition pressure, number of episodes/micturition and bladder weight vs. media control. ■ Amelioration of hypersensitivity to thermal stimuli. ■ Transplantation increased BBB scores (9.4) vs. media (7.1).

Lepore *Exp Neurol* 2005	*Model:* Female SD **rats**, approx 250 g *Injury:* **C3/4** Lateral funiculus **cut**	■ **NRP/GRP** from spinal cord of PLAP transgenic E13.5 embryonic rat ■ The NRP and GRP co-cultured for 3–10 days and resuspended in Type I collagen matrix prior to transplantation ■ 4×10^5 cells injected into the lesion **@ 0 hr PI** □ Whole E14 fetal spinal cord tissue (FSC) **@ 0 hr PI** □ **MP** postoperatively ■ **CsA** 10 mg/kg/d s.c. **ACUTE**	SCI + **1**. NRP/GRP, 4 d survival **2**. NRP/GRP, 3 wk survival **3**. NRP/GRP, 5 wk survival **4**. E14 FSC, 4 d survival **5**. E14 FSC, 3 wk survival **6**. E14 FSC, 5 wk survival No indications of "n"	*Histology:* At the moment of transplantation, all cells in the mixture expressed the early neural marker, nestin. ■ Both E14/FSC and NRP/GRP filled the cavity by 3 wk PI and migrated in white matter. At 5 wk PI almost no cells (both groups) expressed nestin, but cells expressed NeuN, synaptophysin, as well as oligodendrocyte and astrocyte markers. ■ Graft-derived cells that had migrated into the host spinal cord expressed markers of neurons (close to injury site), astrocytes, and oligodendrocytes. *Behavior:* Not reported
Lepore *Brain Res* 2005	*Model:* Female SD **rats**, approx 250 g *Injury:* **C3/4** Lateral funiculus **cut**	■ **NRP/GRP** from spinal cord of PLAP transgenic embryonic rat. The NRP and GRP were co-cultured for 5–10 days. ■ 1×10^6 cells or 4×10^6 NRP/GRP or 1×10^6 beads **intrathecally** into L3-L5 **@ 24 hr or 1 or 2 or 3 wk PI** □ **MP** postoperatively. ■ **CsA** 10 mg/kg.day s.c. **SUBACUTE**	**SCI+** **1**. 1×10^6 cells at 24 hr PI n = 9 **2**. 4×10^6 cells at 1, 2 and 3 wk PI n = 8 **3**. Flourescent beads at 1 wk PI n = 3 Survival: 4 d, 2 and 5 wk after transplantation	*Histology:* In both group1 and 2 found cells at the lesion site, with more cells animals that were in group 2. ■ The cells displayed a mature morphology ■ Injected cells also found in the brain at the interface of the ventricles and parenchyma. Cells found as far as 10 mm away from the injury site in the white matter. Very few fluorescent beads made it into the lesion site. ■ Cells co-localize with astrocyte (GFAP), oligodendrocyte (RIP) and possibly neural (NeuN, synaptophysin) markers *Behavior:* Not reported
Neuhuber *J Neurosurg Spine* 2008	*Model:* Female SD **rats**, approx 250 g *Injury:* **T8-T9 contusion NYU/ MASCIS** (25 mm)	■ **NRPs** and **GRPs** from embryonic rat spinal cord grown in **FGF/ NT-3** for 9–13 days; labeled with hPLAP. • 5×10^4 cells/μl (intrathecal **i.t.** delivery) or • 1×10^5 cells/μl (direct injection) **@ 9 d PI** • **i.t.** delivery: 40μl injected into spinal canal from L4–5 • Direct injection: 5μl into lesion site in liquid collagen and 2.5μl rostral/caudal of lesion site in media • **CsA** 10 mg/kg/d **SUBACUTE**	**SCI+** **1**. NRPs/GRPS via direct injection n = 10 **2**. NRPs/GRPS via i.t. delivery n = 10 **3**. Control n = 10	*Histology, Physiology:* Graft volume of direct injection NRPs/GRPs (5.9 ± 1.3 mm^3) much greater than i.t. NPC (0.3 ± 0.08 mm^3). i.t. injected cells found only dorsally • Both direct injection and i.t. injection of NRPs/GRPs reduced % of injured tissue and increased % of spared tissue compared to controls *Behavior:* Only direct injection (9.4 ± 0.5) of had improved function on BBB compared to control (7.2 ± 0.4). i.t. NRPs/GRPs (8.1 ± 0.5) *(Comment: there were small group differences prior to transplant)* • Direct spinal cord injection of NRPs/GRPs resulted in lower micturition pressure (vs. i.t.-neuronal /glial precursors or controls) • Both direct and i.t. injected NRPs/GRPs animals had less detrusor hyper-reflexia • i.t. injected NRPs/GRPS animals had a decreased bladder capacity

(*Continued*)

Table 8. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
4. Oligodendrocyte Precursor Cells Derived From Newborn Rodents				
Bambakidis *Spine J* 2004	*Model:* Female SD **rats**, 170–220 g *Injury:* **T9/10 NYU** Impactor, 10g×12.5 mm	■ **OPCs** from **P0** SD rats and mmunopanned for A2B5; labeled with a fluorescein diacetate fluorescent dye. 1.5×10^5 cells into the spinal cord injury site **@ 5 d PI** □ Injection of recombinant glycoprotein molecule Sonic hedgehog (**Shh**) (6μl of 50ng/ml) **@ 0 hr PI** **@ 1d + 3 d PI** ■ No mention of immunosuppression **SUBACUTE**	**SCI+** **1**. OPCs n = 8 **2**. OPCs + Shh n = 10 **3**. Shh n = 9 **4**. Saline n = 12 **5**. Laminectomy only n = 8 Survival: 28 d PT	*Histologic/Biochemical/Physiologic:* Significantly more spared white matter in treated groups. • Trend of improved latencies in motor evoked potential (MEP) in the same groups that correlated with the improvements in BBB scores and spared tissue. • Transplanted animals with and without Shh had high nestin and O4, but those animal that received only Shh had relatively few O4 (normal nestin). *Behavior:* A significant improvement of transplanted animals with and without Shh at 28 d post injury (**BBB: control = 13.6, OPCs = 18.3, OPCs + Shh = 19.4**). No significant difference with Shh alone (BBB = 16.4)
Lee *J Neurotrauma* 2005	*Model:* Male SD **rats**, 300–350 g *Injury:* **T9 contusion NYU** Impactor 10g×25 mm	■ **Oligodendrocyte-type-2 astrocyte** O-2A progenitors **from P2 rats. Dissocated primary cells cultured for 10–15 days to induce differentiation.** ■ **Cells were labeled with Brdu** **5×10^5 cells were into the spinal cord injury site** @ 7 days PI ■ **CsA** 10 mg/kg/day s.c. **SUBACUTE**	**SCI +** **1**. O-2A progenitors n = 21 **2**. Culture media control n = 19	*Histology:* Colocalization of Brdu and oligodendrocyte marker (CNPase), *(comment: Brdu is believed to lack the specificity required for tracking transplanted cells (Iwashita, 2000 Neuroreport).* ■ A significant increase in reterogradely labeled (FG) red nuclei and reticular nuclei in the transplanted group relative to the controls. ■ No difference in SSEPs latency between transplanted group and media. ■ A significant decrease in MEP latency for the transplanted group vs. the media control group. *Behavior:* A significant improvement in BBB scores of transplanted animals vs. control from 7 wk PI **At 9 week BBB: transplant = 12.2, control = 9.8**

5. Oligodendrocyte Precursor Cells Derived From Human Embryonic Stem Cells

Keirstead *J Neurosci* 2005	*Model:* Female SD **rats**, 200–220 g *Injury:* **T8–11 contusion, IH** 200 kdyn acutely; **IH** 150, 200, 250 kdyn chronically	■ **Human ESC** (H7)-derived pre-differentiated **OPCs** (passage 32) □ **Human fibroblasts** (hFBs) In total 1.5 10^6 cells (**high**) or 2.5 10^5 (**low**) injected both rostral/caudal (one site each). **@ 7 d PI** **@ 10 months PI** • **CsA** s.c. 10 mg/kg/d **SUBACUTE & CHRONIC**	Subacute: **SCI+** **1**. Low + cell hESC-d-OPC n = 8 **2**. High + cell hESC-d-OPC n = 3×8 **3**. hFBs controls n = 8 **4**. Media n = 2×8 Chronic: **SCI+** **5**. 150, 200, 250 Kdyn, + hESC-d-OPCs **6**. 150, 200 250 Kdyn, + media n = 6 in each group	*Histology:* 7 d PI transplanted hESC cells resided primarily around the lestion, at times up to 7 mm rostral the injury site. • 7 d PI transplanted hESC significantly increased oligodendrocyte remyelination and decreased the density of demyelinated axons. • In chronic transplants, hESC were present but did not increased remyelination. *Behavior:* Subacutely transplanted hESC (both 2.5×10^5 cells or 1.5×10^6 cells) promotes significant recovery ($p < 0.01$) fromt 3 wk PI At 8 wk PI the hESC high cell group had **a BBB score of 16 vs. 13** and 12 for hFBs and media. **hESC-d-OPCs improved stride length, stride width, toe spread and paw rotation.** • No improvement in chronic groups
Cloutier *Regen Med* 2006	*Model:* Female SD **rats**, 200–220 g *Injury:* **T8–11 contusion, IH** 50 kdyn or 200 kdyn	■ **Human ESC** (H7) pre-differentiated OPCs or □ **Human fibroblast** controls (hFBs) In total 1.5×10^6 cells in 15μl injected rostral/caudal (one site each) @ **7 d PI** • **CsA** s.c. 10 mg/kg/d **SUBACUTE**	**SCI+** **1**. 50 kdyn + hESC-d-OPCs n = 10 **2**. 200 kdyn + hESC-d-OPCs n = 7 **3**. 50 kdyn + hFBs n = 9 **4**. 200 kdyn + hFBs n = 7	*Histologic/Biochemical/Physiologic:* 2 months hESC derived OPCs survival in the site of implantation & up to 6 mm cranial and caudal in 200 kdyn group, but failed to migrate in 50 kdyn group. • Extensive oligodendrocyte remyelination in 200 kdyn group. No remyelination with hFBs. • No evidence of demyelination or remyelination following 50 kdyn injury. *Behavior:* BBB data presented only for the 50 kdyn group. At time of transplant both hFb and hESC had BBB scores 19**. hESC had no detrimental affect during the following 7 wk.**

d: day, days; hr: hour, hours; IH – Infinite Horizon Impactor; PI: post-injury; PT: post-transplant; s.c.: subcutaneous; SCI: spinal cord injury; SD: Sprague–Dawley; Tx: transection; wk: week, weeks; + ve: positive; -ve: negative.

5HT: serotonin; BBB: Basso, Beattie and Bresnahan locomotor test; BDNF – brain-derived neurotrophic factor; ChAT: choline acetyltransferase; CRF: corticotrophin releasing factor; CsA: Cyclosporine; CSPG - chondroitin sulfate proteoglycan; CST: corticospinal tract; eNCAM: embryonic neural cell adhesion molecule; EGFP: enhanced green fluorescent protein; DH:dorsal horn; DLF: dorsolateral funiculus; ESC: embryonic stem cell; FACS: fluorescence activated cell sorting; FG: fluorogold; FGF: fibroblast growth factor; GFAP: glial fibrillary acidic proteins; GFP – green fluorescent protein; GRP: glial restricted precursor cell; LF: lateral funiculus; LP: lumbar puncture; MEP: motor-evoked potentials; MP: methylprednisolone; NCAM: neural cell adhesion molecule; NPC: precursor cell; NRP: neural restricted precursor cell; PLAP: placental alkaline phosphatase; Shh: sonic hedgehog; SPN: spinal parasympathetic nucleus; SSEP: somatosensory evoked potentials.

Table 9. Summary Statement for Fate-Restricted Neural and Glial Precursors

Pros	*Cons*
Several studies (3) indicate more white matter sparing and (re)myelination of host axons after transplantation of rodent and human oligodendrocyte precursors at one week after thoracic contusions, correlating with behavioral improvements.	Convincing behavioral improvements with GRPs was only seen in conjunction with overexpression of a (BDNF/NT-3) neurotrophin or with co-transplantation as GRP/NRP mixtures.
Preclinical characterization for embryonic stem-cell derived oligodendrocyte precursors has reached the level of FDA consideration for Phase 1 trials	Logistics of GRPs/NRPs as fetal-derived cells will raise ethical concerns and safety issues.
	It is not known whether embryonic stem cell-derived OPCs bear a tumor-risk due to possible contamination with pluripotent stem cells.
Knowledge Gaps	
The overall number of studies with these cells is still small and independent replications would be highly desirable to allow firmer conclusions.	
Demyelinated axons as a therapeutic target for remyelination strategies in SCI remain controversial, and behavioral improvements seen are only correlated to observations of remyelination.	
Efficacy in chronically injured spinal cords has yet to be demonstrated. One initial study using a one year delay in intervention failed to demonstrate behavioral efficacy.	

Unfortunately, the rodent-to-rodent transplantations do not provide more insights regarding the cell characteristics. Twenty-two studies with rodent BMSCs employed blunt injury models (17 contusions) in mostly rat studies (only two murine studies). Given the perceived relevance of blunt models to support a rationale for clinical translation, these studies will be discussed. A large proportion of these 22 studies reported positive behavioral effects (12 studies), while six did not show behavioral data and four failed to see benefits. It must not be overlooked that the transplantation of any cell may confer benefits over saline or media injections, and this is rarely controlled for by using additional cell types as controls (e.g., fibroblasts). In most studies, the cells were injected directly into or next to the SCI site, yet, in some hands, intrathecal (Ohta et al., 2004) and even intravenous (Urdzikova et al., 2006) delivery seems successful. Other researchers did not have success with intravenous delivery (Fan et al., 2008). Most studies used a subacute or acute timing for the transplantation, except Zurita and Vaquero (2004, 2006) and Vaquero and colleagues (2006) who delayed the treatment to 3 months after spinal-cord contusion by weight drop at T6–8. These authors allowed the rats to survive for up to 12 months, and reported improvement to a BBB of around 17, whereas the control animals were completely paralyzed throughout (BBB = 0). Such poor performance in controls is highly surprising, as even rats with completely transected spinal cords typically score a few points on the BBB. Nevertheless, these long survival times should be considered, as most rodent studies are terminated around 6–8 weeks when the performance is deemed to reach a "plateau."

In the light of the widely observed behavioral benefits, it is somewhat surprising that the histological data are very divergent. Histological observations of these cells range from good survival and differentiation of BMSC into neural cells, to poor survival and no differentiation into neural cells. Claims of differentiation are less credible when *in-vitro* dyes have been used to label the transplanted cells (e.g., the chromatin stain Hoechst; see Guest et al., 2008, in OEC section). Still, the heterogeneity of histological results once more underlines that beneficial behavioral effects can be brought about by multiple factors. These range from neuroprotection (via secretion of trophic factors and modification of inflammation) to the recruitment of endogenous cells, including stem cells and remyelinating cells, and – although hotly debated – the differentiation and integration of neural cells originating from the transplant. Indeed, several studies reported more preserved white matter or less cell death, indicative of neuroprotection (Ankeny et al., 2004; Bakshi et al., 2006; Dasari et al., 2007; Ohta et al., 2004; Urdzikova et al., 2006), while another could not confirm these effects (Yoshihara et al., 2006).

The claims of axonal regeneration in contusion studies can only be partially interpreted within the site of the lesion but not the host spinal cord itself where spared axons and regenerated axons are not easily distinguishable. Such questions are better addressed in sharp models of SCI, and 10 studies transplanted BMSCs into fully ($n = 3$) or partially ($n = 7$) transected spinal cords. Interestingly, two of the three studies with full transection reported behavioral improvements on the BBB score from 3 to 7 (Kamada et al., 2005; Koda et al., 2007). While this may be due to some axonal regeneration, other mechanisms like trophic effects on spinal circuits below the level of the injury site cannot be ruled out. BMSCs do promote axonal growth and these effects may be attributed to invading SCs; axon growth can be greatly enhanced with co-expression of trophic factors by the transplanted BMSCs (Lu et al., 2007).

From a translational perspective, BMSCs are the most widely studied cells using rodents, large mammals, and primates. This and the easy access to BMSCs for autotransplantation explains their use in several human treatment studies using a mixture of BMSCs and hematopoietic (mononuclear) cells (Callera et al., 2006; Chernykh et al., 2007; Saito et al., 2008; Yoon et al., 2007). Unfortunately, these reports included small patient cohorts only, used uncharacterized mixtures of bone-marrow cells, and were mostly not controlled. Hence, a systematic clinical validation is needed. A summary of the pros and cous and knowledge gaps of BMSC transplantation for SCI is given in Table 11.

Table 10. Bone Marrow-Derived Stromal Cells – BMSC – Mesenchymal Stem Cells

The stromal cells from the bone marrow are isolated and separated from the hematopoetic cell fraction of the bone marrow by their predilection to adhere to plastic. Some authors exclude the contamination by hematopoetic cells (which are CD 34 positive) by FACS. BMSCs are hence a crude mixture of stromal cells which support the growth of hematopoetic stem cells and mesenchymal stem cells. Some authors provide some additional (somewhat unspecific) markers to characterize these mesenchymal stem cells. Hence the actual stromal versus mesenchymal stem cell nature of the transplanted cells is unclear in many studies.

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
1. Human Bone Marrow Stromal Cells (BMSCs)				
Sheth *J Neurosurg Spine* 2008	*Model:* **Adult** female SD nude **rats**, 160–180 g *Injury:* **T9 contusion**, **NYU** weight drop, 10 g×12.5 mm	■ 6 μl of media with or without 600,000 **HUMAN BMSCs** (shipped frozen, thawed & expanded prior to transplant) injected directly into injury epicenter **@ 1 wk PI** Some BMSCs were labeled with GFP using lentiviral vectors prior to transplant. • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. Human BMSCs (n = 24) **2**. GFP-labeled human BMSCs (n = 5) **3**. Media control (n = 14) Survival: 6 wk	*Histology:* The number of surviving BMSCs was relatively low and varied considerably from rat to rat (not quantified). **None of the transplanted cells expressed human-specific NSE, neurofilament H, or GFAP.** Abundant neurofilament H labeled axons were found bridging the lesion in some of the BMSC animals. Average cavity volume in the BMSC group was sig smaller (~2 mm^3) than in the control group (~4 mm^3) –neuroprotective effects. *Behavior:* BBB Scores – Most rats recovered to a BBB score of 11 or 12. **No significant difference was found in BBB scores between groups at any timepoint.**
Lee *Acta Neurobiol Exp (Wars)* 2007	*Model:* **Adult** male SD **rats,** 300–350 g *Injury:* **T9** moderate **contusion NYU** Impactor, 10 g×25 mm	■ **HUMAN BMSCs** (3rd or 4th passage, adherent cells from the iliac bone marrow of 10–15 year old human donors; retrovirally labeled with β-Gal) 5μl of media with or without 500,000 BMSCs injected **directly into lesion** epicenter **@ 1 wk PI** • **CsA** (10 mg/kg/d, i.p.) from 2 d prior to transplant until endpoint. **SUBACUTE**	**SCI+** **1**. Media group (n = 11) **2**. hBMSC group (n = 18) Rats randomly assigned to groups Survival: 9 wk	*Histology:* hBMSCs survival demonstrated in the spinal cord up to 3 wk PT. Very few cells claimed to express neuronal (Tau) or astrocytic (GFAP) markers *Behavior:* Weekly testing for 2 months PI BBB Scores – **modest (but sig) improvement in hBMSC-treated group** compared to control group by 8 wk PT, ~11.5 versus ~10 Von Frey testing the hBMSC-treated group showed sig decreased withdrawal thresholds by 8 wk PT SSEPs –hBMSC-treated group (n = 10) had shorter latencies than control group (n = 8), Motor Evoked Potentials (MEPs) **no significant differences** between the two treatment groups.
Kim *Acta Neurochir Suppl* 2006	*Model:* **Adult** male SD **rats,** 200–300 g *Injury:* **T9 contusion NYU** weight drop, 10g×25 mm	■ **HUMAN BMSCs** (adherent cells from iliac crest bone marrow) labeled with BrdU and injected directly into the lesion site (in 5 μl PBS) **@ 1 wk PI** • For 7 d PT each rat also received 5 μl/d of PBS infused into CSF via **intrathecal** catheter, and that PBS contained **bFGF** (0.36 ng/μl) for some of the BMSC-treated rats. • **CsA** (10 mg/kg/day; from 2 d prior to transplant until endpoint. **SUBACUTE**	**SCI+** **1**. PBS transplant & infusion (control) **2**. BMSC transplant & PBS infusion **3**. BMSC transplant & bFGF infusion n = 10 per group Survival: 8 wk PT	*Histology:* Estimated cavity volume in control > BMSC > BMSC&bFGF group. BrdU + /human mito + cells were found in vicinity of the lesion and some BrdU + ve cells were + ve for GFAP (suggesting astrocytic differentiation), but –ve for MAP2 (i.e., no evidence for neuronal differentiation). *Behavior:* BBB Scores –From 5–8 wk PT BMSC-treated groups outperformed controls and by 8 wk PT BMSC **& bFGF (~13) > BMSCs (~11); > control (~10)**

(*Continued*)

Table 10. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Deng *Cytotherapy* 2008	*Model:* **Adult** female SD **rats**, 240–270 g *Injury:* **T10 contusion** weight drop, 10g 50 mm	■ **HUMAN BMSCs** (adherent CD34- cells from mono-nuclear fraction of human BM; 5–7 passages) labeled with BrdU. 250,000 cells in 5 μl injected into the spinal cord 'at 3 spots on average': injury center, 2 mm rostral, and 2 mm caudal to lesion **@ 30 min PI** □ **Human OECs** (from outer layers of aborted/dead fetal olfactory bulbs; 5–7 months of gestation; 3–4 passages) labeled with Hoechst 33342 @ 30 min PI • **CsA** (10 mg/kg/day, s.c.) for 2 weeks following transplant. **ACUTE**	**SCI+** **1.** 5 μl of BMSCs **2.** 5 μl of OECs **3.** 3.4 μl of BMSCs & 1.6 μl of OECs **4.** 5 μl of DMEM-F12 (control) n = 15 per group Survivalt: 5 wk PT	*Histologic/Biochemical/Physiologic:* All cell groups had sig more axons in the lesion than the control group, and the **BMSC & OEC cografted group had sig. more axons in the lesion site than all other groups**. Authors claim neuronal and glial differention of the transplanted BMSCs *Behavior:* BBB Scores – At 5 wk PT the **BMSC & OEC** group showed coordinated weight-supported stepping (**BBB = ~15**), the **BMSC** group frequent coordination (**BBB = ~13**), the OEC group occasional coordination (**BBB = ~11**) and the **control** group failed to gain weight support at all (**BBB = ~6**). tcMMEP at 5 wk PT:. BMSC & OEC group (7.3 ± 0.7); OECs (8.0 ± 1.6); BMSCs alone (9.6 ± 2.6), all were sig better than control (13.4 ± 3.7)
Himes *Neurorehabil Neural Repair* 2006	*Model:* **Adult** female SD **rats**, 225–250 g *Injury:* **Contusion** using **MASCIS** Impactor with 10 g rod dropped from the following heights: 12.5 mm – mild injury 25 mm – moderate injury 50 mm – severe injury	■ **HUMAN BMSCs** (adherent cells; 5×10^5 cells in 10μl injected directly into lesion epicenter in the mild and severely injured group. Moderately injured rats received two additional injections of 2.5×10^5 hBMSCs in 5μl (with or without cells) at rostral and caudal ends of the lesion **@ 1 wk PI** □ **MP** (62.5 mg/ml) i.v. immediately after contusion plus 2 hr later. • **CsA** 10 mg/kg/day starting 3 d before transplantation and continuing for 2 wk PT, after 2 wk PT the drinking water (Neoral, 50 μg/ml) **SUBACUTE**	Expt 1 - **Mild SCI+** **1.** hBMSCs n = 7 **2.** Vehicle alone n = 8 Expt 2 - **Severe SCI+** **1.** hBMSCs n = 3 **2.** Vehicle alone n = 5 Expt 3 - **Moderate SCI+** **1.** hBMSCs into epicenter and both rostral and caudal n = 5 **2.** Vehicle alone, same sites n = 8 Survival: 11 wk	*Histology:* Severe SCI: 50% died before 11 wk endpoint. BMSCs detected at 2 wk PT, but very few remained at 11 wk PT. Moderate SCI: Numerous axons passed through lesion in BMSC-grafted tissue, (not quantified). More S100 + ve Schwann cells and RIP + ve oligodendrocytes within the injury zone of BMSC-treated rats *Behavior:* BBB: Mild SCI – BMSC group improved (BBB = **15 ± 1 vs. 12.9 ± .5** at 11 wk PT) Severe SCI – BMSC-treated **transient improvement only** at 3 weeks. Moderate SCI – BMSC group was only sig higher at 1, 3, and 7 wk PT –**no different long-term functional recovery**. Exploratory Rearing – **improved in BMC group**. Grid Walking – **no sig difference** between groups Thermal Sensitivity – BMSCs did not worsen
Cizkova *Cell Mol Neurobio* 2006	*Model:* **Adult** male Wistar **rats,** 300–320 g *Injury:* **T8–9** modified **balloon compression** injury (12.5μl of saline for 5 min)	■ **HUMAN BMSCs,** mononuclear, adherent cells from adult male & female bone marrow prelabeled with BrdU (~64%) • non-adherent CD34 + hematopoietic cells excluded by passaging • 1 million hMSCs in 0.5 ml DMEM delivered by IV injection **@ 7 d PI** • *Immunosuppression:* none **SUBACUTE**	**SCI+** **1.** hBMSC group n = 15 **2.** Control 0.5 ml DMEM n = 15 Survival: 28 d PI	*Histology:* Transplanted cells mainly infiltrated ventrolateral white matter tracts, and spread to segments up to 3 mm rostral/caudal to the injury epicenter – (NUMA (human nuclei marker) and BrdU). Differentiation: • Neuronal – BrdU/MAP2 colabeled cells in ventral horn or lamina VII; no colabeling with NeuN • Glial - ~2% of BrdU + cells colabeled with APC (oligo marker); no colabeling with GFAP or NeuN. • More GAP-43 + axons found within damaged white matter tracts in treated rats (not quantified). *Behavior:* **BBB** rats receiving hMSCs had sig higher scores than control at 21 and 28 d PI (**15.7 ± 1.5 vs. 11.0 ± 1.3 at 28 d PI**)

Neuhuber *Brain Res* 2005	*Model:* **Adult** female SD **rats**, 225–250 g Gender? *Injury:* **C3–4 Subtotal** right cervical **hemisection**	■ **Human BMSCs** (plastic-adherent) 4 different donors prelabeled with PKH26 membrane dye. • **Gelfoam** soaked in media alone or containing **5×10^5 BMSCs** was implanted into the cavity, followed by an injection of 5–10 μl of media or BMSCs (5×10^4 cells/μl) **@ 0 hr PI** □ **MP** 30 mg/kg, i.v. was administered 10 min after injury and 2 hr later • **CsA** 10 mg/kg/day, initially s.c.; after 2 wk via drinking water **ACUTE**	Rats receiving hBMSCs from the 4 different donors made up 4 different treatment groups (n = 8 per group). In addition there was a gelfoam transplant control group (n = 8) and an uninjured behavioral control group (n = 10). 2 rats from each BMSC group were sacrificed at 2 w PI, 4 additional animals died leaving n = 5 or 6 for all BMSC groups and n = 7 for the gelfoam group, all of which lasted until the 11 wk PI endpoint.	*Histology:* BMSCs, identified by PKH26 labeling and antibodies against human mitochondria (hMITO), integrated well into lesion site with little migration out of the graft at 2 wk PI; many NF + ve and GAP-43 + ve axons were observed in the grafts at 2 wk PI as well. After 11 wk PI the grafts were devoid of BMSCs – hMITO negative. Only 3 of the 4 BMSC-donor groups showed sig greater average axon growth (NF) into the graft than the gelfoam control group, demonstrating donor-dependent variability of axonal regeneration. BMSCs from different donors produced differing amounts of certain growth factors (e.g., VEGF) and cytokines (e.g., SDF-1a and IL-6) *Behavior:* BBB – **no sig results** - almost complete recovery of all rats after this type of lesion. Limb preference test – **no sig recovery or group differences.** The behavioral outcomes also varied sig between groups of animals treated with BMSCs from different donors and donor groups reached significicance in different tests (cyinder; grid walking; thermal sensitivity).
Zhao *Cell Transplant* 2004	*Model:* **Adult** male Wistar **rats**, 250–350 g *Injury:* **Left** spinal cord **hemisection** at **T9**	■ **HUMAN BMSCs** adherent cells from mononuclear fraction of bone marrow of healthy human adults or □ **CD34 + UCBCs** = CD34 + cells from mononuclear fraction of blood from healthy **human full-term neonates.** • Two injections of 5μl of PBS (one rostral and one caudal to injury site (5×10^4 cells/μl for a total of 500,000 cells) **@ 0 hr PI** • All cells labeled with BrdU prior to transplant • Immunosuppression not mentioned **ACUTE**	**SCI+** **1**. BMSCs **2**. CD34 + UCBCs **3**. PBS control n = 20 per group Survival: 7 d PT (n = 2/group) 14 d PT (n = 2/group) 21 d PT (n = 2/group) 28 d PT (all remaining rats)	*Histology:* A small number of BrdU + cells were also GFAP + (~7%) or NeuN + (1–2%) at 28 d PT in samples of tissue from both cell-treated groups – (confocal IHC). *Behavioral:* Modified Tarlov Scores – Cell-treated groups showed sig higher scores than control at 7, 14, 21, and 28 d PT, (controls ~4–5; **rats with cell treatments (6–7) CD34 + UCBCs sig better than BMSCs at 7 & 14 d PT, but not thereafter.** Survival – no rats in either cell-treated group died prior to the final endpoint, whereas 7 of the 20 rats (35%) in the control group died prior to 28 d PT final endpoint.. *COMMENT: A large percentage of control rats died were 'LESS ACTIVE AND BARELY RESPONSIVE' – that means ENDPOINT HAS BEEN REACHED by Western Ethics. Blinding was likely compromised.*

(*Continued*)

Table 10. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
2. Primate and Pig MSCs				
Deng *Cytotherapy* 2006	***PRIMATE** Model:* Rhesus **monkeys** (gender?) *Injury:* **T9–10 Contusion** (50g weight dropped from 12cm through guide tube onto 10 mm^2 impact plate)	■ **MSCs from rhesus monkeys** (mononuclear cells from male rhesus monkey bone marrow aged 2–3 yrs). • **Pre-differentiated to neural lineage cells** • and labeled with Hoechst 33342 prior to transplant. • 2.5 million MSC-derived cells in 0.2 mL of PBS injected directly into lesion **@ 2 wk PI** • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. BMSC-derived neuron treatment group (n = 5) **2**. No treatment control (n = 5) Survival: 90 d PT True blue tracer was injected 2–3 cm caudal to spinal cord injury 1 week prior.	*Histology:* The authors claim that ~10% of the Hoechst-labeled cells are co-labeled with neural markers (NF, NSE, or GFAP). Spared long axonal tracts in the transplanted group Electrophysiology: CSEP – recovered to near normal latency and amplitude in MSC-treated animals, but did not recover at all in control animals. MEP – substantial recovery of latency and amplitude in MSC-treated animals with no recovery in control animals at all. *Behavior:* • Sensory: Toe pinch reflex on both sides returned by 3–4 wk PT in treated monkeys but, only one monkey in the control group regained "a slight sensory response". • Motor: **Tarlov scores: Control animals show absolutely no recovery** (all 0 scores) and 3 of 5 die prior to the endpoint. **4 out of 5 experimental animals showed recovery of active joint movement (2–2.5 on Tarlov) by 3 months PT, and one weight support (3).**
Zurita *Transplantation* 2008	**PIGS** *Model:* **Adult** female **minipigs,** 20 kg *Injury:* **T12-T13 compression** injury (using 2 surgical Heifetz's clips for 30 min)	■ **Autologous BMSCs** (adherent cells from iliac crest) labeled with BrdU. 100 μl of autologous plasma with or without 15 million BMSCs injected into the lesion site **@ 3 months PI** • An additional 25 μl of plasma with or without 10 million BMSCs was injected at 4 points adjacent to the lesion cavity and another 500 μl with or without 15 million BMSCs were infused into the subarachnoid space. All pigs underwent 15 mins of passive mobilization of hindlimbs daily. • No immunosuppression – autologous transplants **3 month CHRONIC**	**SCI+** **1**. BMSCs (n = 7) **2**. Plasma control (n = 3) Survival: 3 months PT No statistics.	*Histologic/Biochemical/Physiologic:* • MRI: reduced lesion cavities from 0.18 to 0.13 cm^3 with BMSCs • IHC – Lesion cavities were partially bridged by bundles of myelinated (Luxol fast blue) NF + axons in the BMSC-treated group. BrdU + cells were reported in those bridges and claimed to express glial (p75, GFAP, and S100) and neuronal (NeuN and NF). *Behavioral:* Motor function scores – All pigs remained paraplegic prior to treatment. Pigs **receiving cells showed progressive functional recovery** starting with signs of movement in hindlimbs at 1 months PT and ending with an average of ~6 on the motor function scale (indicating weight support in stance but no stepping) at 3 months PT. SSEPs –SSEPs were absent following SCI prior to treatment. Control pigs failed to recover SSEP by 3 months PT, but **SSEPs were recovered in BMSC-treated pigs**.

3a. Rodent Bone Marrow Stromal Cells *(Blunt Injury Models)*

Bakshi J Neurotrauma 2006	*Model:* **Adult** female SD **rats,** 250–300 g *Injury:* "Midthoracic" **contusion** (**MASCIS** Impactor; 10 g weight from 25 mm height)	■ **BMSCs** from hPAP + Fischer 344 **rats** 1, 2, or 4 million BMSCs (50,000 cells/μl of media) • All cells delivered via lumbar puncture with flushing of syringe with 10μl saline **@ 4,7,9,13,20,or 27 d PI** • **CsA** (10mg/kg/d; from 3 d prior transpalnt) **SUBACUTE & CHRONIC**	**SCI+** **1**. 2 million BMSCs 1 week post injury with endpoints at 1 wk PT (n = 3), 2 wk PT (n = 2), or 6 wk PT (n = 6); **2**. 2 million BMSCs at 4, 9, 13, 20, or 27 days post injury (n = 5 each) endp. = 2 wk PT **3**. 1, 2, or 4 million BMSCs or vehicle 9 d PI n = 4; endpoint = 2 wk PT. **4a**. Single dose of 2 million BMSCs at 9 d PI n = 6 **4b**. 3 doses of 2 million BMSCs at weekly intervals starting 9d PI. 30 d PI endpoint n = 6 **5**. "no treatment" control n = 6	*Histology:* Exp 1) BMSCs congregated along the dorsal surface of the injured cord above the lesion site in a dense network (AP histochem). BMSCs accumulation is selective in damaged tissue, not random. BMSC group showed: Sig smaller cyst (empty areas) volume – Sig smaller injury volume - Sig more spared white matter Exp 2) cell volume was sig greater for early transplants < 2 wk PT, but later transplants did survive to the endpoint. Exp 3) No sig difference in cell volume was seen when comparing groups transplanted with 1, 2, or 4 million BMSCs *Exp 4)* trend towards more cells with multiple injections *Behavior:* Not reported
Ankeny *Exp Neurol* 2004	*Model:* **Adult** female Wistar **rats**, 200–250 g *Injury:* **T8 contusion** **OSU** Impactor; 1 mm displacement	■ **BMSCs** from adult male Wistar **rats,** pre-labeled with BrdU (10 μg/ml; 48 hr) • ~300,000 cells in 5 μl of PBS directly into lesion (T8) with or without 60,000 in 1 μl at T11 (near presumed site of hindlimb CPG) **@ 2 d PI** • *Immunosuppression:* None **SUBACUTE**	Preliminary Expt: **SCI+** **1**. BMSCs at T8 (n = 6) **2**. No Treatment (n = 5) Second Expt: **SCI+** **3**. BMSCs at T8 only (n = 6) **4**. BMSCs at T8 and T11 (n = 7) **5**. PBS (control) at T8 and T11 (n = 3) Survival: 8 wk	*Histology:* Most BMSCs at 8 wk PI were undifferentiated/unidentified. Some laminin/fibronectin or GFAP expression, no neuronal differentiation of the BMSCs Spared tissue area was sig greater rostrally and caudally BMSC-treated animals showed sig greater NF200 + area in lesion site *Behavior:* BBB and BBB Subscore (Popovich version) **- No sig difference** between BMSC and no treatment groups at any time point Hindlimb Airstepping - Sig more rats receiving **BMSC-transplants** (either at T8 or at T8 and T11) **exhibited spontaneous airstepping** behavior when weight was removed from their hindlimbs.
Fan *Chin J Traumatol* 2008	*Model:* Wistar **rats** *Injury:* **T11 contusion** modified **Allen's** method; 10 g from 15 cm	■ **BMSCs** (adherent cells from BM) of adult male Wistar **rats** [250–300 g] passaged 3 times) labeled with CM-DiI; 50 μl of PBS containing 5 million BMSCs • Different routes of administration • Intervention timing unclear – assumed to be immediately post-injury • *Immunosuppression:* None.	**SCI+** **1**. BMSC intra-ventricular injection **2**. BMSC injected into lesion (intrathecal) **3**. BMSC injected at L3-L4 (remote intrathecal) **4**. BMSC i.v. n = 10 per group Survival: 24 hr, 1, 2, 3, and 4 wk PT; n = 2 per timepoint.	*Histology:* Labeled cells were present in injured spinal cord parenchyma 24 hours post-transplant only with BMSC injected into lesion. Labeled cells did not migrate into the injured cord in substantial numbers in the i.v. administered group. Labeled cells started migrating into the cord at 1 wk PT in the **intraventricular and remote intrathecal injected groups and reached numbers similar to direct injection into lesion by 2 wk PT**. The number of labeled cells in those 3 groups was lower at 3 and 4 wk PT, with intraventricular and remote intrathecal animals having sig more cells (~30–44) than the group injected into the lesion (~19–25) at 4 wk PT. **i.v. delivery is not effective** *Behavior:* Not reported.

(*Continued*)

Table 10. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Hofstetter *PNAS* 2002	*Model:* **Adult** female Lewis **rats**, 250–260 g *Injury:* **T9 contusion NYU** Impactor, 25 mm height)	■ **BMSCs** (plastic-adherent) from adult male Lewis **rats** labeled with GFP by retrovirus. • 5 μl of BMSC (30,000 cells/μl) suspension injected directly into lesion and 2.5 μl injected 2 mm rostral & 2 mm caudal to lesion for a total of 300,000 BMSCs. Control = media alone, same injection locations and volumes **@ 0 hr** **@ 1 wk PI** • *Immunosuppression:* not required **ACUTE and SUBACUTE**	**SCI+** **1**. BMSC injections immediately pi (n = 8) **2**. Media injections immediately pi (n = 8) **3**. BMSC injections 1 wk PI (n = 12) **4**. Media injections 1 wk PI (n = 10) Survival: 5 weeks PI	*Histology:* Expression of some neuronal markes in vitro, but no action potentials or voltage-gated Na + or K + currents (whole-cell recordings). Cell counts at 5 wk PI revealed sig more BMSCs survived in animals treated 1 wk PI (2,966 ± 681) than in those treated immediately after injury (518 ± 106). IHC: BMSC were tightly associated with longitudinally arranged immature astrocytes, formed bundles bridging the epicenter of the injury, and bundles of NF and 5-HT + ve fibers were found at the interface between graft and scar tissue. *Behavior:* ACUTE: No difference between BMSC and control groups on BBB SUBACUTE: **BMSC group improved to BBB from 7.9 + 0.1 in controls to 9.2 ± 0.5**. Control animals did not regain weight support in stance, but 9/12 in the BMSC group did, 2 regained weighted supported stepping.
Khalatbary *Neurol Res* 2007	*Model:* **Adult** female SD **rats,** 250–300 g *Injury:* **L1 contusion** weight drop 10g x 25 mm)	■ **BMSCs** labeled with BrdU adherent, fibronectin + ve cells cultured from adult female SD **rats** • 2.5×106 cells in 0.5 ml saline delivered **intravenously @ 1 wk PI** • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. BMSCs – killed 5 or 6 wk PI **2**. Media control – killed 5 or 6 wk PI (unclear) **3**. No treatment – killed immediately after injury **4**. No treatment – killed 5 wk PI Group sizes not stated	*Histologic/Biochemical/Physiologic:* BMSCs vs. media at 6 wk PI: BMSC group showed reduced cavitation compared to media (lacking statistical values, but authors claim 'significance'). BMSCs: BrdU-labeled BMSCs migrated into the spinal cord from the bloodstream, survived 4–5 wk, and showed highest density at centre of lesion and decreasing density both rostral and caudal. *The data suggest that IV delivered BMSCs get into the injured spinal cord as a result of blood-spinal cord barrier compromise following injury.* *Behavior:* BBB ran for 5 wk, but results not reported
Bi *Acta Pharmacol Sin* 2008	*Model:* Female SD **rats,** 220–250 g *Injury:* **T9 contusion** weight drop 10g×25 mm	■ **BMSCs**, labeled with BrdU (adherent cells from bone marrow of adolescent male SD **rats** [60–80 g]) **@ 1 wk PI** □ 8 mg/kg **Salvianoic Acid B** (Sal B) or PBS (equal volume) injected i.p. for 4 d immediately after injury or 3 d after transplant. • 10μl of PBS with or without 1 million BMSCs injected directly into lesion • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. BMSCs & PBS for 3 d PT n = 9 **2**. BMSC & Sal B for 3 d PT n = 9 **3**. Sal B i.p. for 4 d PI n = 6 **4**. PBS injected into lesion n = 6 **5**. PBS i.p. for 4 d PI n = 6	*Histology – qualitative:* Sal B i.p. alone provided a sig reduction in cavity area compared to PBS i.p. BMSCs seemed protected by Sal B – but this was not quantified to provide an estimate of total survival The authors also claim less cavitation in the BMSC & Sal B group compared to BMSCs alone or controls, but quantification, is entirely lacking. *Behavior:* **Sal B i.p. group had sig improved BBB scores at 28 d PI compared to PBS i.p. control (10.5 vs. 7.3,** respectively). Both BMSC treated groups had sig. higher BBB scores compared to PBS control from 14 d PT to endpoint.BMSC & Sal B treated group was sig better than BMSC alone at 21 and 28 d PT.

Jendelova *J Neurosci Res* 2004	*Model:* Wistar **rats**, 6–8 weeks old; gender? *Injury:* **T8–9 balloon compression** (15 μl of fluid for 5 min)	■ **BMSCs**, adherent cells from BM of 4 week old Wistar **rats,** labeled with BrdU and at 6–10 passages these were colabeled with iron oxide nanoparticles prior to transplant. BMSC were CD90 + & fibronectin + and CD11b & CD45 negative. □ mouse embryonic stem cells (ESCs) • Injected i.v. into femoral vein (0.5 μl? of PBS with or without ~2 million cells **@ 1 wk PI** • **Depo-Medrol** administered weekly for immunosuppression (dose not provided) **SUBACUTE**	**SCI+** **1**. BMSCs (n = 8) **2**. PBS (n = 6) **3**. ESC *n = ?* Survival: 4 wk PT	*Ex vivo* MRI: Nanoparticle-labeled cells populated the lesion 4 wk PT. *Histology:* Prussian blue + cells partially filled the lesion after BMSC transplant. As a result, the lesions appeared smaller in the BMSC group than in control rats (not quantified). Authors Claimed < 3% of BMSCs differentiated into neurons and none into astrocytes - *Behavior:* BMSC group reported to do better in open field test (BBB scores?) but data not shown.
Lee *Neuropathology* 2003	*Model:* **Adult** C57BL/6 **mice**, 15–20 g *Injury:* **T10 contusion,** 0.25 mm at 40 ms using pneumatic impact device	■ Adherent BMSCs from 5–8 week old **C57BL/6 male mice**; $2^{nd}/3^{rd}$ passage; Hoechst 33342 labeled cells suspended in DMEM at 1×10^3 cells per μl) and injected directly into (1.5 μl) & 2 mm rostral to the lesion (1.5 μl) **@ 1 wk PI** • *Immunosuppression:* None **SUBACUTE**	1. SCI + BMSC-treated mice (n = 3) Survival: 10 d PT (n = 1) and 4 wk PT (n = 2).	*Histologic/Biochemical/Physiologic:* GFAP expression in close proximity to Hoechst-labeled nuclei at 4 wk PT – suggested possible astrocytic differentiation of BMSCs. **No evidence for neuronal differentiation** using MAP2 or NeuN immunostain. *Behavior:* Not reported
Ohta *Exp Neurol* 2004	*Model:* 4 week old wildtype **SD rats, 70–90 g**, *gender?* Or 4 wk old Wistar **rats**, **70–90g** *Injury:* **T8–9 contusion** **NYU** Impactor 10 g × 12.5 mm or 10g × 25 mm	■ **BMSCs** (adherent cells – 4 passages) from 8 wk old male GFP + ve **Sprague Dawley** transgenic **rats** or wildtype **Wistar rats** (transfected with GFP-retrovirus) • 50 μl of PBS with or without (control) 5×10^6 BMSCs infused into CSF of 4^{th} ventricle **@ 0 hr PI** □ **FK506** (dosage not provided) was used in some of the SD rats receiving GFP + ve cells from SD transgenics. • No immunosuppression was used for cells from Wistars (inbred colony) **ACUTE**	Tracking GFP-labeled cells (SD rats): **12.5 mm SCI+** **1**. BMSC & FK506 n = 18 **2**. BMSC alone n = 18 **3**. Control n = 18 Survival: 4, 7, 14, 21, 28, & 35 d PT, n = 3 each Behavior & cavity volume measures (Wistar rats) **1**. 12.5 mm SCI + BMSCs (n = 8) **2**. 12.5 mm SCI control (n = 8) **3**. 25 mm SCI + BMSCs (n = 8). **4**. 25 mm SCI control (n = 8) Survival: rats perfused at 35 d PT	*Histologic/Biochemical/Physiologic:* BMSCs were found attached to spinal surface at 4 days post-transplant – few invaded lesion; by 3 weeks post-transplant no BMSCs could be found. Similar observation with BMSCs from SD or Wistar rats regardless of the use of immunosuppression. No evidence for neural differentiation of BMSCs was found. Lesion cavity was significantly smaller in BMSC-treated rats. *Behavior:* BBB Scores **higher in BMSC-treated than control** rats at all time-points for rats with mild SCI (**14 ± 3 vs. 10 ± 2** at 5 weeks post-op), but only at 5 weeks post-op for rats with severe contusions (**10 ± 3 vs. 8 ± 0**).

(*Continued*)

Table 10. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Dasari *Neurochem Res* 2007	*Model:* **Adult** male Lewis **rats** (250–300g) *Injury:* Moderate **T10 contusion** **NYU** Impactor 10g×12.5 mm	■ Adherent, non-hematopoietic **BMSCs** integrinβ1 + and CD54 + = ICAM-1] adult female Fischer 344 **rats** • **Neural pre-differentiation** induced using RA and hEGF 5 μl of sterile PBS with or without ~250,000 BMSCs injected directly into lesion site **@ 1 wk PI** • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. BMSC post-SCI (n = 13) **2**. No treatment control (SCI only; n = 11) **No SCI:** **3**. Sham control (laminectomy & PBS injection; n = 10) **4**. No injury control (no laminectomy; n = 10)	*Histologic/Biochemical/Physiologic:* BMSCs survived in lesion site and migrated up to 2 mm in white matter, but weren't found in intact tissue. At 5 wk PT, BMSCs had differentiated into neurons (~28%), oligodendrocytes (~53%), and astrocytes (19%) – based on confocal colocalization of integrinβ1with NF200, CNPase, and GFAP, respectively; n = 3. At 2 wk PT, BMSC-treatment significantly reduced apoptosis of neurons and oligodendrocytes – based on decreased TUNEL and caspase expression, and increased FLIP, XIAP, and PARP expression compared to untreated group using immunos and Westerns for quantification *Behavior:* BBB: BMSC group better than no treatment (control). (**Group avg of 13 vs. 10**, respectively, at 2 wk PT – stat sig) Narrow-beam crossing: BMSC better than no treatment (control); **stat sig difference** in scores by 2 wk PT. *Comment: No treatment controls do not control for non-specific effects*
Vaquero *Neurosci Lett* 2006	*Model:* **Adult** female Wistar **rats** *Injury:* **Thoracic *(level?)* contusion** weight drop: custom made device 12 mm^2 surface, 25 g×20 cm - severe	■ **BMSCs** (adherent cells) **adult male Wistar rats**; 12 weeks old, labeled with Hoechst 33342; 3 million suspended in PBS injected i.v. (500μl) or directly into the lesion site (50 μl) **@ 3 months PI** *(comment: 50 ul seems very high)* □ All animals subjected to 15 min of passive mobilization of hindlimbs daily post-SCI. • *Immunosuppression:* None **3 month CHRONIC**	**SCI+** **1**. BMSCs i.v. (n = 20) **2**. BMSCs into lesion (n = 20) **3**. PBS i.v. (n = 5) **4**. PBS into lesion (n = 20) Survival: 6 months PT	*Histologic/Biochemical/Physiologic:* Hoechst-labeled cells in the spinal cords of 7 out of 20 rats in BMSC i.v. group (not counted), more in the BMSC into lesion group where cavities were partially filled and bridged by tissue bundles containing neurofilament + ve structures (axons?). *Behavior:* BBB Scores – assessed daily (odd) **PBS controls all scored 0** *(comment: that is odd)* on the BBB throughout the study. The BMSC i.v. group only reached scores of 0.8 ± 0.4 after 6 months PT, whereas the **BMSC into lesion group** showed sig motor recovery beginning 15 d PT that **reached 12.8 ± 1.3 by 6 months PT**. Cold Spray Test (sensory) Only the BMSC into lesion group showed sig recovery of sensitivity beginning at 1 months PT and was responding near normal by 6 months PT.

Wu *J Neurosci Res* 2003	*Model:* Wildtype SD **rats,** 4 wk of age, 70–90 g *Injury:* **T8–9 contusion NYU** Impactor 10 g×50 mm	■ **BMSCs** (adherent BM cells) from 8 wk old male **GFP-transgenic SD rats** • 20 μl of media with or without 1 million BMCs injected directly into the lesion center **@ 0 hr PI** • *Immunosuppression:* None **ACUTE**	**SCI+** **1**. BMSCs **2**. Media control Survival: 1, 2, 3, & 4 wk PI; n = 6 treated and 6 control rats per endpoint.	*Histology:* GFP + ve BMSCs survived and partially filled the lesion sites, but gradually decreased in number over time (less than 15%) by 3 wk PI. Cavity size – smaller in BMSC group than control at 3 wk PI. ImmunoEM – BMSCs appeared as fibroblast-like cells surrounded by a collagen fiber matrix. *Behavior:* BBB Scores **BMSC-treated rats achieved sig higher scores at 2, 3, and 4 wk PI**. 6 of 24 treated rats showed weight-supported stepping and consistent coordination at 3 wk PI (BBB score of 14), whereas control rats failed to show weight-supported stepping at all.
Satake *Spine* 2004	*Model:* Male Lewis **rats,** 250–350 g *Injury:* **T10 contusion NYU** Impactor, weight drop from a height of 6.25 mm (to produce partial, incomplete SCI)	■ Bone marrow-derived **BMSCs** (stromal cells or a mix of stromal & hematopoietic cells?) From male Lewis **rats** (5w, 100–125g) • Labeled with GFP using recombinant adenovirus (GFP-BMSCs) • 10^6 cells suspended in 100 μl HBSS injected into subarachnoid space at L4–5 **@ 3, 5, or 7 d PI** • *Immunosuppression:* None **SUBACUTE**	Transplants were done at 3, 5, and 7 d PI, with 7 or 14 d PT endpoints, so there were 6 distinct groups each with rats receiving: **1**. SCI + GFP-BMSCs (n = 6), or control injections - β-Gal labeled cells (n = 2–3) or 100 μl of PBS (n = 2–3). **2**. Sham control group all received GFP-MSCs (n = 6); ½ were killed 7 days later, other ½ at 14 days.	*Histology:* GFP + BMSCs delivered via lumbar puncture were found in very small numbers in and around an earlier thoracic contusion – few cells survived and migrated into the spinal cord lesion, as well as intact parenchyma. Overlap of GFP & Nestin signals in fluorescent microscopy suggests some immature neuronal differentiation, but convincing evidence of true colocalization (e.g., confocal) is lacking. *Behavior:* Not reported
Yano *J Neurotrauma* 2005	*Model:* **Adult** Wistar **rats,** 200–250 g *Injury:* **T10 contusion pneumatic** impact device – 1 mm displacement, 40 ms, and 2 ms	■ **BMSCs** from 4–8 week old GFP transgenic **mice;** (adherent CD34– cells) 7 μl with 1×10^4 cells/μl injected into the spinal cord at T9 (5 mm rostral to the injury site) **@ 0 hr PI** • **CsA** (10mg/kg/d; s.c.) for 4 wk PT. **SUBACUTE**	**1**. SCI + BMSCs(n = 3) **2**. SCI – No treatment (n = 11) **3**. No injury (n = 6) Survival: 4 wk PT	*Histology:* At 4 wk PT *in vivo* fluorescent imaging revealed GFP + cells around the transplant site in 3 of the 6 uninjured rats and in all 3 rats with SCI the cells migrated toward the injury site. Some GFP + cells claimed to expressed neuronal (MAP2 and NeuN) and astrocytic (GFAP) markers. *Behavior:* BBB Scores – **No significant difference** between treated and untreated injured animals up to 28 d PI. *Numbers not provided*
Yano *J Neurotrauma* 2006	*Model:* **Adult** SD **rats,** 200–250 g; gender? *Injury:* **T10 contusion pneumatic** impact device – 1 mm displacement, 40 ms, and 2 ms	■ 5 μl of media with or without rat **BMSCs** (1.5×10^4 cells/μl; from 5–8 wk old female SD **rats**; 4th passage) *labeled with Hoechst 33342* and injected at T9 (**8 mm rostral to the injury center** & 2 mm deep) **@ 1 wk PI** • **CsA** (10 mg/kg/d; s.c.) for 4 wk PT **SUBACUTE**	**SCI+** **1**. BMSCs (n = 7) **2**. SCI + Media control (n = 4) Survival: 4 wk PT	*Histology:* Hoechst-labeled BMSCs migrated toward the injury site - *C: unreliable marker* ^{125}I-iomazenil radioactivity indicative of improved GABA receptor function in the area around the injury following BMSC transplant. Some (~10%) GFP + BMSCs were also positive for MAP2 and $GABA_A$ receptor α1-subunit antibodies – suggesting limited neuronal differentiation. *Behavior:* BBB Scores – **no significant difference between BMSC- and media- treated groups**

(*Continued*)

Table 10. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Yoshihara *Brain Res* 2006	*Model:* **Adult** female SD **rats**, 225–250 g *Injury:* Thoracic (?) **contusion MASCIS** device, 10g x 25 mm	■ 10 μl of Vitrogen (collagen matrix) injected into lesion site with or without 500,000 **BMSCs** (from hPLAP transgenic Fischer **rats**; 4th or 5th passage?) and 5 μl of media with or without 250,000 BMSCs injected at both the rostral and caudal edges of the lesion **@ 9 d PI** □ Some rats also received exercise (motorized cycle training) 3 days/week until endpoint • **CsA** (30 mg/kg/d, s.c., for 3 d prior to transplant and 15 mg/kg/d, s.c. following transplantation) **SUBACUTE**	**SCI+** **1**. Vitrogen matrix control (n = 10) **2**. BMSC + Vitrogen (n = 10) **3**. BMSC + Vitrogen & exercise (n = 6) Survival: 3 months after translantation	*Histology:* • No neuroprotective effects of BMSC treatments (lesion size). BMSCs did not migrate away from the lesion. • Survival was highly variable at 3 months PT. Exercise did not alter BMSC survival **no correlation between cell survival and behavioral scores.** Many host macrophages (hPLAP-/ED1+) were present in and around the lesion in all animals at 3 months PT. No indication of neuronal (NeuN), astrocytic (GFAP), or oligodendroglial (RIP) differentiation of BMSCs was found (double-label IHC) *Behavior:* BBB Scores –**but no sig differences among the groups at any timepoint up to 12 wk PI, and none of the animals in any group regained weight support**. Grid Test –**no sig differences** among the groups. Rearing Test –**no sig difference** in attempted rears/min among the 3 groups.
Huang *J Huazhong Univ Sci Tech Med Sci* 2006	*Model:* **Adult** Female SD **rats,** ~200 g *Injury:* **T9 contusion** using a modified Allen's method (weight drop 10 g×5 cm). Only rats with BBB score < 4 @ 1 wk PI were included	■ **BMSCs** from 2 month old adult SD **rats**; (3×10^4/μl; 5th passage, adherent) nuclei pre-labeled with Hoechst, alone, **NGF** (20 AU/μl) alone, or both. 5 μl injected directly into the lesion site, 2.5 μl injected 2 mm rostral and caudal to lesion, all at depth of 1 mm, **@ 1 wk PI** • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. DMEM (control) n = 8 **2**. BMSCs n = 8 **3**. NGF n = 8 **4**. BMSCs & NGF n = 8 Survival: 1 month PI for ½ of each group. 2 month PI for the other ½ of each group.	*Histology:* • Hoechst-labeled cells were seen in the spinal cord at 1 and 2 months PI - - Cavity volume (HE stain) – reported lower in 'experimental' groups - - Reported axonal regeneration and neuronal and glial differentiation of the transplanted cells – *alternative explanations possible* *Behavior:* BBB Scores – Treated groups (average) indicates some degree of weight support by 8 wk PI, whereas the control group did not, and had **statistically significant ($p < .05$) improvements in BBB scores** compared to control rats
Urdzikova *J Neurotrauma* 2006	*Model:* **Wistar rats**, 300–330 g *Injury:* **T8–9 balloon compression** (15 μl of fluid for 5 min)	■ **BMSCs** (stromal, adherent) or ■ **Bone Marrow (**BM) "cells" mix of: mononuclear cells – Hematopoetic SCs, macrophages, lymphocytes, and BMSCs) from 4 week old **Wistar rats @ 1 wk PI**. BMSCs were passaged 6–10 times, labeled with iron oxide nanoparticles and PKH26; **BM** cells were freshly prepared not cultured. • 2 million cells in 0.5 ml PBS injected into **femoral vein**. □ Some rats received **GCSF** to mobilize endogenous BMSCs • **Depo-Medrol** (2mg/rat/wk, i.m.) for immunosuppression **SUBACUTE**	**SCI+** **1**. BMSCs **2**. BM cells **3**. GCSF (5 μg/100 g) via i.v., from 7–11 d PI **4**. Saline n = 15 per group Survival: 35 d PI	*Histology: Ex vivo* MRI BMSCs migrated to the lesion site; Claim little Prussian blue staining colocalized with microglia/macrophages (ED1; not quantified). Lesion size (n = 3/group) was smaller in rats that received cells or GCSF than control (not quantified). BMSC and BM cells- treated groups showed **spared white matter** rotral and caudal vs. control, some spared gray matter. *Behavior:* IR Heat Test – By 35 d PI, average **latency of withdrawal** to hindlimb stimulation was **sig reduced in BMSC, BM cells, and GCSF groups** compared to control. BBB Scores – **BMSC, BM cells, and GCSF groups were sig. higher than controls** from 14–35 d PI, with scores ranging from 10–12 compared to 7–9 for controls.

Zurita *Neuroreport* 2004	*Model:* **Adult** female Wistar **rats** *Injury:* **T6–8 (?) contusion**, weight drop, 25g×20 cm	■ **BMSCs** (adherent cells from adult male Wistar **rat** bone marrow; 12 wk old, 250–300 g) labeled with Hoechst 33342 and 50 μl of PBS with or without 1 million BMSCs injected directly into the lesion site **@ 3 months PI** • *Immunosuppression:* None **3 month CHRONIC**	**SCI+** **1**. BMSC n = 10 **2**. PBS control n = 10 All animals were subjected to 15 min of daily passive mobilization of hindlimbs post-injury. Survival: 4 wk PT	*Histology:* Hoechst-labeled BMSCs were found in tissue bridges in the lesion site at 4 wk PT (not quantified), and some of these cells had neurofilament or GFAP + cytoplasm – suggesting possible neuronal and astrocytic differentiation of transplanted cells – supported by morphological appearances. *Behavior:* BBB Scores – Tested daily but scores only shown for every 5 d PT. BMSC-treated group **showed sig improvement in BBB scores** starting at 15 d PI, eventually reaching an average of ~8 (plantar placement with no weight support). **Control rats showed no recovery (all scores of 0 throughout).** *But see discussion*
Zurita *Neurosci Lett* 2006	*Model:* **Adult** female Wistar **rats** *Injury:* **T6–8 contusion,** weight drop: 25 g×20 cm - severe	■ **BMSCs** (adherent cells) from adult male Wistar **rat** bone marrow; 12 weeks old, 250–300 g) retrovirally labeled with βGal **and 50 μl (odd)** with 5 million injected directly into the lesion site**@ 3 months PI** □ All animals were subjected to 15 min of daily passive mobilization of hindlimbs post-injury. • *Immunosuppression:* None **3 month CRHONIC**	**SCI+** **1**. BMSC (n = 20) **2**. PBS control (n = 10) Survival: 2 months PT (n = 3 BMSC rats), 6 months PT (n = 3 BMSC rats), 12 months PT (remaining rats)	*Histology:* BMSC group had smaller lesion cavities (not quantified) and βGal -labeled BMSCs in tissue bridges at 2, 6, and 12 months PT (not quantified). Descending Serotonin and TH + fibers, as well as CGRP + sensory fibers and BDA + (traced pyramidal tract) fibers were found in the bridges of BMSC-treated rats (not quantified) - suggestive of axonal regeneration, *COMMENT: the possibility of enhanced sparing not ruled out* *Behavior:* **BBB Scores** – BMSC-treated group reached an average of **~17 by 12 months PT**. Control rats showed absolutely no recovery (all scores of **0** throughout). *But see discussion*
de Haro *Neurosci Lett* 2005	*Model:* **Adult** female Wistar **rats** *Injury:* **T7 contusion** (25 g×20 cm) using hollow guide tube.	■ **BMSCs** (adherent cells) from adult (12 wk, 250–300 g) male Wistar **rats**. Cells were labeled with Hoescht 33342 dye and the radioisotope 111In-oxine (63–75% labeling efficiency). • 6×10^6 111In-oxine-labeled BMSCs in PBS injected either i.v. or directly into lesion **@ 3 months PI** • *Immunosuppression:* none **3 month CHRONIC**	**SCI+** **1**. 6×10^6 111In-oxine-labeled BMSCs in 1 ml of PBS administered i.v. n = 10 **2**. 6×10^6 111In-oxine-labeled BMSCs in 100 μl of PBS injected directly into lesion at a rate of 0.25 μl/min n = 10 Survival: 10 days PI	*In vivo Imaging:* Gammagraphic images of 111In-oxine-labeled BMSCs collected from 3–10 d PT. These images revealed distribution of cells throughout the rats, and only scarce activity in the spinal cord, up to 10 days after i.v. administration of BMSCs. In contrast, labeled BMSCs injected directly into the lesion cavity showed persistent gammagraphic activity in the lesion without spread to the rest of the body for up to 10 d PT. *Histology:* Fluorescent microscopy revealed an average of 12 ± 7.3 bisbenzimide-labeled BMSCs in the entire spinal cord at 10 d PT following i.v. injection of cells. In sharp contrast, >100 BMSCs were found per section of spinal cord following intralesion administration. *Behavior:* Assessment unclear, but the authors state that all rats were paraplegic post-injury without signs of functional recovery after 3 months.

(*Continued*)

Table 10. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Chopp *Neuroreport* 2000	*Model:* **Adult** male Wistar **rats**, ~300 g *Injury:* **T9 Contusion** **NYU** Impactor; 10g×25 mm	■ Cultured, plastic-adherent **BMSCs** (Adult male Wistar rats) Cells prelabeled with BrdU (>90%) • 250,000 BMSCs in 4μl PBS injected directly into lesion **@ 1 wk PI** • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. BMSCs - 2 wk PI endpoint n = 6 **2**. BMSCs - 3 wk PI endpoint n = 6 **3**. BMSCs - 5 wk PI endpoint n = 9 **4**. PBS only control – 5 wk PI endpoint n = 10	*Histology:* Survival & migration: 1 wk PT - ~26,000 cells with ~2 mm of rostral/caudal migration 2 wk PT - ~21,000 cells with ≥2 mm migration 4 wk PT - ~15,000 cells with ~5 mm max migration Double immunohistochemical labeling for BrdU and NeuN suggest some neuronal differentiation of transplanted BMSCs. BMSC-treated spinal cords also showed more Nestin and RIP staining near the transplanted cells (not quantified). *Behavior:* Weekly BBB only. **BMSC–treated groups showed an average improvement in BBB score** from 7.0 (1 wk PI) to **15.3** (5 wk PI), whereas PBS-treated rats only improved from 6.7 (1 wk PI) to **11.5** (5 wk PI). The groups were significantly different from 2 wk PI on. BMSC group also showed significantly improved BBB scores over time (repeated measures ANOVA), indicating that they continued to show improve from 2 to 5 wk PI.
Koda *Neuroreport* 2005	*Model:* Female C57BL/6 **mice** (8–9 weeks, 25g avg.) *Injury:* Extradural **compression** (**20 g, 5 min**) at **T8** using a rectangular plastic plate.	■ **BMSCs (plastic adherent cells)** or □ **HSCs** (lineage marker −ve, c-kit- and Sca-I + ve cells) from male GFP transgenic **mouse** bone marrow. • 3 μl of either cell suspension or PBS injected directly into lesion @ **1 wk PI** • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. HSC group (3×10^4 cells in 3μl; n = 10) **2**. BMSC group (3×10^4 cells in 3μl; n = 10) **3**. PBS control group (3μl; n = 10) Survival: 6 wk PI	*Histology:* Some GFP + HSCs also stained with the oligo marker APC, but not the hematopoietic lineage marker CD-45 (not quantified). Some GFP + ve BMSCs also stained for fibronectin, but for neural lineage markers. *Behavior:* Hindlimb motor function score (Farooque et al., J Neurotrauma, 2001) **Both HSC and BMSC groups showed sig improvement compared to control from 3–6 wk PI**. At 6 wk PI the scores indicated partial hindlimb weight bearing in HSC group but no weight bearing in control group. No sig difference between HSC and BMSC groups at any timepoint.
3b. Rodent Bone Marrow Stromal Cells ***(Sharp Injury Models – Complete and Incomplete Transections)***				
Kamada J *Neuropathol Exp Neurol* 2005	*Model:* **Adult** male Wistar rats (8wk, avg weight 200 g) *Injury:* **Complete Tx** with removal of a 4 mm segment at T7-T8	■ **BMSC-derived Schwann cells (BMSC-SCs)** (plastic adherent) collected from femurs of adult male Wistar **rats** and prelabled with GFP using a retrovirus. • A 5 mm tube of ultrafiltration membrane filled with matrigel and 2×10^6 **BMSC-SCs** or **matrigel** (MG) alone was grafted into the gap in the spinal cord @ **0 hr PI** • *Immunosuppression:* None **ACUTE**	**SCI+** **1**. BMSC-SC group (n = 9) **2**. MG only control group (n = 7) Survival: 6 wk PI	*Histology:* Number of NF- and TH- + ve nerve fibers was sig higher in BMSC-SC group (~20) vs. control (~10), 1 mm into the graft from the interface at both ends and in the middle of the graft. No sig difference regarding 5-HT- or CGRP- + ve nerve fibers (very few). GFP + ve BMSC-SCs were S100, p75, and P0 + ve, but endogenous SCs were also noted (+ve for SC markers, but not GFP). *Behavior:* Sig recovery of hindlimb function in the BMSC-SC group compared to control from 4 wk PT until 6 wk PT (**BBB** avg at 6 wk PT **was 7.0 versus 3.6,** respectively). This recovery was abolished in two BMSC-SC rats by retransecting the graft at the mid-point at 6 wk PT and no functional gains were seen for these animals up to 4 wk later.

Koda *Eur Spine J* 2007	*Model:* **Adult** male Wistar **rats,** 8 wk, avg weight 200 g *Injury:* **Complete Tx** with removal of T8 segment (**between T7 and T9**; about 4 mm).	■ **BMSCs** (plastic-adherent cells from bone marrw of adult male Wistar **rats**) infected with adenovirus carrying **BDNF** (BMSC-BDNF) or LacZ (BMSC-LacZ) genes. • A 5 mm tube of ultrafiltration membrane filled with **matrigel** and 1×10^8 BMSC-BDNF or BMSC-LacZ or matrigel (MG) alone was grafted into the gap in the spinal cord **@ 0 hr PI** • *Immunosuppression:* None ACUTE	**SCI+** **1**. BMSC-BDNF **2**. BMSC-LacZ **3**. MG alone control Survival: 6 wk PI *"n" not stated*	*Histology:* Significant increases in the number of GAP43 + ve, TH + ve and CGRP + ve axons in various portions of the grafts in animals treated with BMSC-BDNF less so with BMSC-LacZ. • Few 5-HT fibers in any of the grafts (no sig difference between groups) Double labeling of BMSC groups showed fibronectin + ve cells (one marker for BMSCs) in close contact with GAP-43 + ve fibers. No fibronectin + ve cells in control grafts. *Behavior:* BBB:. Avg BBB scores at 6 wk PI **were 6.1, 6.0, and 3.6 for BMSC-BDNF, BMSC-LacZ, and control**, respectively. There were no sig differences between the two BMSC groups at any time point.
Cao *J Neurosci Res* 2007	*Model:* **Adult** female Wistar **rats** *Injury:* **Complete transection at ~T11**	■ **BMSCs** (adult female Wistar **rats**, 200–250g, n = 5) Cells pre-labeled with CFDA-SE. • 10μl of media with or without 500,000 BMSCs injected directly with gelfoam into lesion site at time of injury **@ 9 d PI** • *Immunosuppression:* None **SUBACUTE**	**SCI+** **1**. BMSCs n = 16 (12 for light microscopy, 4 for immuno-EM) **2**. Media control n = 4 (2 for light microscopy, 2 for immuno-EM) Survival: 1 wk PT	*Histology:* The transection site was bridged by regenerating tissues composed of fibers, cellular elements, and remnants of gelfoam. • Large numbers of CFDA SE-labled BMSCs found in lesion site – many were fragmented and phagocytosed by OX42 + microglia & macrophages. Some CFDA Se-labeled **BMSCs expressed CNP, CXCR4, NGF, and S100** (similar to in culture). • Marked increase in CNP + cells in the lesion site following BMSC transplant, all CNP + cells were also CXCR4 + . Some of these cells were also S100 + . More NF200 and SP + ve fibers found in the lesion sites of BMSC-treated rats; rare in control. • **Some of these cells showed morphological similarities to Schwann cells.** *Behavior:* Not reported
Bakshi *J Neurosurg (Spine)* 2004	*Model:* Inbred Fischer 344 **rats** *Injury:* Partial **right cervical hemisection** (Right lateral funiculus excised at **C3**)	■ **BMSCs** from hPAP + ve adult Fischer 344 **rats** or □ **LRNPs** (Lineage Restricted Neural Precursors [i.e., mixed population of NRPs and GRPs at ratio of ~1:1]) from E13.5 embryos • 2 million BMSCs or LRNPs in 40μl of culture medium & 10μl of saline **delivered i.v., intraventricularly, or intrathecally** (via lumbar puncture, **LP**) **@ 1 d PI** • **Hydrogel matrix** used to fill lesion cavity & dura incision covered with nylon • *Immunosuppression:* None **ACUTE**	**SCI+** **1**. BMSC via LP n = 12 **2**. BMSC via i.v. n = 4 **3**. BMSC via ventricle n = 4 **3**. LRNP via LP n = 6 **No SCI:** **5**. BMSC via LP without SCI n = 4 **5**. BMSC via i.v. without SCI n = 3 Survival: 3, 10, or 14 days post transplantation	*Histology:* • Regardless of delivery method, hPAP + ve BMSCs and LRNPs were found in the injured spinal cord tissue only, not in intact parenchyma or uninjured cords – (assessed by using hPAP histochemistry). • Regardless of delivery method, the number of BMSCs in the injured cord increased over time (from 3 to 10 or 14 days). The number of LRNPs also increased, but over a longer time point (from 10 days to 5 weeks). • LP and intraventricular delivery of BMSCs provided a higher number of BMSCs in the injured cord compared to i.v. infusion. *Behavior:* Not reported

(*Continued*)

Table 10. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Lu *J Neurosci* 2004	*Model:* **Adult** female F344 Fischer 344 **rats** – for injury and BMSC harvest. *Injury:* **C4 dorsal column transection** (wire knife)	■ Autologous **rat** GFP-labeled **BMSC**s (2×10^5 cells in 2μl) into lesion site @ **0 hr PI** □ **Dibutyryl-cAMP**, 50μg in 2μl injected into L4 DRG @ 5 d PRIOR injury □ Injection of **NT-3** within (0.6 μg in 2 μl) into lesion @ 0 hr PI & 1.5 mm rostral (1μg in 2μl) @ 1 wk PI (PI stimulus). • *Immunosuppression:* None **ACUTE**	**SCI + BMSCs plus:** **1**. cAMP & NT-3 injections n = 12 **2**. cAMP & PBS (instead of NT-3) injections n = 12 **3**. PBS (instead of cAMP) & NT-3 injections n = 12 **4**. PBS (instead of cAMP and rostral NT-3) & NT-3 in lesion only n = 12 **5**. PBS (instead of cAMP and NT-3) at all 3 sites n = 12 Survival: 1 & 3 months The dorsal column axons were traced with CTB (1%, 2μl into sciatic nerve) prior to sacrifice.	*Histology:* Grafts survived and integrated well with host tissue and rostral injection resulted in minimal parenchymal disruption (Nissl staining). BMSCs remained within the lesion site or migrated only short distances from the lesion. Growth of sensory axons into the BMSC graft differed between groups, but not over time, so the 1 and 3 month endpoint data were combined. This growth was sig augmented by either cAMP or NT-3 treatment compared to control (i.e., all cAMP & NT-3 treated groups were sig > group 5). Sensory axons extend beyond the lesion-graft boundary only in subjects that received rostral NT-3 injections. That growth was sig enhanced with the addition of cAMP treatment. *Behavior:* **No sig. functional differences were found** among the treatment groups at any time point on tape-removal task, horizontal ladder, or rope task.
Lu *Exp Neurol* 2005	*Model:* **Adult** female **Fischer 344 rats**, 160–200 g *Injury:* **C3 dorsal column transection** (wire knife).	■ **BMSCs** plastic-adherent cells isolated from Fischer 344 adult female **rats, some 'neurally induced'** by chemical treatment (BMSC-N), with or without retroviral transduction to overexpress human **BDNF** (BMSC-BDNF and BMSC-N-BDNF). • 2μl injected into the epicenter @ **0 hr PI**. All cell suspensions were $1\times10^5/\mu$l (i.e., ~200,000 cells total) • No immunosuppression – syngenic **ACUTE**	**SCI+** **1**. BMSCs (n = 22) **2**. BMSC-Ns (n = 4) **3**. BMSC-BDNF (n = 22) **4**. BMSC-N-BDNF (n = 4) **5**. PBS (n = 6) **6**. Sham (C3 laminectomy without lesion; n = 6) Survival: •1 month PT for histology and ELISA • 3 months PT behavioral and histological	*Histology:* All BMSC grafts survived well from 1–3 months PT, filling the lesion cavity and integrating with host tissue. BMSC-BDNF grafts tended to be larger and more densely packed with cells (especially endogenous Schwann cells) than BMSC grafts. BDNF-treated grafts were also larger at 3 months than at 1 month PT. BMSCs 'neurally induced' in vitro did not sustain a neural phenotype in vivo and these grafts were indistinguishable from those of non-induced BMSCs in terms of graft size, integration, and axonal growth at 1 month PT (IHC for NF quantified). At both 1 and 3 months PT, transduction of BMSCs to overexpress BDNF (compared to normal BMSCs) resulted in sig increase in the extent and diversity of host axonal growth, sig enhancing graft penetration by CTB-traced sensory axons as well as axons positive for NF, 5-HT, TH, ChAT, and CGRP. • 1 month PT ELISA: BDNF transduction of BMSCs sig enhanced production of all of neurotrophins. *Behavior:* Functional recovery **was not** observed for any of the groups (tape removal and rope-walking tasks).

Lu *Exp Neurol* 2007	*Model:* **Adult** female Fischer 344 **rats**, 160–200 g *Injury:* **C3 dorsal column transection** (wire knife)	■ Syngenic **BMSCs** from Fisher rats genetically modified to express NT-3 and/or GFP. 2.0×10^5 cells in 2.0 μl injected directly into the lesion **@ 6 wk PI** **CHRONIC**	**SCI+** **1**. BMSC (n = 10) **2**. BMSC-NT-3 (n = 10) **3**. No treatment (lesion only) n = 7 Ascending dorsal column sensory axons were traced in all animals with CTB (1%, 2 μl injected into sciatic nerve) 3 d prior to sacrifice. Survival: 6 wk and 3 months	*Histology:* IHC: At 6 wk PI the no treatment group showed cystic cavities with extensive astrocytosis (GFAP + ve) and dense deposition of NG2 surrounding the lesion. At 3 months PI the no treatment group showed similar cavitation, whereas the lesions were largely filled by cells in in 5/10 BMSC, and 8/10 BMSC-NT-3 transplants (Nissl stain; remaining 7 graft recipients had no GFP + ve cells in the lesion and were excluded from further study). More endogenous Schwann cells (27C7 antibody) were found in BMSC-NT-3 grafts). Strong NG2 expression within the grafts; NF + ve and CTB-traced axons were found in the lesion site in both cell transplant groups; the NT-3 expressing graft showed sig more of this axonal growth. Regenerating axons preferentially associated with Schwann cell surfaces expressing both NG2 and L1 and NCAM. Cell grafts were well vascularized (RECA1). *Behavior:* Not reported
Stokols *Tissue Eng* 2006	*Model:* **Adult** Female Fischer 344 rats, 160–200 g *Injury:* **C4 dorsal column aspiration** (2 mm long, 1.5 mm wide, 1.4 mm deep)	■ **Autologous BMSCs** (Adult female Fischer 344 **rats**) engineered to express GFP (**GFP-BMSCs**) or BDNF as well (**BDNF-GFP-BMSCs**) Cells suspended in **Matrigel (MG) or Fibrin** implanted into templated agarose scaffolds; ~150,000 BMSCs in MG. ~210,000 MSCs in Fibrin **@ 0 hr PI** Scaffolds were custom sized to fit lesion • No immunosuppression, autologous **ACUTE**	**SCI+** Agarose scaffold filled + **1**. BDNF-GFP-BMSCs in Matrigel n = 7 **2**. BDNF-GFP-BMSCs in Fibrin (n = 13) 3. GFP-BMSCs in Matrigel (n = 6) **4**. GFP-BMSCs in Fibrin (n = 6) **5**. Agarose scaffold alone (n = 5) **6**. No treatment control (n = 3) **7**. BDNF-GFP-BMSCs without scaffold (n = 3) Survival: 1 month PI.	*Histologic/Biochemical/Physiologic:* Agarose scaffolds were biocompatible and stable in vivo 1 month after implant. Most channels contained cells. • Axons grew into all templated agarose scaffolds in an organized and linear manner • BDNF-GFP-MSCs (secrete BDNF) had significantly enhanced axon penetration compared to GFP-MSCs • **Fibrin filled scaffolds had significantly greater axon penetration compared to Matrigel-filled scaffolds** (regardless of type of cell transplanted) • Myelinated (by Schwann cells) and unmyelinated axon bundles were found in the scaffold channels tEM, as were some astrocytes, blood vessels, and microglia, but not oligodendrocytes (IHC). *Behavior:* Not reported
Taylor *J Neurosci* 2006	*Model:* **Adult** Female Fischer 344 **rats**, 150–200 g *Injury:* **C3 dorsal column transection** (wire knife)	■ **Autologous BMSCs** (150,000 in 2 μl, prelabeled with BrdU) injected directly into lesion with (BMSC-NT-3) or without (BMSC) retroviral transduction of **NT-3** expression @ 0 hr PI □ Some animals also received injection of lentiviral vectors (2.5 μl) expressing **NT-3 (LV-NT-3)** or GFP (LV-GFP; control) 2.5 mm rostral to the lesion. • Immunosuppression: None **ACUTE**	**SCI+** **1**. BMSC-NT-3 & LV-NT-3 (n = 11 & 6 for ELISA) **2**. BMSC & LV-NT-3 (n = 10 & 6 ELISA) **3**. BMSC-NT-3 & LV-GFP (n = 8 & 6 ELISA) **4**. BMSC & LV-GFP (n = 8 & 6 ELISA) **5**. BMSC-NT-3 (no LV; n = 6 for ELISA) **6**. BMSC (no LV; n = 6 for ELISA) **7**. No lesion/cells & no LV (n = 4 & 3) Survival: 1 month PI.	*Histology:* **BMSC were used as a bridge of the lesion site – the study tests the effects of virally expressed NT3** • CTB-traced sensory axon profile quantification revealed avg axonal growth up to 250 μm beyond the lesion. This was **sig. greater for the BMSC-NT-3 & LV-NT-3 group than all others**. These animals also showed sig. greater average growth into the lesion and up to 500 μm beyond the lesion compared to groups not receiving LV-NT-3. It was noted that this enhanced axonal growth was only seen if the zone of NT-3 vector transduction reached within 100 μm of the lesion border. Upon reaching areas of high NT-3 expression beyond the lesion, the regenerating axons sprout locally rather than continuing to grow despite the continued presence of the growth factor gradient. *Behavior:* Not reported

(*Continued*)

Table 10. Continued

Reference	*Model/Injury*	*Intervention/Intervention timing*	*Experimental groups*	*Outcomes*
Papers that include the transplantation of BMSCs but are dealt with in the other tables				
Zhao *Cell Transplant.* 2004	Used human BMSCs and CD34 + UCBCs – reviewed in the section on BMSCs above.			
Lu *J Neurosci* 2006	OEC study that just uses fibroblasts and BMSCs for comparison of axonal growth in a blunt model. SEE OEC TABLES.			
Parr *Neuroscience* 2008	This paper focuses on transplants of neural stem/progenitor cells with and without BMSCs. Hence it is in the NSPC section.			

d: day, days; hr: hour, hours; PI: post-injury; IH: Infinite Horizon Impactor; i.v.: intravenous; PT: post-transplant; s.c.: subcutaneous; SCI: spinal cord injury; SD: Sprague–Dawley; Tx: transection; wk: week, weeks; + ve: positive; -ve: negative.

5HT: serotonin; APC: adenomatous polyposis coli gene protein; BBB: Basso, Beattie and Bresnahan locomotor test; BDA: biotinylated dextran amine; bFGF: basic fibroblast growth factor; BMSC: Human Bone Marrow Stromal Cell; CFDA-SE: carboxy fluorescein diacetate; CGRP - calcitonin gene-related peptide; ChAT: choline acetyltransferase; CNP: 2′, 3′-cyclic nucleotide 3′-phosphodiesterase/phosphohydrolase; CsA: Cyclosporine; CSEP: cortical somatosensory-evoked potential; CSPG - chondroitin sulfate proteoglycan; CST: corticospinal tract; EGF: epidermal growth factor; DMEM: Dulbecco's modified Eagle's medium; DRG: dorsal root ganglion; FLIP: FLICE-inhibitory protein; GAP-43 - growth associated protein-43; G-CSF: granulocyte colony stimulating factor; GFAP: glial fibrillary acidic proteins; GFP – green fluorescent protein; hPAP: human placental alkaline phosphatase; HSCs: hematopoetic stem cells; ICAM-1: intercellular adhesion molecule-1 (CD54); IHC: immunohistocemistry; LacZ – beta-galactosidase; LV: lentiviral vectors; MAP-2: microtubule-associated protein-2; MG: Matrigel; MP: methylprednisolone; MRI: magnetic resosnance imaging; NCAM: neural cell adhesion molecule; NF: neurofilament; NSE: neuron specific enolase; NGF: neural growth factor; OEC: olfactory ensheahting cell; NGF: nerve growth factor; PARP: poly [ADP- ribose] polymerase; RA: retinoic acid; SC: stem cells; Sca-1: stem cell antigen 1; SSEP: somatosensory evoked potentials; tcMMEP: transcranial magnetic motor evoked potentials; TH – tyrosine hydroxylase; UCBCs: umbilical cord blood cells; XIAP: X-linked inhibitor of apoptosis protein.

Table 11. Summary Statement for Bone Marrow Stromal Cells

Pros	*Cons*
Easily harvested for autotransplantations	Integration in the injured spinal cord very is limited
Both human and rodent BMSCs have been studied and demonstrate behavioral efficacy in many rodent SCI studies (18 of 25)	No convincing differentiation into neural cells – despite claims to the contrary.
Large animal and primate studies have been done, as well as studies in chronic contusion injuries.	BMSCs are a somewhat ill-defined populations of cells – most likely containing several subpopulations of mesenchymal stem cells.
BMSCs have some bridging capacity in sharp transection models which get populated by endogenous Schwann cells.	
Knowledge Gaps	
Little is known about the exact mechanisms by which BMSCs provide neuroprotection and improve behavioral outcomes. This information might allow screens for more homogeneous BMSCs with more effective properties.	
There are no studies assessing the benefits of BMSCs in acute or chronic cervical contusion injuries.	

Discussion

The potential to bridge the injured spinal cord and repopulate the area of injury with cells that might restore axonal continuity, bridge the area for axonal regeneration, and promote axonal growth back to its distal targets has fascinated SCI researchers for decades. At first, it would seem to be a relatively simple task: fill the inhospitable area of parenchymal devastation with growth-promoting cells, and let them "do their thing." The exciting discovery that CNS axons regenerated quite robustly into peripheral nerve grafts and SC environments was followed by the difficult realization that they were also rather reticent about reentering the host CNS. Excitement around the potential to overcome this barrier with OECs was accompanied by explosive interest in "stem cell" candidates that might have this and other therapeutic capabilities. Around the world, the enthusiasm of clinicians and desperation of patients thrust cell transplantation approaches into the translational spotlight, as individuals with cord injuries traveled around the globe to receive these technologies (at substantial financial cost). The announcement of FDA approval to conduct a stem-cell-based trial in North America in January 2009 ignited tremendous excitement, only to be put on hold some 6 months later for safety concerns.

Along the way, a great deal has been learned, and this should not be overlooked. It has become appreciated that the cells themselves may be the source of growth factors that can positively influence the environment. The initial goal of inducing rampant long-distance axonal regeneration has been dampened to a large degree by the realization that remyelination of demyelinated axons may be the most realistic therapeutic objective, although some authors believe that endogenous remyelination is effective albeit somewhat slower. Advances in cell biology have led to more sophisticated strategies of modifying cells with certain genetic traits, and developing purified sources of cells. The field has learned a great deal about evaluating what cells do once implanted into the cord: *How many survive? Do they integrate and migrate? What do they associate with? How do they influence the host environment?*

Alas, amongst this undeniable progress are significant questions, and a systematic review of the literature on this therapeutic approach exposes many gaps in our current knowledge. As an overview, one can quickly appreciate that while many labs around the world are studying cell transplantation therapies, substantial heterogeneity and uncertainty exists around the very nature of the cells that they are studying. This is perhaps best exemplified in the BMSC literature, but is equally perplexing in the discussion of precursor cells and stem-cell-based approaches. While our approach in this systematic review groups these under "umbrella" subtypes, the differences in source and culture conditions between a cell from one lab and the "same" cell from another may make them quite distinct in important ways. It is, unfortunately, far more complex than obtaining minocycline from Sigma, preparing it according to the manufacturer's protocol, and infusing a standard dose into a rodent after an acute SCI.

In this regard, while this review found many studies under each "umbrella" cell type, the actual pre-clinical substantiation for many cells within those subtypes seems quite modest. The "knowledge gaps" are fairly applicable across all of the cell types: the lack of independent replication (although this is inherently more difficult for cells than for pharmacologic treatments), the relative majority of laceration-type injuries over the more clinically relevant contusion injuries, the paucity of work in large animal models, and importantly, the near absence of chronic injury work. Given that patients with chronic injuries are the typically the most vocal proponents of cell transplantation therapies, and are the ones travelling around the world to be subjected to these unproven treatments, it is remarkable that so little has been reported in models of chronic injury. For those who have tackled this problem in pre-clinical studies, the results have generally not been very promising. This remains a daunting yet important task for the future, as chronically injured individuals with SCI will continue to be the most vocal "consumers" of this technology. Many of these chronically injured people seeking a cell transplantation treatment are likely to be unaware that the pre-clinical studies done at chronic time points on that particular cell have not been performed, or have shown negative results. Ironically, even for clinicians and scientists, they themselves might be surprised to note the gap between what they think "should" be done in pre-clinical studies before moving a cell transplant treatment into clinical trials and what is actually occurring in the field (or has happened in the past) (Kwon et al., 2009).

Despite these misgivings, the interest in cell transplantation for SCI will remain high, and it seems quite likely that FDA-sanctioned and closely regulated trials will be initiated in the foreseeable future. It is hoped that this systematic review has illustrated the pervasive interest in cell transplantation treatments for SCI, the experimental heterogeneity that inherently comes from such widespread interest, and has revealed both the promise and the knowledge gaps in these approaches, as they stood in the summer of 2008. The field will obviously continue to evolve, with hopes that further refinement and understanding will increase the chances that cell transplantation will someday emerge as a fruitful treatment for patients.

Acknowledgment

This review was supported by the Canadian SCI Solutions Network (Now Rick Hansen Institute). BKK holds a CIHR New Investigator Award, and WT is the Rick Hansen Man in Motion Chair in Spinal Cord Injury Research.

Author Disclosure Statement

No competing financial interests exist.

References

Schwann cells

Agudo, M., Woodhoo, A., Webber, D., Mirsky, R., Jessen, K.R., and McMahon, S.B. (2008). Schwann cell precursors transplanted into the injured spinal cord multiply, integrate and are permissive for axon growth. Glia 56, 1263–1270.

Azanchi, R., Bernal, G., Gupta, R., and Keirstead, H.S. (2004). Combined demyelination plus Schwann cell transplantation therapy increases spread of cells and axonal regeneration following contusion injury. J. Neurotrauma 21, 775–788.

Bamber, N.I., Li, H.Y., Lu, X.B., Oudega, M., Aebischer, P., and Xu, X.M. (2001). Neurotrophins BDNF and NT-3 promote axonal re-entry into the distal host spinal cord through Schwann cell-seeded mini-channels. Eur. J. Neurosci. 13, 257–268.

Barakat, D.J., Gaglani, S.M., Neravetla, S.R., Sanchez, A.R., Andrade, C.M., Pressman, Y., Puzis, R., Garg, M.S., Bunge, M.B., and Pearse, D.D. (2005). Survival, integration, and axon growth support of glia transplanted into the chronically contused spinal cord. Cell Transplant. 14, 225–240.

Biernaskie, J., Sparling, J.S., Liu, J., Shannon, C.P., Plemel, J.R., Xie, Y., Miller, F.D., and Tetzlaff, W. (2007). Skin-derived precursors generate myelinating Schwann cells that promote remyelination and functional recovery after contusion spinal cord injury. J. Neurosci. 27, 9545–9559.

Brierley, C.M., Crang, A.J., Iwashita, Y., Gilson, J.M., Scolding, N.J., Compston, D.A., and Blakemore, W.F. (2001). Remyelination of demyelinated CNS axons by transplanted human Schwann cells: The deleterious effect of contaminating fibroblasts. Cell Transplant. 10, 305–315.

Chau, C.H., Shum, D.K.Y., Li, H., Pei, J., Lui, Y.Y., Wirthlin, L., Chan, Y.S., and Xu, X.M. (2004). Chondroitinase ABC enhances axonal regrowth through Schwann cell-seeded guidance channels after spinal cord injury. FASEB J. 17, 194–196.

Chen, A., Xu, X.M., Kleitman, N., and Bunge, M.B. (1996). Methylprednisolone administration improves axonal regeneration into Schwann cell grafts in transected adult rat thoracic spinal cord. Exp. Neurol. 138, 261–276.

Duncan, I.D., Aguayo, A.J., Bunge, R.P., and Wood, P.M. (1981). Transplantation of rat Schwann cells grown in tissue culture into the mouse spinal cord. J. Neurol. Sci. 49, 241–252.

Duncan, I.D., and Milward, E.A. (1995). Glial cell transplants: Experimental therapies of myelin diseases. Brain Pathol. 5, 301–310.

Firouzi, M., Moshayedi, P., Saberi, H., Mobasheri, H., Abolhassani, F., Jahanzad, I., and Raza, M. (2006). Transplantation of Schwann cells to subarachnoid space induces repair in contused rat spinal cord. Neurosci. Lett. 402, 66–70.

Fouad, K., Schnell, L., Bunge, M.B., Schwab, M.E., Liebscher, T., and Pearse, D.D. (2005). Combining Schwann cell bridges and olfactory-ensheathing glia grafts with chondroitinase promotes locomotor recovery after complete transection of the spinal cord. J. Neurosci. 25, 1169–1178.

Golden, K.L., Pearse, D.D., Blits, B., Garg, M.S., Oudega, M., Wood, P.M., and Bunge, M.B. (2007). Transduced Schwann cells promote axon growth and myelination after spinal cord injury. Exp. Neurol. 207, 203–217.

Guest, J.D., Hesse, D., Schnell, L., Schwab, M.E., Bunge, M.B., and Bunge, R.P. (1997a). Influence of IN-1 antibody and acidic FGF-fibrin glue on the response of injured corticospinal tract axons to human Schwann cell grafts. J. Neurosci. Res. 50, 888–905.

Guest, J.D., Rao, A., Olson, L., Bunge, M.B., and Bunge, R.P. (1997b). The ability of human Schwann cell grafts to promote regeneration in the transected nude rat spinal cord. Exp. Neurol. 148, 502–522.

Halfpenny, C., Benn, T., and Scolding, N. (2002). Cell transplantation, myelin repair, and multiple sclerosis. Lancet Neurol. 1, 31–40.

Hill, C.E., Hurtado, A., Blits, B., Bahr, B.A., Wood, P.M., Bartlett, B.M., and Oudega, M. (2007). Early necrosis and apoptosis of Schwann cells transplanted into the injured rat spinal cord. Eur. J. Neurosci. 26, 1433–1445.

Hill, C.E., Moon, L.D.F., Wood, P.M., and Bunge, M.B. (2006). Labeled Schwann cell transplantation: Cell loss, host Schwann cell replacement, and strategies to enhance survival. Glia 53, 338–343.

Hood, B., Levene, H.B., and Levi, A.D. (2009). Transplantation of autologous Schwann cells for the repair of segmental peripheral nerve defects. Neurosurg. Focus 26, E4.

Hsu, J.Y., and Xu, X.M. (2005). Early profiles of axonal growth and astroglial response after spinal cord hemisection and implantation of Schwann cell-seeded guidance channels in adult rats. J. Neurosci. Res. 82, 472–483.

Hurtado, A., Moon, L.D.F., Maquet, V., Blits, B., Jerome, R., and Oudega, M. (2006). Poly (D,L-lactic acid) macroporous guidance scaffolds seeded with Schwann cells genetically modified to secrete a bi-functional neurotrophin implanted in the completely transected adult rat thoracic spinal cord. Biomaterials 27, 430–442.

Iannotti, C., Li, H.Y., Stemmler, M., Perman, W.H., and Xu, X.M. (2002). Identification of regenerative tissue cables using in vivo MRI after spinal cord hemisection and Schwann cell bridging transplantation. J. Neurotrauma 19, 1543–1554.

Iannotti, C., Li, H.Y., Yan, P., Lu, X., Wirthlin, L., and Xu, X.M. (2003). Glial cell line-derived neurotrophic factor-enriched bridging transplants promote propriospinal axonal regeneration and enhance myelination after spinal cord injury. Exp. Neurol. 183, 379–393.

Imaizumi, T., Lankford, K.L., and Kocsis, J.D. (2000). Transplantation of olfactory ensheathing cells or Schwann cells restores rapid and secure conduction across the transected spinal cord. Brain Res. 854, 70–78.

Kamada, T., Koda, M., Dezawa, M., Yoshinaga, K., Hashimoto, M., Koshizuka, S., Nishio, Y., Morlya, H., and Yamazaki, M. (2005). Transplantation of bone marrow stromal cell-derived Schwann cells promotes axonal regeneration and functional recovery after complete transection of adult rat spinal cord. J. Neuropathol. Exp. Neurol. 64, 37–45.

Keirstead, H.S., Morgan, S.V., Wilby, M.J., and Fawcett, J.W. (1999). Enhanced axonal regeneration following combined demyelination plus Schwann cell transplantation therapy in the injured adult spinal cord. Exp. Neurol. 159, 225–236.

Kohama, I., Lankford, K.L., Preiningerova, J., White, F.A., Vollmer, T.L., and Kocsis, J.D. (2001). Transplantation of cryopreserved adult human Schwann cells enhances axonal conduction in demyelinated spinal cord. J. Neurosci. 21, 944–950.

Martin, D., Robe, P., Franzen, R., Delree, P., Schoenen, J., Stevenaert, A., and Moonen, G. (1996). Effects of Schwann cell transplantation in a contusion model of rat spinal cord injury. J. Neurosci. Res. 45, 588–597.

Martin, D., Schoenen, J., Delree, P., Leprince, P., Rogister, B., and Moonen, G. (1991). Grafts of syngenic cultured, adult dorsal root ganglion-derived Schwann cells to the injured spinal cord of adult rats: preliminary morphological studies. Neurosci. Lett. 124, 44–48.

Martin, D., Schoenen, J., Delree, P., Rigo, J.M., Rogister, B., Leprince, P., and Moonen, G. (1993). Syngeneic grafting of adult rat DRG-derived Schwann cells to the injured spinal cord. Brain Res. Bull. 30, 507–514.

Meijs, M.F.L., Timmers, L., Pearse, D.D., Tresco, P.A., Bates, M.L., Joosten, E.A.J., Bunge, M.B., and Oudega, M. (2004). Basic fibroblast growth factor promotes neuronal survival but not behavioral recovery in the transected and Schwann cell implanted rat thoracic spinal cord. J. Neurotrauma 21, 1415–1430.

Menei, P., Montero-Menei, C., Whittemore, S.R., Bunge, R.P., and Bunge, M.B. (1998). Schwann cells genetically modified to secrete human BDNF promote enhanced axonal regrowth across transected adult rat spinal cord. Eur. J. Neurosci. 10, 607–621.

Moon, L.D.F., Leasure, J.L., Gage, F.H., and Bunge, M.B. (2006). Motor enrichment sustains hindlimb movement recovered after spinal cord injury and glial transplantation. Restor. Neurol. Neurosci. 24, 147–161.

Oudega, M., Xu, X.M., Guenard, V., Kleitman, N., and Bunge, M.B. (1997). A combination of insulin-like growth factor-I and platelet-derived growth factor enhances myelination but diminishes axonal regeneration into Schwann cell grafts in the adult rat spinal cord. Glia 19, 247–258.

Papastefanaki, F., Chen, P., Lavdas, A.A., Thornaidou, D., Schachner, M., and Matsas, R. (2007). Grafts of Schwann cells engineered to express PSA-NCAM promote functional recovery after spinal cord injury. Brain 130, 2159–2174.

Pearse, D.D., Marcillo, A.E., Oudega, M., Lynch, M.P., Wood, P.M., and Bunge, M.B. (2004a). Transplantation of Schwann cells and olfactory ensheathing glia after spinal cord injury: Does pretreatment with methylprednisolone and interleukin-10 enhance recovery? J. Neurotrauma 21, 1223–1239.

Pearse, D.D., Pereira, F.C., Marcillo, A.E., Bates, M.L., Berrocal, Y.A., Filbin, M.T., and Bunge, M.B. (2004b). cAMP and Schwann cells promote axonal growth and functional recovery after spinal cord injury. Nat. Med. 10, 610–616.

Pearse, D.D., Sanchez, A.R., Pereira, F.C., Andrade, C.M., Puzis, R., Pressman, Y., Golden, K., Kitay, B.M., Blits, B., Wood, P.M., and Bunge, M.B. (2007). Transplantation of Schwann cells and/or olfactory ensheathing glia into the contused spinal cord: Survival, migration, axon association, and functional recovery. Glia 55, 976–1000.

Pinzon, A., Calancie, B., Oudega, M., and Noga, B.R. (2001). Conduction of impulses by axons regenerated in a Schwann cell graft in the transected adult rat thoracic spinal cord. J. Neurosci. Res. 64, 533–541.

Plant, G.W., Bates, M.L., and Bunge, M.B. (2001). Inhibitory proteoglycan immunoreactivity is higher at the caudal than the rostral Schwann cell graft-transected spinal cord interface. Mol. Cell Neurosci. 17, 471–487.

Ramon-Cueto, A., Plant, G.W., Avila, J., and Bunge, M.B. (1998). Long-distance axonal regeneration in the transected adult rat spinal cord is promoted by olfactory ensheathing glia transplants. J. Neurosci. 18, 3803–3815.

Saberi, H., Moshayedi, P., Aghayan, H.R., Arjmand, B., Hosseini, S.K., Emami-Razavi, S.H., Rahimi-Movaghar, V., Raza, M., and Firouzi, M. (2008). Treatment of chronic thoracic spinal cord injury patients with autologous Schwann cell transplantation: An interim report on safety considerations and possible outcomes. Neurosci. Lett. 443, 46–50.

Schaal, S.M., Kitay, B.M., Cho, K.S., Lo, T.P., Barakat, D.J., Marcillo, A.E., Sanchez, A.R., Andrade, C.M., and Pearse, D.D. (2007). Schwann cell transplantation improves reticulospinal axon growth and forelimb strength after severe cervical spinal cord contusion. Cell Transplant. 16, 207–228.

Takami, T., Oudega, M., Bates, M.L., Wood, P.M., Kleitman, N., and Bunge, M.B. (2002). Schwann cell but not olfactory ensheathing glia transplants improve hindlimb locomotor performance in the moderately contused adult rat thoracic spinal cord. J. Neuroscience 22, 6670–6681.

Vavrek, R., Pearse, D.D., and Fouad, K. (2007). Neuronal populations capable of regeneration following a combined treatment in rats with spinal cord transection. J. Neurotrauma 24, 1667–1673.

Vroemen, M., Caioni, M., Bogdahn, U., and Weidner, N. (2007). Failure of Schwann cells as supporting cells for adult neural progenitor cell grafts in the acutely injured spinal cord. Cell Tissue Res. 327, 1–13.

Weidner, N., Blesch, A., Grill, R.J., and Tuszynski, M.H. (1999). Nerve growth factor-hypersecreting Schwann cell grafts augment and guide spinal cord axonal growth and remyelinate central nervous system axons in a phenotypically appropriate manner that correlates with expression of L1. J. Comp Neurol. 413, 495–506.

Wirth, E.D., III, Reier, P.J., Fessler, R.G., Thompson, F.J., Uthman, B., Behrman, A., Beard, J., Vierck, C.J., and Anderson, D.K. (2001). Feasibility and safety of neural tissue transplantation in patients with syringomyelia. J. Neurotrauma 18, 911–929.

Xu, X.M., Chen, A., Guenard, V., Kleitman, N., and Bunge, M.B. (1997). Bridging Schwann cell transplants promote axonal regeneration from both the rostral and caudal stumps of transected adult rat spinal cord. J. Neurocytol. 26, 1–16.

Xu, X.M., Guenard, V., Kleitman, N., Aebischer, P., and Bunge, M.B. (1995a). A combination of BDNF and NT-3 promotes supraspinal axonal regeneration into Schwann cell grafts in adult rat thoracic spinal cord. Exp. Neurol. 134, 261–272.

Xu, X.M., Guenard, V., Kleitman, N., and Bunge, M.B. (1995b). Axonal regeneration into Schwann cell-seeded guidance

channels grafted into transected adult rat spinal cord. J. Comp. Neurol. 351, 145–160.

Xu, X.M., Zhang, S.X., Li, H., Aebischer, P., and Bunge, M.B. (1999). Regrowth of axons into the distal spinal cord through a Schwann-cell-seeded mini-channel implanted into hemisected adult rat spinal cord. Eur. J. Neurosci. 11, 1723–1740.

Olfactory ensheathing glial cells

Au, E., Richter, M.W., Vincent, A.J., Tetzlaff, W., Aebersold, R., Sage, E.H., and Roskams, A.J. (2007). SPARC from olfactory ensheathing cells stimulates Schwann cells to promote neurite outgrowth and enhances spinal cord repair. J. Neurosci. 27, 7208–7221.

Barakat, D.J., Gaglani, S.M., Neravetla, S.R., Sanchez, A.R., Andrade, C.M., Pressman, Y., Puzis, R., Garg, M.S., Bunge, M.B., and Pearse, D.D. (2005). Survival, integration, and axon growth support of glia transplanted into the chronically contused spinal cord. Cell Transplant. 14, 225–240.

Barnett, S.C., Alexander, C.L., Iwashite, Y., Gilson, J.M., Crowther, J., Clark, L., Dunn, L.T., Papanastassiou, V., Kennedy, P.G., and Franklin, R.J. (2000). Identification of a human olfactory ensheathing cell that can effect transplant mediated remyelination of demyelinated CNS axons. Brain 123, 1581–1588.

Boyd, J.G., Lee, J., Skihar, V., Doucette, R., and Kawaja, M.D. (2004). LacZ-expressing olfactory ensheathing cells do not associate with myelinated axons after implantation into the compressed spinal cord. Proc. Natl. Acad. Sci. USA 101, 2162–2166.

Bretzner, F., Liu, J., Currie, E., Roskams, A.J., and Tetzlaff, W. (2008). Undesired effects of a combinatorial treatment for spinal cord injury – Transplantation of olfactory ensheathing cells and BDNF infusion to the red nucleus. Eur. J. Neurosci. 28, 1795–1807.

Cao, L., Liu, L., Chen, Z.Y., Wang, L.M., Ye, J.L., Qiu, H.Y., Lu, C.L., and He, C. (2004). Olfactory ensheathing cells genetically modified to secrete GDNF to promote spinal cord repair. Brain 127, 535–549.

Chuah, M.I., Choi-Lundberg, D., Weston, S., Vincent, A.J., Chung, R.S., Vickers, J.C., and West, A.K. (2004). Olfactory ensheathing cells promote collateral axonal branching in the injured adult rat spinal cord. Exp. Neurol. 185, 15–25.

Deng, Y.B., Liu, Y., Zhu, W.B., Bi, X.B., Wang, Y.Z., Ye, M.H., and Zhou, G.Q. (2008). The co-transplantation of human bone marrow stromal cells and embryo olfactory ensheathing cells as a new approach to treat spinal cord injury in a rat model. Cytotherapy 10, 551–564.

Deumens, R., Koopmans, G.C., Honig, W.M., Maquet, V., Jerome, R., Steinbusch, H.W., and Joosten, E.A. (2006). Chronically injured corticospinal axons do not cross large spinal lesion gaps after a multifactorial transplantation strategy using olfactory ensheathing cell/olfactory nerve fibroblast-biomatrix bridges. J. Neurosci. Res. 83, 811–820.

Doucette, R. (1991). PNS-CNS transitional zone of the first cranial nerve. J. Comp Neurol. 312, 451–466.

Guest, J.D., Herrera, L., Margitich, I., Oliveria, M., Marcillo, A., and Casas, C.E. (2008). Xenografts of expanded primate olfactory ensheathing glia support transient behavioral recovery that is independent of serotonergic or corticospinal axonal regeneration in nude rats following spinal cord transection. Exp. Neurol. 212, 261–274.

Imaizumi, T., Lankford, K.L., Burton, W.V., Fodor, W.L., and Kocsis, J.D. (2000a). Xenotransplantation of transgenic pig olfactory ensheathing cells promotes axonal regeneration in rat spinal cord. Nat. Biotechnol. 18, 949–953.

Imaizumi, T., Lankford, K.L., and Kocsis, J.D. (2000b). Transplantation of olfactory ensheathing cells or Schwann cells restores rapid and secure conduction across the transected spinal cord. Brain Res. 854, 70–78.

Kubasak, M.D., Jindrich, D.L., Zhong, H., Takeoka, A., McFarland, K.C., Munoz-Quiles, C., Roy, R.R., Edgerton, V.R., Ramon-Cueto, A., and Phelps, P.E. (2008). OEG implantation and step training enhance hindlimb-stepping ability in adult spinal transected rats. Brain 131, 264–276.

Li, Y., Field, P.M., and Raisman, G. (1997). Repair of adult rat corticospinal tract by transplants of olfactory ensheathing cells. Science 277, 2000–2002.

Li, Y., Field, P.M., and Raisman, G. (1998). Regeneration of adult rat corticospinal axons induced by transplanted olfactory ensheathing cells. J. Neurosci. 18, 10514–10524.

Lima, C., Pratas-Vital, J., Escada, P., Hasse-Ferreira, A., Capucho, C., and Peduzzi, J.D. (2006). Olfactory mucosa autografts in human spinal cord injury: a pilot clinical study. J. Spinal Cord. Med. 29, 191–203.

Lima, C., Escada, P., Pratas-Vital, J., Branco, C., Arcangeli, C.A., Lazzeri, G., Maia, C.A., Capucho, C., Hasse-Ferreira, A., and Peduzzi, J.D. (2010). Olfactory mucosal autografts and rehabilitation for chronic traumatic spinal cord injury. Neurorehabil. Neural Repair 24, 10–22.

Lopez-Vales, R., Fores, J., Navarro, X., and Verdu, E. (2007). Chronic transplantation of olfactory ensheathing cells promotes partial recovery after complete spinal cord transection in the rat. Glia 55, 303–311.

Lopez-Vales, R., Fores, J., Verdu, E., and Navarro, X. (2006). Acute and delayed transplantation of olfactory ensheathing cells promote partial recovery after complete transection of the spinal cord. Neurobiol. Dis. 21, 57–68.

Lu, J., Feron, F., Ho, S.M., kay-Sim, A., and Waite, P.M. (2001). Transplantation of nasal olfactory tissue promotes partial recovery in paraplegic adult rats. Brain Res. 889, 344–357.

Lu, J., Feron, F., Kay-Sim, A., and Waite, P.M. (2002). Olfactory ensheathing cells promote locomotor recovery after delayed transplantation into transected spinal cord. Brain 125, 14–21.

Lu, P., Yang, H., Culbertson, M., Graham, L., Roskams, A.J., and Tuszynski, M.H. (2006). Olfactory ensheathing cells do not exhibit unique migratory or axonal growth-promoting properties after spinal cord injury. J. Neurosci. 26, 11120–11130.

Moreno-Flores, M.T., Bradbury, E.J., Martin-Bermejo, M.J., Agudo, M., Lim, F., Pastrana, E., Avila, J., Az-Nido, J., McMahon, S.B., and Wandosell, F. (2006). A clonal cell line from immortalized olfactory ensheathing glia promotes functional recovery in the injured spinal cord. Mol. Ther. 13, 598–608.

Nash, H.H., Borke, R.C., and Anders, J.J. (2002). Ensheathing cells and methylprednisolone promote axonal regeneration and functional recovery in the lesioned adult rat spinal cord. J. Neurosci. 22, 7111–7120.

Pearse, D.D., Sanchez, A.R., Pereira, F.C., Andrade, C.M., Puzis, R., Pressman, Y., Golden, K., Kitay, B.M., Blits, B., Wood, P.M., and Bunge, M.B. (2007). Transplantation of Schwann cells and/or olfactory ensheathing glia into the contused spinal cord: Survival, migration, axon association, and functional recovery. Glia 55, 976–1000.

Ramon-Cueto, A., Cordero, M.I., Santos-Benito, F.F., and Avila, J. (2000). Functional recovery of paraplegic rats and motor axon regeneration in their spinal cords by olfactory ensheathing glia. Neuron 25, 425–435.

Ramon-Cueto, A., Plant, G.W., Avila, J., and Bunge, M.B. (1998). Long-distance axonal regeneration in the transected adult rat

spinal cord is promoted by olfactory ensheathing glia transplants. J. Neurosci. 18, 3803–3815.
Richter, M.W., Fletcher, P.A., Liu, J., Tetzlaff, W., and Roskams, A.J. (2005). Lamina propria and olfactory bulb ensheathing cells exhibit differential integration and migration and promote differential axon sprouting in the lesioned spinal cord. J. Neurosci. 25, 10700–10711.
Ruitenberg, M.J., Levison, D.B., Lee, S.V., Verhaagen, J., Harvey, A.R., and Plant, G.W. (2005). NT-3 expression from engineered olfactory ensheathing glia promotes spinal sparing and regeneration. Brain 128, 839–853.
Ruitenberg, M.J., Plant, G.W., Hamers, F.P., Wortel, J., Blits, B., Dijkhuizen, P.A., Gispen, W.H., Boer, G.J., and Verhaagen, J. (2003). Ex vivo adenoviral vector-mediated neurotrophin gene transfer to olfactory ensheathing glia: Effects on rubrospinal tract regeneration, lesion size, and functional recovery after implantation in the injured rat spinal cord. J. Neurosci. 23, 7045–7058.
Steward, O., Sharp, K., Selvan, G., Hadden, A., Hofstadter, M., Au, E., and Roskams, J. (2006). A re-assessment of the consequences of delayed transplantation of olfactory lamina propria following complete spinal cord transection in rats. Exp. Neurol. 198, 483–499.
Takami, T., Oudega, M., Bates, M.L., Wood, P.M., Kleitman, N., and Bunge, M.B. (2002). Schwann cell but not olfactory ensheathing glia transplants improve hindlimb locomotor performance in the moderately contused adult rat thoracic spinal cord. J. Neurosci. 22, 6670–6681.
Toft, A., Scott, D.T., Barnett, S.C., and Riddell, J.S. (2007). Electrophysiological evidence that olfactory cell transplants improve function after spinal cord injury. Brain 130, 970–984.

Neural stem/progenitor cells

Alexanian, A.R., Crowe, M.J., and Kurpad, S.N. (2006). Efficient differentiation and integration of lineage-restricted neural precursors in the traumatically injured adult cat spinal cord. J. Neurosci. Methods 150, 41–46.
Bottai, D., Madaschi, L., Di Giulio, A.M., and Gorio, A. (2008). Viability-dependent promoting action of adult neural precursors in spinal cord injury. Mol. Med. 14, 634–644.
Cao, Q.L., Zhang, Y.P., Howard, R.M., Walters, W.M., Tsoulfas, P., and Whittemore, S.R. (2001). Pluripotent stem cells engrafted into the normal or lesioned adult rat spinal cord are restricted to a glial lineage. Exp. Neurol. 167, 48–58.
Chen, J., Bernreuther, C., Dihne, M., and Schachner, M. (2005). Cell adhesion molecule l1-transfected embryonic stem cells with enhanced survival support regrowth of corticospinal tract axons in mice after spinal cord injury. J. Neurotrauma 22, 896–906.
Cummings, B.J., Uchida, N., Tamaki, S.J., Salazar, D.L., Hooshmand, M., Summers, R., Gage, F.H., and Anderson, A.J. (2005). Human neural stem cells differentiate and promote locomotor recovery in spinal cord-injured mice. Proc. Natl. Acad. Sci. USA 102, 14069–14074.
Fujiwara, Y., Tanaka, N., Ishida, O., Fujimoto, Y., Murakami, T., Kajihara, H., Yasunaga, Y., and Ochi, M. (2004). Intravenously injected neural progenitor cells of transgenic rats can migrate to the injured spinal cord and differentiate into neurons, astrocytes and oligodendrocytes. Neurosci. Lett. 366, 287–291.
Guo, J.S., Zeng, Y.S., Li, H.B., Huang, W.L., Liu, R.Y., Li, X.B., Ding, Y., Wu, L.Z., and Cai, D.Z. (2007). Cotransplant of neural stem cells and NT-3 gene modified Schwann cells promote the recovery of transected spinal cord injury. Spinal Cord 45, 15–24.
Hains, B.C., Johnson, K.M., Eaton, M.J., Willis, W.D., and Hulsebosch, C.E. (2003). Serotonergic neural precursor cell grafts attenuate bilateral hyperexcitability of dorsal horn neurons after spinal hemisection in rat. Neuroscience 116, 1097–1110.
Hasegawa, K., Chang, Y.W., Li, H., Berlin, Y., Ikeda, O., Kane-Goldsmith, N., and Grumet, M. (2005). Embryonic radial glia bridge spinal cord lesions and promote functional recovery following spinal cord injury. Exp. Neurol. 193, 394–410.
Hofstetter, C.P., Holmstrom, N.A., Lilja, J.A., Schweinhardt, P., Hao, J., Spenger, C., Wiesenfeld-Hallin, Z., Kurpad, S.N., Frisen, J., and Olson, L. (2005). Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat. Neurosci. 8, 346–353.
Ikegami, T., Nakamura, M., Yamane, J., Katoh, H., Okada, S., Iwanami, A., Watanabe, K., Ishii, K., Kato, F., Fujita, H., Takahashi, T., Okano, H.J., Toyama, Y., and Okano, H. (2005). 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. 22, 3036–3046.
Ishii, K., Nakamura, M., Dai, H., Finn, T.P., Okano, H., Toyama, Y., and Bregman, B.S. (2006). Neutralization of ciliary neurotrophic factor reduces astrocyte production from transplanted neural stem cells and promotes regeneration of corticospinal tract fibers in spinal cord injury. J. Neurosci. Res. 84, 1669–1681.
Iwanami, A., Kaneko, S., Nakamura, M., Kanemura, Y., Mori, H., Kobayashi, S., Yamasaki, M., Momoshima, S., Ishii, H., Ando, K., Tanioka, Y., Tamaoki, N., Nomura, T., Toyama, Y., and Okano, H. (2005). Transplantation of human neural stem cells for spinal cord injury in primates. J. Neurosci. Res. 80, 182–190.
Karimi-Abdolrezaee, S., Eftekharpour, E., Wang, J., Morshead, C.M., and Fehlings, M.G. (2006). Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J. Neurosci. 26, 3377–3389.
Kimura, A., Ohmori, T., Ohkawa, R., Madoiwa, S., Mimuro, J., Murakami, T., Kobayashi, E., Hoshino, Y., Yatomi, Y., and Sakata, Y. (2007). Essential roles of sphingosine 1-phosphate/S1P1 receptor axis in the migration of neural stem cells toward a site of spinal cord injury. Stem Cells 25, 115–124.
Lee, S.H., Chung, Y.N., Kim, Y.H., Kim, Y.J., Park, J.P., Kwon, D.K., Kwon, O.S., Heo, J.H., Kim, Y.H., Ryu, S., Kang, H.J., Paek, S.H., Wang, K.C., Kim, S.U., and Yoon, B.W. (2009). Effects of human neural stem cell transplantation in canine spinal cord hemisection. Neurol. Res. 31, 996–1002.
Lowry, N., Goderie, S.K., Adamo, M., Lederman, P., Charniga, C., Gill, J., Silver, J., and Temple, S. (2008). Multipotent embryonic spinal cord stem cells expanded by endothelial factors and Shh/RA promote functional recovery after spinal cord injury. Exp. Neurol. 209, 510–522.
Lu, P., Jones, L.L., Snyder, E.Y., and Tuszynski, M.H. (2003). Neural stem cells constitutively secrete neurotrophic factors and promote extensive host axonal growth after spinal cord injury. Exp. Neurol. 181, 115–129.
Macias, M.Y., Syring, M.B., Pizzi, M.A., Crowe, M.J., Alexanian, A.R., and Kurpad, S.N. (2006). Pain with no gain: Allodynia following neural stem cell transplantation in spinal cord injury. Exp. Neurol. 201, 335–348.
McDonald, J.W., Liu, X.Z., Qu, Y., Liu, S., Mickey, S.K., Turetsky, D., Gottlieb, D.I., and Choi, D.W. (1999). Transplanted embryonic stem cells survive, differentiate and promote recovery in injured rat spinal cord. Nat. Med. 5, 1410–1412.

Meng, X.T., Li, C., Dong, Z.Y., Liu, J.M., Li, W., Liu, Y., Xue, H., and Chen, D. (2008). Co-transplantation of bFGF-expressing amniotic epithelial cells and neural stem cells promotes functional recovery in spinal cord-injured rats. Cell Biol. Int. 32, 1546–1558.

Ogawa, Y., Sawamoto, K., Miyata, T., Miyao, S., Watanabe, M., Nakamura, M., Bregman, B.S., Koike, M., Uchiyama, Y., Toyama, Y., and Okano, H. (2002). Transplantation of in vitro-expanded fetal neural progenitor cells results in neurogenesis and functional recovery after spinal cord contusion injury in adult rats. J. Neurosci. Res. 69, 925-933.

Okada, S., Ishii, K., Yamane, J., Iwanami, A., Ikegami, T., Katoh, H., Iwamoto, Y., Nakamura, M., Miyoshi, H., Okano, H.J., Contag, C.H., Toyama, Y., and Okano, H. (2005). In vivo imaging of engrafted neural stem cells: its application in evaluating the optimal timing of transplantation for spinal cord injury. FASEB J. 19, 1839–1841.

Pan, H.C., Cheng, F.C., Lai, S.Z., Yang, D.Y., Wang, Y.C., and Lee, M.S. (2008). Enhanced regeneration in spinal cord injury by concomitant treatment with granulocyte colony-stimulating factor and neuronal stem cells. J. Clin. Neurosci. 15, 656–664.

Parr, A.M., Kulbatski, I., and Tator, C.H. (2007). Transplantation of adult rat spinal cord stem/progenitor cells for spinal cord injury. J. Neurotrauma 24, 835–845.

Parr, A.M., Kulbatski, I., Zahir, T., Wang, X., Yue, C., Keating, A., and Tator, C.H. (2008). Transplanted adult spinal cord-derived neural stem/progenitor cells promote early functional recovery after rat spinal cord injury. Neuroscience 155, 760–770.

Pfeifer, K., Vroemen, M., Blesch, A., and Weidner, N. (2004). Adult neural progenitor cells provide a permissive guiding substrate for corticospinal axon growth following spinal cord injury. Eur. J. Neurosci. 20, 1695–1704.

Pfeifer, K., Vroemen, M., Caioni, M., Aigner, L., Bogdahn, U., and Weidner, N. (2006). Autologous adult rodent neural progenitor cell transplantation represents a feasible strategy to promote structural repair in the chronically injured spinal cord. Regen. Med. 1, 255–266.

Setoguchi, T., Nakashima, K., Takizawa, T., Yanagisawa, M., Ochiai, W., Okabe, M., Yone, K., Komiya, S., and Taga, T. (2004). Treatment of spinal cord injury by transplantation of fetal neural precursor cells engineered to express BMP inhibitor. Exp. Neurol. 189, 33–44.

Takeuchi, H., Natsume, A., Wakabayashi, T., Aoshima, C., Shimato, S., Ito, M., Ishii, J., Maeda, Y., Hara, M., Kim, S.U., and Yoshida, J. (2007). Intravenously transplanted human neural stem cells migrate to the injured spinal cord in adult mice in an SDF-1- and HGF-dependent manner. Neurosci. Lett. 426, 69–74.

Tarasenko, Y.I., Gao, J., Nie, L., Johnson, K.M., Grady, J.J., Hulsebosch, C.E., McAdoo, D.J., and Wu, P. (2007). Human fetal neural stem cells grafted into contusion-injured rat spinal cords improve behavior. J. Neurosci. Res. 85, 47–57.

Teng, Y.D., Lavik, E.B., Qu, X., Park, K.I., Ourednik, J., Zurakowski, D., Langer, R., and Snyder, E.Y. (2002). Functional recovery following traumatic spinal cord injury mediated by a unique polymer scaffold seeded with neural stem cells. Proc. Natl. Acad. Sci. USA 99, 3024–3029.

Vroemen, M., Aigner, L., Winkler, J., and Weidner, N. (2003). Adult neural progenitor cell grafts survive after acute spinal cord injury and integrate along axonal pathways. Eur. J. Neurosci. 18, 743–751.

Vroemen, M., Caioni, M., Bogdahn, U., and Weidner, N. (2007). Failure of Schwann cells as supporting cells for adult neural progenitor cell grafts in the acutely injured spinal cord. Cell Tissue Res. 327, 1–13.

Wu, S., Suzuki, Y., Kitada, M., Kataoka, K., Kitaura, M., Chou, H., Nishimura, Y., and Ide, C. (2002). New method for transplantation of neurosphere cells into injured spinal cord through cerebrospinal fluid in rat. Neurosci. Lett. 318, 81–84.

Ziv, Y., Avidan, H., Pluchino, S., Martino, G., and Schwartz, M. (2006). Synergy between immune cells and adult neural stem/progenitor cells promotes functional recovery from spinal cord injury. Proc. Natl. Acad. Sci. USA 103, 13174–13179.

Neural and glial restricted precursors

Bambakidis, N.C., and Miller, R.H. (2004). Transplantation of oligodendrocyte precursors and sonic hedgehog results in improved function and white matter sparing in the spinal cords of adult rats after contusion. Spine J. 4, 16–26.

Cao, Q.L., Howard, R.M., Dennison, J.B., and Whittemore, S.R. (2002). Differentiation of engrafted neuronal-restricted precursor cells is inhibited in the traumatically injured spinal cord. Exp. Neurol. 177, 349–359.

Cao, Q.L., Xu, X.M., Devries, W.H., Enzmann, G.U., Ping, P., Tsoulfas, P., Wood, P.M., Bunge, M.B., and Whittemore, S.R. (2005). Functional recovery in traumatic spinal cord injury after transplantation of multineurotrophin-expressing glial-restricted precursor cells. J. Neurosci. 25, 6947–6957.

Cloutier, F., Siegenthaler, M.M., Nistor, G., and Keirstead, H.S. (2006). Transplantation of human embryonic stem cell-derived oligodendrocyte progenitors into rat spinal cord injuries does not cause harm. Regen. Med. 1, 469–479.

Enzmann, G.U., Benton, R.L., Woock, J.P., Howard, R.M., Tsoulfas, P., and Whittemore, S.R. (2005). Consequences of noggin expression by neural stem, glial, and neuronal precursor cells engrafted into the injured spinal cord. Exp. Neurol. 195, 293–304.

Han, S.S., Liu, Y., Tyler-Polsz, C., Rao, M.S., and Fischer, I. (2004). Transplantation of glial-restricted precursor cells into the adult spinal cord: Survival, glial-specific differentiation, and preferential migration in white matter. Glia 45, 1–16.

Hill, C.E., Proschel, C., Noble, M., Mayer-Proschel, M., Gensel, J.C., Beattie, M.S., and Bresnahan, J.C. (2004). Acute transplantation of glial-restricted precursor cells into spinal cord contusion injuries: Survival, differentiation, and effects on lesion environment and axonal regeneration. Exp. Neurol. 190, 289–310.

Keirstead, H.S., Nistor, G., Bernal, G., Totoiu, M., Cloutier, F., Sharp, K., and Steward, O. (2005). Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J. Neurosci. 25, 4694–4705.

Lee, K.H., Yoon, D.H., Park, Y.G., and Lee, B.H. (2005). Effects of glial transplantation on functional recovery following acute spinal cord injury. J. Neurotrauma 22, 575–589.

Lepore, A.C., and Fischer, I. (2005). Lineage-restricted neural precursors survive, migrate, and differentiate following transplantation into the injured adult spinal cord. Exp. Neurol. 194, 230–242.

Lepore, A.C., Bakshi, A., Swanger, S.A., Rao, M.S., and Fischer, I. (2005). Neural precursor cells can be delivered into the injured cervical spinal cord by intrathecal injection at the lumbar cord. Brain Res. 1045, 206–216.

Mitsui, T., Shumsky, J.S., Lepore, A.C., Murray, M., and Fischer, I. (2005). Transplantation of neuronal and glial restricted precursors into contused spinal cord improves bladder and

motor functions, decreases thermal hypersensitivity, and modifies intraspinal circuitry. J. Neurosci. 25, 9624–9636.
Neuhuber, B., Barshinger, A.L., Paul, C., Shumsky, J.S., Mitsui, T., and Fischer, I. (2008). Stem cell delivery by lumbar puncture as a therapeutic alternative to direct injection into injured spinal cord. J. Neurosurg. Spine 9, 390–399.

Bone marrow-derived stromal cells – mesenchymal stem cells

Ankeny, D.P., McTigue, D.M., and Jakeman, L.B. (2004). Bone marrow transplants provide tissue protection and directional guidance for axons after contusive spinal cord injury in rats. Exp. Neurol. 190, 17–31.
Bakshi, A., Barshinger, A.L., Swanger, S.A., Madhavani, V., Shumsky, J.S., Neuhuber, B., and Fischer, I. (2006). Lumbar puncture delivery of bone marrow stromal cells in spinal cord contusion: a novel method for minimally invasive cell transplantation. J. Neurotrauma 23, 55–65.
Bakshi, A., Hunter, C., Swanger, S., Lepore, A., and Fischer, I. (2004). Minimally invasive delivery of stem cells for spinal cord injury: Advantages of the lumbar puncture technique. J. Neurosurg. Spine 1, 330–337.
Bi, X.B., Deng, Y.B., Gan, D.H., and Wang, Y.Z. (2008). Salvianolic acid B promotes survival of transplanted mesenchymal stem cells in spinal cord-injured rats. Acta Pharmacol. Sin. 29, 169–176.
Callera, F., and do Nascimento, R.X. (2006). Delivery of autologous bone marrow precursor cells into the spinal cord via lumbar puncture technique in patients with spinal cord injury: A preliminary safety study. Exp. Hematol. 34, 130–131.
Cao, Q., Ding, P., Lu, J., Dheen, S.T., Moochhala, S., and Ling, E.A. (2007). 2′, 3′-Cyclic nucleotide 3′-phosphodiesterase cells derived from transplanted marrow stromal cells and host tissue contribute to perineurial compartment formation in injured rat spinal cord. J. Neurosci. Res. 85, 116–130.
Chernykh, E.R., Stupak, V.V., Muradov, G.M., Sizikov, M.Y., Shevela, E.Y., Leplina, O.Y., Tikhonova, M.A., Kulagin, A.D., Lisukov, I.A., Ostanin, A.A., and Kozlov, V.A. (2007). Application of autologous bone marrow stem cells in the therapy of spinal cord injury patients. Bull. Exp. Biol. Med. 143, 543–547.
Chopp, M., Zhang, X.H., Li, Y., Wang, L., Chen, J., Lu, D., Lu, M., and Rosenblum, M. (2000). Spinal cord injury in rat: Treatment with bone marrow stromal cell transplantation. Neuroreport 11, 3001–3005.
Cizkova, D., Rosocha, J., Vanicky, I., Jergova, S., and Cizek, M. (2006). Transplants of human mesenchymal stem cells improve functional recovery after spinal cord injury in the rat. Cell. Mol. Neurobiol. 26, 1167–1180.
Dasari, V.R., Spomar, D.G., Cady, C., Gujrati, M., Rao, J.S., and Dinh, D.H. (2007). Mesenchymal stem cells from rat bone marrow downregulate caspase-3-mediated apoptotic pathway after spinal cord injury in rats. Neurochem. Res. 32, 2080–2093.
de Haro, J., Zurita, M., Ayllon, L., and Vaquero, J. (2005). Detection of 111In-oxine-labeled bone marrow stromal cells after intravenous or intralesional administration in chronic paraplegic rats. Neurosci. Lett. 377, 7–11.
Deng, Y.B., Liu, X.G., Liu, Z.G., Liu, X.L., Liu, Y., and Zhou, G.Q. (2006). Implantation of BM mesenchymal stem cells into injured spinal cord elicits de novo neurogenesis and functional recovery: Evidence from a study in rhesus monkeys. Cytotherapy 8, 210–214.
Deng, Y.B., Liu, Y., Zhu, W.B., Bi, X.B., Wang, Y.Z., Ye, M.H., and Zhou, G.Q. (2008). The co-transplantation of human bone marrow stromal cells and embryo olfactory ensheathing cells as a new approach to treat spinal cord injury in a rat model. Cytotherapy 10, 551–564.
Fan, L., DU, F., Cheng, B.C., Peng, H., and Liu, S.Q. (2008). Migration and distribution of bone marrow stromal cells in injured spinal cord with different transplantation techniques. Chin. J. Traumatol. 11, 94–97.
Himes, B.T., Neuhuber, B., Coleman, C., Kushner, R., Swanger, S.A., Kopen, G.C., Wagner, J., Shumsky, J.S., and Fischer, I. (2006). Recovery of function following grafting of human bone marrow-derived stromal cells into the injured spinal cord. Neurorehabil. Neural Repair 20, 278–296.
Hofstetter, C.P., Schwarz, E.J., Hess, D., Widenfalk, J., El Manira, A., Prockop, D.J., and Olson, L. (2002). Marrow stromal cells form guiding strands in the injured spinal cord and promote recovery. Proc. Natl. Acad. Sci. USA 99, 2199–2204.
Huang, F., Wang, J., and Chen, A. (2006). Effects of co-grafts mesenchymal stem cells and nerve growth factor suspension in the repair of spinal cord injury. J. Huazhong Univ. Sci. Technol. Med. Sci. 26, 206–210.
Jendelova, P., Herynek, V., Urdzikova, L., Glogarova, K., Kroupova, J., Andersson, B., Bryja, V., Burian, M., Hajek, M., and Sykova, E. (2004). Magnetic resonance tracking of transplanted bone marrow and embryonic stem cells labeled by iron oxide nanoparticles in rat brain and spinal cord. J. Neurosc. Res. 76, 232–243.
Kamada, T., Koda, M., Dezawa, M., Yoshinaga, K., Hashimoto, M., Koshizuka, S., Nishio, Y., Morlya, H., and Yamazaki, M. (2005). Transplantation of bone marrow stromal cell-derived Schwann cells promotes axonal regeneration and functional recovery after complete transection of adult rat spinal cord. J. Neuropathol. Exp. Neurol. 64, 37–45.
Khalatbary, A.R., and Tiraihi, T. (2007). Localization of bone marrow stromal cells in injured spinal cord treated by intravenous route depends on the hemorrhagic lesions in traumatized spinal tissues. Neurol. Res. 29, 21–26.
Kim, K.N., Oh, S.H., Lee, K.H., and Yoon, D.H. (2006). Effect of human mesenchymal stem cell transplantation combined with growth factor infusion in the repair of injured spinal cord. Acta Neurochir., Suppl. 99, 133–136.
Koda, M., Kamada, T., Hashimoto, M., Murakami, M., Shirasawa, H., Sakao, S., Ino, H., Yoshinaga, K., Koshizuka, S., Moriya, H., and Yamazaki, M. (2007). Adenovirus vector-mediated ex vivo gene transfer of brain-derived neurotrophic factor to bone marrow stromal cells promotes axonal regeneration after transplantation in completely transected adult rat spinal cord. Eur. Spine J. 16, 2206–2214.
Koda, M., Okada, S., Nakayama, T., Koshizuka, S., Kamada, T., Nishio, Y., Someya, Y., Yoshinaga, K., Okawa, A., Moriya, H., and Yamazaki, M. (2005). Hematopoietic stem cell and marrow stromal cell for spinal cord injury in mice. Neuroreport 16, 1763–1767.
Lee, J., Kuroda, S., Shichinohe, H., Ikeda, J., Seki, T., Hida, K., Tada, M., Sawada, K., and Iwasaki, Y. (2003). Migration and differentiation of nuclear fluorescence-labeled bone marrow stromal cells after transplantation into cerebral infarct and spinal cord injury in mice. Neuropathology 23, 169–180.
Lee, K.H., Suh-Kim, H., Choi, J.S., Jeun, S.S., Kim, E.J., Kim, S.S., Yoon do, H., and Lee, B.H. (2007). Human mesenchymal stem cell transplantation promotes functional recovery following acute spinal cord injury in rats. Acta Neurobiol. Exp. (Wars.) 67, 13–22.
Lu, P., Jones, L.L., and Tuszynski, M.H. (2005). BDNF-expressing marrow stromal cells support extensive axonal growth at sites of spinal cord injury. Exp. Neurol. 191, 344–360.
Lu, P., Jones, L.L., and Tuszynski, M.H. (2007). Axon regeneration through scars and into sites of chronic spinal cord injury. Exp. Neurol. 203, 8–21.

Lu, P., Yang, H., Jones, L.L., Filbin, M.T., and Tuszynski, M.H. (2004). Combinatorial therapy with neurotrophins and cAMP promotes axonal regeneration beyond sites of spinal cord injury. J. Neurosci. 24, 6402–6409.

Neuhuber, B., Timothy Himes, B., Shumsky, J.S., Gallo, G., and Fischer, I. (2005). Axon growth and recovery of function supported by human bone marrow stromal cells in the injured spinal cord exhibit donor variations. Brain Res. 1035, 73–85.

Ohta, M., Suzuki, Y., Noda, T., Ejiri, Y., Dezawa, M., Kataoka, K., Chou, H., Ishikawa, N., Matsumoto, N., Iwashita, Y., Mizuta, E., Kuno, S., and Ide, C. (2004). Bone marrow stromal cells infused into the cerebrospinal fluid promote functional recovery of the injured rat spinal cord with reduced cavity formation. Exp. Neurol. 187, 266–278.

Parr, A.M., Tator, C.H., and Keating, A. (2007). Bone marrow-derived mesenchymal stromal cells for the repair of central nervous system injury. Bone Marrow Transplant. 40, 609–619.

Saito, F., Nakatani, T., Iwase, M., Maeda, Y., Hirakawa, A., Murao, Y., Suzuki, Y., Onodera, R., Fukushima, M., and Ide, C. (2008). Spinal cord injury treatment with intrathecal autologous bone marrow stromal cell transplantation: the first clinical trial case report. J. Trauma 64, 53–59.

Satake, K., Lou, J., and Lenke, L.G. (2004). Migration of mesenchymal stem cells through cerebrospinal fluid into injured spinal cord tissue. Spine 29, 1971–1979.

Sheth, R.N., Manzano, G., Li, X., and Levi, A.D. (2008). Transplantation of human bone marrow-derived stromal cells into the contused spinal cord of nude rats. J. Neurosurg. Spine 8, 153–162.

Stokols, S., Sakamoto, J., Breckon, C., Holt, T., Weiss, J., and Tuszynski, M.H. (2006). Templated agarose scaffolds support linear axonal regeneration. Tissue Eng. 12, 2777–2787.

Taylor, L., Jones, L., Tuszynski, M.H., and Blesch, A. (2006). Neurotrophin-3 gradients established by lentiviral gene delivery promote short-distance axonal bridging beyond cellular grafts in the injured spinal cord. J. Neurosci. 26, 9713–9721.

Urdzikova, L., Jendelova, P., Glogarova, K., Burian, M., Hajek, M., and Sykova, E. (2006). Transplantation of bone marrow stem cells as well as mobilization by granulocyte-colony stimulating factor promotes recovery after spinal cord injury in rats. J. Neurotrauma 23, 1379–1391.

Vaquero, J., Zurita, M., Oya, S., and Santos, M. (2006). Cell therapy using bone marrow stromal cells in chronic paraplegic rats: systemic or local administration? Neurosci. Lett. 398, 129–134.

Wu, S., Suzuki, Y., Ejiri, Y., Noda, T., Bai, H., Kitada, M., Kataoka, K., Ohta, M., Chou, H., and Ide, C. (2003). Bone marrow stromal cells enhance differentiation of cocultured neurosphere cells and promote regeneration of injured spinal cord. J. Neurosci. Res. 72, 343–351.

Yano, S., Kuroda, S., Lee, J.B., Shichinohe, H., Seki, T., Ikeda, J., Nishimura, G., Hida, K., Tamura, M., and Iwasaki, Y. (2005). In vivo fluorescence tracking of bone marrow stromal cells transplanted into a pneumatic injury model of rat spinal cord. J. Neurotrauma 22, 907–918.

Yano, S., Kuroda, S., Shichinohe, H., Seki, T., Ohnishi, T., Tamagami, H., Hida, K., and Iwasaki, Y. (2006). Bone marrow stromal cell transplantation preserves gammaaminobutyric acid receptor function in the injured spinal cord. J. Neurotrauma 23, 1682–1692.

Yoon, S.H., Shim, Y.S., Park, Y.H., Chung, J.K., Nam, J.H., Kim, M.O., Park, H.C., Park, S.R., Min, B.H., Kim, E.Y., Choi, B.H., Park, H., and Ha, Y. (2007). Complete spinal cord injury treatment using autologous bone marrow cell transplantation and bone marrow stimulation with granulocyte macrophage-colony stimulating factor: Phase I/II clinical trial. Stem Cells 25, 2066–2073.

Yoshihara, H., Shumsky, J.S., Neuhuber, B., Otsuka, T., Fischer, I., and Murray, M. (2006). Combining motor training with transplantation of rat bone marrow stromal cells does not improve repair or recovery in rats with thoracic contusion injuries. Brain Res. 1119, 65–75.

Zhao, Z.M., Li, H.J., Liu, H.Y., Lu, S.H., Yang, R.C., Zhang, Q.J., and Han, Z.C. (2004). Intraspinal transplantation of CD34+ human umbilical cord blood cells after spinal cord hemisection injury improves functional recovery in adult rats. Cell Transplant. 13, 113–122.

Zurita, M., and Vaquero, J. (2004). Functional recovery in chronic paraplegia after bone marrow stromal cells transplantation. Neuroreport 15, 1105–1108.

Zurita, M., and Vaquero, J. (2006). Bone marrow stromal cells can achieve cure of chronic paraplegic rats: functional and morphological outcome one year after transplantation. Neurosci. Lett. 402, 51–56.

Zurita, M., Vaquero, J., Bonilla, C., Santos, M., de Haro, J., Oya, S., and Aguayo, C. (2008). Functional recovery of chronic paraplegic pigs after autologous transplantation of bone marrow stromal cells. Transplantation 86, 845–853.

Discussion

Kwon, B.K., Hillyer, J., and Tetzlaff, W. (2009). Translational research in spinal cord injury: A survey of opinion from the SCI community. J. Neurotrauma DOI:10.1089/neu.2009.1048.

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