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RESEARCH ARTICLE

Transplantation of human embryonic stem cell-derived oligodendrocyte progenitors into rat spinal cord injuries does not cause harm

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Demyelination contributes to loss of function following spinal cord injury. We have shown previously that transplantation of human embryonic stem cell-derived oligodendrocyte progenitors into adult rat 200 kD contusive spinal cord injury sites enhances remyelination and promotes recovery of motor function. Previous studies using oligodendrocyte lineage cells have noted a correlation between the presence of demyelinating pathology and the survival and migration rate of the transplanted cells. The present study compared the survival and migration of human embryonic stem cell-derived oligodendrocyte progenitors injected 7 days after a 200 or 50 kD contusive spinal cord injury, as well as the locomotor outcome of transplantation. Our findings indicate that a 200 kD spinal cord injury induces extensive demyelination, whereas a 50 kD spinal cord injury induces no detectable demyelination. Cells transplanted into the 200 kD injury group survived, migrated, and resulted in robust remyelination, replicating our previous studies. In contrast, cells transplanted into the 50 kD injury group survived, exhibited limited migration, and failed to induce remyelination as demyelination in this injury group was absent. Animals that received a 50 kD injury displayed only a transient decline in locomotor function as a result of the injury. Importantly, human embryonic stem cell-derived oligodendrocyte progenitor transplants into the 50 kD injury group did not cause a further decline in locomotion. Our studies highlight the importance of a demyelinating pathology as a prerequisite for the function of transplanted myelinogenic cells. In addition, our results indicate that transplantation of human embryonic stem cell-derived oligodendrocyte progenitor cells into the injured spinal cord is not associated with a decline in locomotor function.

Keywords: demyelination, niche, oligodendrocyte progenitor, pre-differentiation, rat, remyelination, transplantation

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Spinal cord injury (SCI) leads to the interruption of long tracts that carry information to and from the brain and distal regions of the spinal cord. Animal models of contusive SCI reveal that moderate lesions are incomplete and that the injury site is surrounded by spared tissue containing myelinated and demyelinated axons [1–4]. Following the initial trauma, spared tissue is subject to a secondary degenerative phenomenon that includes demyelination, in which the myelin sheath is lost. This problem, first reported in 1906 by Homes, has slowly gained interest and has been reported in different species including monkey [5], cat [6–8], guinea pig [9] and rat [10,11]. Demyelination is attributed to the initial mechanical insult and ensues within hours as a result of the death of oligodendrocytes by necrosis and apoptosis [12–16]. Electrophysiological and morphological studies have demonstrated that demyelination has detrimental functional effects, such as disruption of action potentials, rendering it a significant component of the SCI pathology [7,17]. Remyelination occurs following SCI [18,19], but is a marginal event in comparison to demyelination, which we have previously shown to be a chronic and progressive phenomenon that continues well beyond a year following SCI in rat [11]. There is little information regarding demyelination and remyelination following SCI in humans, however, a growing body of morphological evidence suggests that the same phenomena occurs in humans [20–23].

Over the last two decades, several studies have demonstrated that myelination of host axons by transplanted oligodendrocyte-lineage cells is a pragmatic approach to recovering function. Transplant-mediated remyelination has been demonstrated in focal, gliotoxin-induced regions of demyelination in adult rat [24–26] and in neonatal myelin-deficient animals [27–31].

Efforts to translate this approach to humans are increasingly utilizing human embryonic stem cells (hESCs), which are capable of generating a seemingly unlimited cell supply for transplantation. Our laboratory has developed a method to differentiate hESCs into

oligodendroglial progenitor cells (OPCs) in high purity [32]. We have shown that transplantation of these cells into either the *shiverer* mouse model of dysmyelination or adult rat 200 kD contusive SCIs results in the integration of transplanted cells, their differentiation into mature oligodendrocytes, the formation of compact myelin by transplanted cells and increased locomotor recovery [32,33]. Although transplantation of hESC-derived OPCs has been show to improve locomotor function, it is not known whether the transplant volume or the cells themselves may have deleterious effects that may have been masked by the severity of the SCI, or the subsequent benefit conferred by the transplant. Furthermore, it is not known whether demyelination is a pre-requisite to transplant-mediated recovery of locomotion.

In this study, we compared the survival and migration of hESC-derived OPCs injected 7 days after a 200 kD or 50 kD contusive SCI, as well as the locomotor outcome of transplantation. Our data indicate that a 50 kD SCI induces no demyelination, and that OPC transplantation in this injury group resulted in the survival and limited migration of cells, with no remyelination or alteration of locomotor outcome. These findings indicate that demyelination provides a pathological niche for the function of transplanted myelinogenic cells. Furthermore, our results indicate that transplantation of hESC-derived OPCs into the injured spinal cord is not associated with a decline in locomotor function.

Materials & methods

Cell culture

High purity populations of OPCs were prepared from the H7 hESC line and were maintained and amplified as previously described [32,34]. The H7 hESC line at passage 32 and the human fibroblast line at passage 48 were obtained from Geron Corporation (CA, USA). Cells were expanded in hESC growth media [35] and differentiated as previously described [32]. Dissociated cells were placed for 2 days in 50% hESC growth media, and 50% glial restriction media (GRM). GRM consisted of DMEM:F12, B27 supplement (Gibco-Invitrogen, CA, USA), 25 µg/ml insulin, 6.3 ng/ml progesterone, 10 µg/ml putrescin, 50 ng/ml sodium selenite, 50 µg/ml holotransferin, 40 ng/ml tri-iodo-thyroidin, 4 ng/ml basic fibroblast growth factor (bFGF) and 10 ng/ml epidermal growth factor (EGF; Sigma-Aldrich, St. Louis, MO, USA). The transition media was supplemented for 1 day with 4 ng/ml bFGF and 20 ng/ml EGF (Sigma-Aldrich). Cells were then exposed to transition media supplemented with 2 ng/ml bFGF, 20 ng/ml EGF (Sigma-Aldrich), and 10 µM/ml all-*trans*-retinoic acid (RA; Sigma-Aldrich) in dimethyl sulphoxide (DMSO; Sigma-Aldrich) for 1 day.

This media was then replaced with 100% GRM supplemented with 20 ng/ml EGF (Sigma-Aldrich) and 10 µM/ml all-*trans*-RA in DMSO for a further 7 days. RA was then omitted from media for the duration of the differentiation protocol. Cells were exposed to GRM supplemented with 20 ng/ml EGF for 25 days. Cultures were then exposed to Trypsin-ethylenediaminetetraacetic acid (EDTA; Gibco-Invitrogen, CA, USA) for 2–5 min, plated on 1:30 Matrigel substrate, and cultured for 1 week in GRM supplemented with 20 ng/ml EGF. Thus, the differentiation protocol took 42 days.

Immunocytochemical staining

Cultures were fixed 1 week later by exposure to 4% paraformaldehyde (Fisher Scientific, Pittsburgh, PA, USA) in phosphate buffer (PB) for 10 min. Nuclear staining was conducted by exposing cultures to Mayer's hematoxylin for 10 min and blue staining in ammonia water for 10 min. Immunocytochemical staining was performed using standard protocols. Imaging slides were blocked with 20% serum for 30 min at room temperature. Primary antiserum (rabbit anti-NG-2, 1:100; rabbit anti-SOX10, 1:200 [Chemicon, Temecula, CA, USA]; mouse anti-Tuj1, 1:200, rabbit anti-Pax6, 1:100 [not shown; CRP Incorporation, Denver, PA, USA]; rabbit anti-cow GFAP, 1:500 [DAKO, Denmark]; mouse anti-SSEA4 supernatant, 1:5 [a kind gift from Geron Corporation]; rabbit anti-PDGFR-α, 1:200 [Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA]) were diluted in 4% serum and applied to imaging chambers overnight at 4°C. Imaging chambers were rinsed three times with phosphate buffered saline (PBS), incubated for 30 min in 20% serum, and biotinylated antisera (rabbit anti-goat, goat anti-mouse or goat anti-rabbit biotinylated immunoglobulin G (IgG) H+L, 1:200 [Vector Laboratories, CA, USA]) was applied and incubated for 1 h at room temperature. For immuno-fluorescence, goat anti-rabbit or goat anti-mouse secondary antibodies (Alexa Fluor 488 or 594, Molecular Probes, Eugene, OR, USA) were used at a dilution of 1:200 in 10% NGS in PBS. Cell quantification was conducted using an Olympus AX-80 light

microscope. The percentage of immunopositive cells was determined by dividing the total number of immunopositive cells by the total number of hematoxylin positive cells in each imaging chamber, and averaging the results from three imaging chambers per marker.

Spinal cord injury

Female Sprague Dawley adult rats (200–220 g, 6–8 weeks old) were anesthetized with an intraperitoneal injection of 7.5 mg/kg Xylazine (Phoenix Pharmaceutical Inc., MO, USA) and 60 mg/kg Ketamine (Phoenix Pharmaceutical Inc.). The dorsal area between the neck and hindlimbs, and extending approximately 2 cm bilaterally from the spine, was shaved and disinfected with serial provodone and 70% ethanol scrubs. A midline incision exposed the spinal column at the level of T8–T11 and the paravertebral muscles were dissected bilaterally to visualize the transverse apophyses. Laminectomy was performed at the 10th thoracic vertebra. The exposed vertebral column was stabilized by clamping the rostral T9 and caudal T11 vertebral bodies with Adson delicate stabilizing forceps (Miltex no. 6–120; Miltex Instruments Co., NY, USA). Contusion injury was induced using the Infinite Horizon Impactor™ (Precision Systems and Instrumentation, LLC) with a force of 50 kD (n = 19) or 200 kD (n = 14) defined by the level of force applied to the exposed spinal cord. The deep and superficial muscle layers were sutured and the skin was closed with stainless steel wound clips. Immediately following surgery, animals were administered subcutaneous saline and prophylactic Baytril™ (Bayer, KS, USA; 2.5 mg/kg/day, subcutaneously) and maintained on an isothermic pad until alert and mobile. Animals receive manual bladder expression twice daily and were inspected for weight loss, dehydration, discomfort and autophagia, with appropriate veterinary care as needed.

Behavioral testing

Before injury, each animal was acclimated and scored using the Basso, Beattie, Bresnahan Locomotor Rating Scale (BBB) [36]. Animals were scored once per week for the duration of the study. BBB scores were analyzed by repeated measures analysis of variance with Tukey's multiple comparison test at each time point.

Cell transplantation

Animals received cyclosporin A (10 mg/kg/day, subcutaneously; Bedford Laboratories, OH, USA) beginning 1 day prior to transplantation and every day thereafter until the end of the study. Transplantation surgeries occurred 7 days following contusion surgery. Immediately prior to transplant, the cell transplant population was assayed for viability by assessing trypan blue exclusion and only populations with over 95% viability were transplanted. Animals were anesthetized as above and the laminectomy site re-exposed. After immobilization of the spinal process rostral to the contusion site, a 10 µl Hamilton syringe (Hamilton, NV, USA) with a silicon-coated pulled glass tip was lowered into the spinal cord using a stereotactic manipulator arm. Cell suspensions were injected along the midline of the spinal cord into one site at the cranial end of the laminectomy and one site at the caudal end of the laminectomy, in a total volume of 15 µl (1,500,000 cells) (n = 10 for 50 kD injuries; n = 7 for 200 kD injuries). The needle was removed after 5 minutes. Control animals (n = 9 for 50 kD injuries; n = 7 for 200 kD injuries) received human fibroblasts (a gift from Geron Corporation) at similar concentrations. In order to determine how far cells are distributed as a result of the implantation procedure itself, some animals (n = 4) received 200 kD injuries followed by transplantation of a total volume of 15 µl (1,500,000 cells) of hESC-derived OPCs, and were killed the following day.

Histology

Animals were terminally anesthetized 7 weeks after cell transplantation and transcardially perfused with 50 ml of 0.1 M PBS containing 28 IU/ml heparin (Acros, NY, USA) followed by 250 ml of 4% paraformaldehyde (Fisher Scientific, PA, USA) in 0.1 M PB pH 7.4. Spinal cord regions extending 1 cm above and 1 cm below the injection/injury site were post fixed overnight. Spinal cords were cut into 1 mm transverse blocks and processed so as to preserve the cranio–caudal sequence and orientation.

Tissue for cryostat processing was transferred to 27% sucrose (Fisher Scientific, NH, USA) for 24 h prior to transverse sectioning at 20 µm, and was then mounted on gelatin-coated slides (Fisher Scientific). Mouse anti-human nuclei antibodies (Chemicon) were used at a dilution of 1:200 in 0.5% normal goat serum (NGS) in PBS (Chemicon International Inc., CA, USA). Primary antibodies were incubated overnight at 4°C. Goat anti-rabbit secondary antibodies (Alexa Fluor 594) were used at a dilution of 1:200 in 10% NGS in PBS. Following 2 h of

incubation, all sections were washed in PBS. Standard immunohistochemical controls were included in each run. Sections were viewed and digitally photographed using an Olympus AX-80 microscope using OLYMPUS MicroSuite B3SV software (Olympus America Inc., NY, USA).

Resin sections were used for the examination of remyelinated and demyelinated axons, and the gross pathology of the transplant environment. For resin processing, odd-numbered spinal cord blocks were post fixed for 24 h in 4% glutaraldehyde (Fisher Scientific) then exposed to 1% osmium tetroxide (OsO_4) (Electron Microscopy Sciences, Fort Washington, PA, USA), dehydrated in ascending alcohols, and embedded in Spurr resin (Electron Microscopy Sciences, PA, USA) according to standard protocols. Transverse semi-thin (1 µm) sections were cut from the rostral face, stained with alkaline toluidine blue, cover slipped and examined by light microscopy on an Olympus AX-80 microscope using OLYMPUS MicroSuite B3SV software.

Results

Myelin histopathology

Analysis of toluidine blue-stained sections revealed no demyelination pathology following 50 kD injuries (Figure 1A–E), whereas demyelination was a prominent pathological feature following 200 kD injuries (Figure 1F–J). Low magnification (40×) imaging revealed minimal pathology following a 50 kD injury, both at the injury epicenter (Figure 1A) and away from the injury epicenter (Figure 1B). Higher magnification (200×) imaging of the dorsal columns of 50 kD injuries also revealed minimal pathology with numerous healthy myelinated axons (Figure 1C). High magnification (1000×) imaging revealed that structural integrity within the dorsal column was maintained with no detectable loss of myelin sheaths (Figure 1D). Very high magnification (2000×) imaging confirmed no detectable loss of myelin sheaths (Figure 1E). In contrast, low magnification (40×) imaging of 200 kD injuries revealed widespread pathology with a clear region of demyelination in the dorsal column as well as in the lateral and ventral white matter, both at the injury epicenter (Figure 1F) and away from the injury epicenter (Figure 1G). Higher magnification (200×) imaging of 200 kD injuries revealed hypercellularity, swelling, and demyelination of axons within the dorsal column (Figure 1H). High magnification (1000×) imaging revealed extensive demyelination of axons as evidenced by the lack of dark staining myelin sheaths (Figure 1I). Very high magnification imaging confirmed extensive demyelination of axons (Figure 1J). The presence of axons without their myelin sheaths indicates that some axons survive injury, despite loss of their myelin sheath. Macrophage and immune cell infiltration were consistent features of 200 kD injuries.

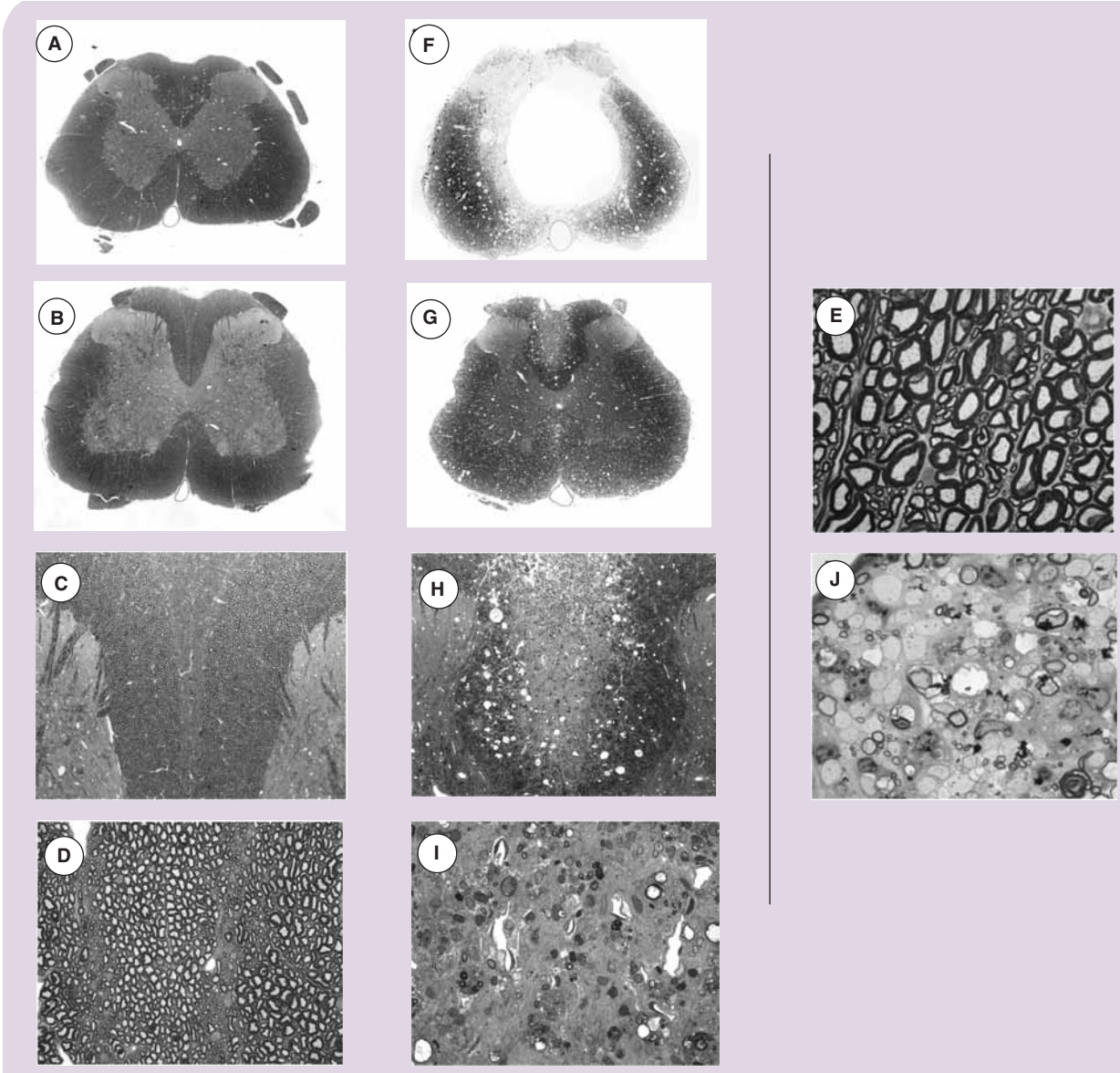
Generation of OPCs from hESCs

The differentiation protocol generated a high percentage of OPCs, confirming our previous findings [32,33]. Cultures consisted of high purity yellow spheres by 10 days of the differentiation protocol (Figure 2A). At the end of the 42-day differentiation protocol, cells had a bipolar morphology (Figure 2B) characteristic of immature oligodendroglial cells and a typical antigenic profile, with a high percentage of $NG2^+$ cells (>80%; Figure 2C), $SOX10^+$ cells (>45%; Figure 2D), and $PDGFR\alpha^+$ cells (>85%; Figure 2E). Cells that did not label with oligodendroglial markers were primarily $GFAP^+$ or $Tuj1^+$ (Figure 2F). This panel of markers demonstrates oligodendroglial lineage commitment. No $SSEA4^+$ cells could be detected at this point in the differentiation protocol indicating that undifferentiated stem cells could not be detected in the transplant population.

Survival & migration of transplanted OPCs

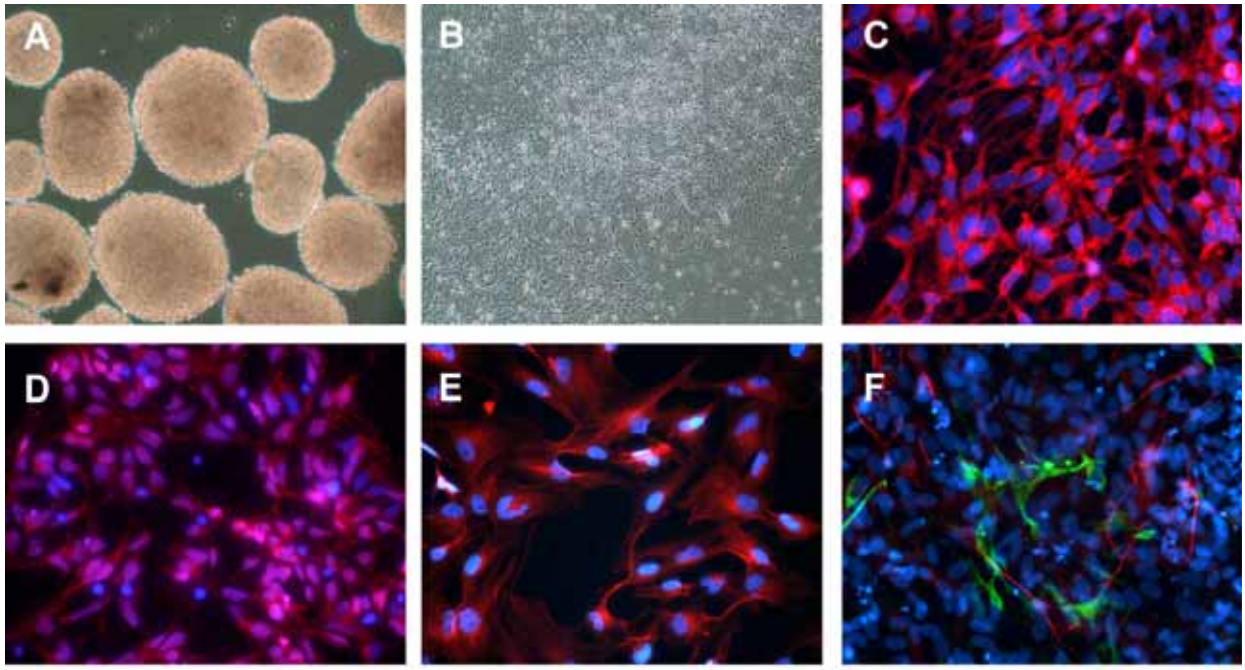
Analysis of transplanted animals 2 months after implantation indicated that hESC-derived OPCs survived and migrated when transplanted into 200 kD injuries, confirming our previous findings [33]. Anti-human nuclear staining revealed clusters (arrow head) of anti-human nuclei-positive cells in all transplanted animals (Figure 3A). In all animals in the 200 kD injury group, anti-human nuclei-positive cells (arrows) spread from the site of implantation into the adjacent white matter. The greatest density of anti-human nuclei-positive cells was detected at the site of implantation and the number rapidly decreased cranially and caudally; cells were detected up to 6 mm cranial or caudal to the implantation site in the 200 kD injury group. In contrast, although anti-human nuclei-positive cells were detected in all animals in the 50 kD injury group, they failed to migrate from the site of transplantation (Figure 3B). Transplanted cells consistently remained clustered at the site of implantation with very limited spread into the surrounding tissue (Figure 3B), or cranial or caudal to the site of implantation (not shown).

Figure 1. Comparative analysis of toluidine blue-stained sections from 50 kD and 200 kD spinal cord contusion injuries.



Histopathology following a 50 kD SCI at 40× **(A)**, 40× **(B)**, 200× **(C)**, 1000× **(D)**, and 2000× **(E)** magnification. **(A)** Low magnification imaging reveals minimal pathology, with minor swelling of the central canal at the lesion epicenter. **(B)** Low magnification imaging reveals minimal pathology 2 mm caudal to the injury epicenter. **(C)** Higher magnification imaging of the dorsal column also reveals minimal pathology 2 mm caudal to the injury epicenter. **(D)** High magnification imaging reveals that structural integrity was maintained with no detectable loss of myelin sheaths 2 mm caudal to the injury epicenter. **(E)** Very high magnification imaging reveals no detectable loss of myelin sheaths 2mm caudal to the injury epicenter. Histopathology following a 200 kD contusive SCI at 40× **(F)**, 40× **(G)**, 200× **(H)**, 1000× **(I)**, and 2000× **(J)** magnification. **(F)** Low magnification imaging reveals widespread pathology with vast tissue necrosis and cavity formation at the lesion epicenter. **(G)** Low magnification imaging reveals widespread pathology with a clear region of demyelination in the dorsal column, 2 mm caudal to the injury epicenter. **(H)** Higher magnification imaging reveals hypercellularity, swelling, and a loss of myelin within the dorsal column, 2 mm caudal to the injury epicenter. **(I)** High magnification imaging reveals extensive demyelination of axons as evidenced by the lack of dark stained myelin sheaths, 2 mm caudal to the injury epicenter. **(J)** Very high magnification imaging reveals extensive demyelination 2 mm caudal to the injury epicenter.

Figure 2. Characterization of human embryonic stem cell-derived oligodendrioglial progenitor cells.



(A) Yellow spheres appeared within 5 days of exposure to retinoic acid (RA) and grew rapidly in the presence of glial restriction media (GRM), evidenced by an increase in their size and proportion relative to other culture components. **(B)** Cells migrated from plated neurospheres, allowing visualization of the bipolar cellular morphology, which is typical of early oligodendroglial lineage cells. **(C)** A high percentage of cells expressed NG2 (red), a marker for oligodendrioglial progenitor cells (OPCs). **(D)** A high percentage of cells expressed SOX10 (red), a marker for OPCs. **(E)** A high percentage of cells expressed platelet-derived growth factor receptor (PDGFR)α (red), a marker for OPCs. **(F)** The few cells that did not label with oligodendroglial markers were primarily GFAP (green) positive or Tuj1 (red) positive. Hoechst nuclear staining is shown in blue in C, D, E, and F. 4× **(A)**, 40× **(B)**, and 400× **(C)**, 400× **(D)**, 400× **(E)**, and 400× **(F)**.

Analysis of transplanted animals 1 day after transplantation revealed transplanted cells only within the lesion epicenter. The lack of human cells in tissue cranial or caudal to the implantation site in these animals indicates that the implantation procedure itself did not propel cells throughout the spinal cord.

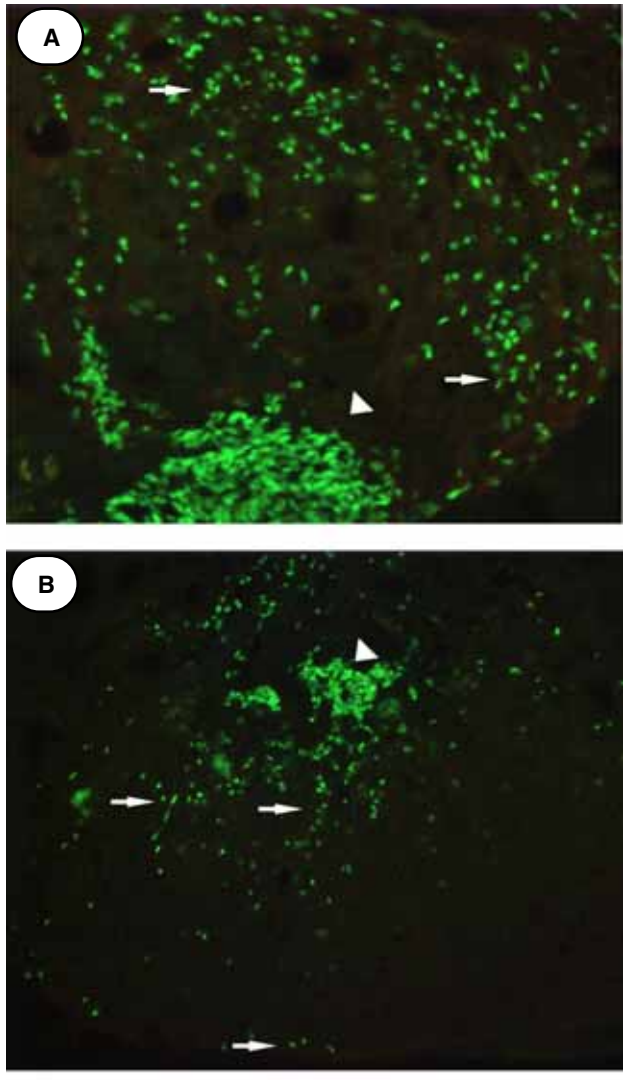
Locomotor outcome following OPC transplantation

Animals in the 50 kD injury group demonstrated a minor and transient loss of locomotor function, with a low point of 18–19 (range: 14–21) on the BBB score scale at day 1 post injury (Figure 4). Animals returned to a normal behavioral score of 21 around 2 weeks following transplantation and maintained normal behavioral scores for the remainder of the 2 months of behavioral assessment. Transplantation of either human fibroblasts or hESC-derived OPCs 7 days post-injury did not decrease locomotor outcome resulting from the 50 kD injury. These observations suggest that cell transplantation does not cause substantial damage that can be detected with this assessment of locomotor function.

Remyelination following OPC transplantation

Comparative analysis of toluidine blue-stained sections from the 50 kD injury group and the 200 kD injury group after transplantation of hESC-derived OPCs are depicted in Figure 5. Low magnification (40×) imaging of transplanted animals with a 50 kD injury revealed minimal pathology (Figure 5A). Higher magnification (200×) imaging of the dorsal column of transplanted animals with a 50 kD injury also revealed minimal pathology (Figure 5B). High magnification (1000×) imaging of these regions revealed that axonal and myelin structural integrity was maintained; no demyelination or remyelination was detected (Figure 5C). Very high magnification imaging (2000×) confirmed no detectable loss of myelin sheaths, and no remyelination (Figure 5D).

Figure 3. Immunostaining for anti-human nuclei showing the distribution of anti-human-positive cells at the site of implantation 2 months following a 200 kD (A) and 50 kD (B) contusive spinal cord injury.



Whereas a cluster (arrow head) of anti-human-positive cells and spread (arrows) of anti-human-positive cells were detected in all rats in the 200 kD injury group, anti-human-positive cells were restricted to the site of implantation in all animals within the 50 kD injury group. 200× **(A)**, 400× **(B)**.

Gross pathological analysis indicated that the 50 kD injury group consistently contained enlarged central canals, with occasional cavities contained to the gray matter.

Low magnification (20×) imaging of hESC-derived OPC transplanted animals with a 200 kD injury revealed widespread pathology with a loss of normal myelin as a consistent feature within the dorsal and ventrolateral columns (Figure 5E). Higher magnification (200×) imaging of transplanted animals with a 200 kD injury revealed regions of pathology in the dorsal column, suggestive of remyelination (Figure 5F). High magnification (1000×) imaging of these regions revealed extensive oligodendrocyte remyelination, as evidenced by the thin myelin sheaths relative to the axon diameter (Figure 5G). Very high magnification (2000×) imaging confirmed extensive remyelination (Figure 5H). These data confirm previous findings using a similar injury and transplant regime [33]. Our previous studies have indicated that remyelination following hESC-derived OPC transplantation is conducted by the transplanted cells [33].

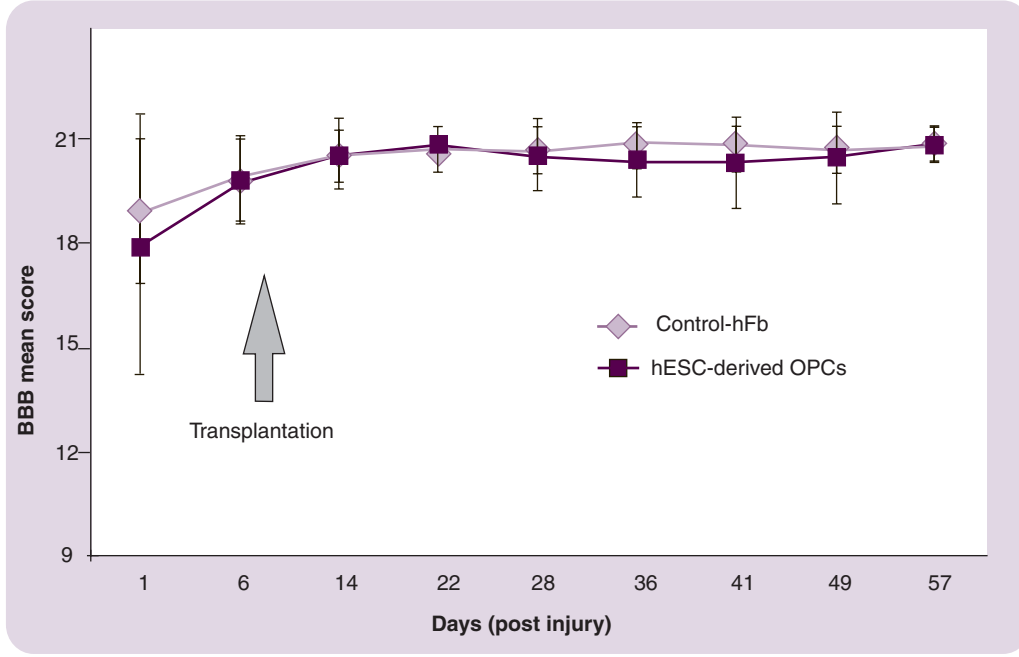
Remyelination was exceedingly rare in human fibroblast-transplanted animals with a 200 kD injury, and absent in animals with a 50 kD injury.

Discussion

Our laboratory has recently shown that transplantation of pre-differentiated hESC-derived OPCs into moderate (200 kD) contusion injuries in adult rats results in integration of transplanted cells, their differentiation into mature oligodendrocytes, the formation of compact myelin by transplanted cells, and increased locomotor recovery [32,33]. In this study, we compared the survival and migration of hESC-derived OPCs injected 7 days after a 200 kD or 50 kD contusive SCI, as well as the locomotor outcome of transplantation.

This study is the first to examine demyelination following a 50 kD SCI, and the fate of transplanted myelinogenic cells in this model. Our analyses revealed minimal pathology in the 50 kD contusion model with no detectable demyelination. Following transplantation of hESC-derived OPCs into the 50 kD injury group, major histopathologic changes were not detected. Survival of transplanted cells was evident in all transplanted animals, although it is likely that the number of surviving cells was far less than the number of cells transplanted. Oligodendrocyte lineage death has been described in developing animals as a means to adjust the number of myelinating cells to the available axons [37,38]. In the developing rat optic nerve, approximately half of the oligodendrocyte progenitors generated differentiate into myelin-forming oligodendrocytes, while the majority of the remainder undergo apoptotic cell death [37,39,40]. In the developing rat cerebral cortex, the same phenomenon is observed, with a loss of approximately 20% of pre-myelinating oligodendrocytes between postnatal days 7–11 and 37% by day 28 [41]. Interestingly, the oligodendrocytes that die are those

Figure 4. Behavioral assessment following a 50 kD contusive injury using the BBB locomotor rating scale.



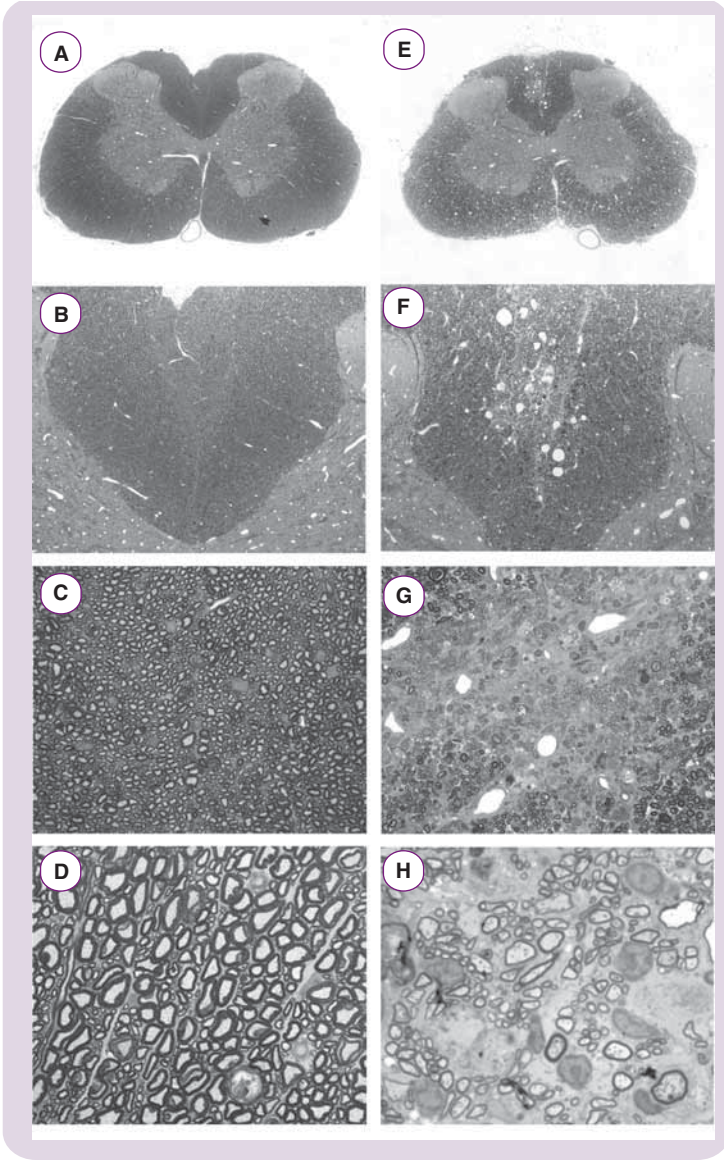
Spinal cord contusion injuries caused a minor and transient loss of locomotor function corresponding to 18–19 on the BBB scale. Animals returned to a normal behavior score around 2 weeks following transplantation and remained at this level for the duration of behavioral assessment (2 months). Importantly, transplantation did not cause impairment of locomotor function.
BBB: Basso, Beattie and Bresnahan; hESC: Human embryonic stem cells; OPCs: Oligodendrioglial progenitor cells.

that fail to contact axons, suggesting that axons affect oligodendrocyte survival. A striking decrease in the total number of oligodendrocytes and their precursors is observed following transection of neonatal rat optic nerves, whereas animals in which the number of axons is increased by genetic manipulation show a proportional increase in oligodendrocyte numbers [37,42,43]. These studies and others [44,45] support the hypothesis that axonal contact is necessary for oligodendrocytes to receive survival signals.

Growth factors associated with the extra-axonal compartment of the CNS also affect survival and myelinogenic capacity of oligodendrocytes and their progenitors. Numerous oligodendrocyte growth factors have been identified, including insulin growth factor (IGF)-1 [46–48], neurotrophin-3 [49–52], neurogulins [53], platelet-derived growth factor (PDGF) [37,48] and ciliary-neurotrophic factor (CNTF) [54–56]. The naturally occurring loss of oligodendrocytes during development of the rat optic nerve can be prevented by delivery of exogenous PDGF and CNTF [37,48]. Several experimental models of demyelination that support transplant-mediated remyelination express growth factors with known effects on the oligodendroglial lineage. Thus, IGF-1 levels are elevated in cuprizone-induced demyelination [57], and PDGF is upregulated in the lysolecithin model of demyelination [58,59]. It is likely that such factors are either not present or are insufficiently expressed following a 50 kD injury, but are expressed following the more severe 200 kD injury that supports transplant-mediated remyelination.

A growing body of evidence supports the concept that inflammatory responses within the CNS promote the survival, proliferation, migration, and differentiation of transplanted OPCs. The survival and migratory capacity of transplanted OPCs is far greater in the rat experimental autoimmune encephalomyelitis model than in normal tissue [60]. These authors have speculated that cytokines expressed in active demyelinating foci might have a positive effect on the survival and migration of OPCs. Recently, Foote and Blakemore have shown that inflammation is conducive to OPC proliferation and differentiation into myelinating oligodendrocytes [63]. In addition, their studies

Figure 5. Comparative analysis of toluidine blue-stained sections from 50 kD and 200 kD spinal cord contusion injuries after transplantation of hESC-derived OPCs.



These images are from tissue sections 2 mm caudal to the injury epicenter. Histopathology following transplantation of human embryonic stem cell (hESC)-derived oligodendroglial progenitor cells (OPCs) after a 50 kD spinal cord injury (SCI) at 40× **(A)**, 200× **(B)**, 1000× **(C)**, and 2000× **(D)** magnification. **(A)** Low magnification imaging revealed minimal pathology. **(B)** Higher magnification imaging of the dorsal column also revealed minimal pathology. **(C)** High magnification imaging revealed that structural integrity was maintained with no detectable loss of myelin sheaths or remyelination. **(D)** Very high magnification imaging reveals no detectable loss of myelin sheaths. Histopathology following a 200 kD SCI at 40× **(E)**, 200× **(F)**, 1000× **(G)**, and 2000× **(H)** magnification. **(E)** Low magnification imaging revealed widespread pathology with a region of demyelination in the dorsal column. **(F)** Higher magnification imaging revealed toluidine blue-stained myelin sheaths within the region of demyelination within the dorsal column, suggestive of remyelination. **(G)** High magnification imaging reveals extensive oligodendrocyte remyelination, as evidenced by the thin myelin sheaths relative to the axon diameter. **(H)** Very high magnification imaging reveals extensive remyelination.

demonstrate that inflammation results in subsequent remyelination in areas of chronic demyelination. Thus, an increased inflammatory response in 200 kD injures as compared with 50 kD injures may have contributed to the superior migration in that experimental group.

The presence of endogenous OPCs may also contribute to the limited migration of transplanted cells in the 50 kD group. Indeed, it has been shown that transplanted OPCs fail to populate normal tissue in adult animals, but will populate OPC-depleted tissue [61–63]. Although all animals in our 50 kD injury group contained surviving cells, in all cases those cells were restricted to within 1mm of the site of injection. As has been previously shown by Foote and Blakemore [63], injection of OPCs into tissue containing endogenous OPCs results in restricted migration. Due to the minimal pathology following a 50 kD contusive SCI, it is plausible that the transplanted cells were restricted to the site of injection due to the presence of endogenous OPCs, oligodendrocytes, or other cells.

Although transplantation of hESC-derived OPCs has been shown to improve locomotor function [33], it remained a possibility that the transplant volume or the cells themselves may have had deleterious effects that may have been masked by the severity of the SCI, or the subsequent benefit conferred by the transplant. Our data indicate that transplantation of hESC-derived OPCs into a minor SCI is not associated with histopathology or a decline in locomotor function.

Conclusion

This study demonstrates that transplantation of hESC-derived OPCs is a safe procedure. We propose that the lack of significant pathology induced by a 50 kD SCI resulted in the absence of remyelination and limited migration by transplanted cells. Our data support the use of hESC-derived OPC transplantation as a therapeutic strategy for human SCI.

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