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Repair of the injured spinal cord by transplantation of neural stem cells in a hyaluronan-based hydrogel

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ABSTRACT

Traumatic injury to the spinal cord causes cell death, demyelination, axonal degeneration, and cavitation resulting in functional motor and sensory loss. Stem cell therapy is a promising approach for spinal cord injury (SCI); however, this strategy is currently limited by the poor survival and uncontrolled differentiation of transplanted stem cells. In an attempt to achieve greater survival and integration with the host tissue, we examined the survival and efficacy of adult brain-derived neural stem/progenitor cells (NSPCs) injected within a hydrogel blend of hyaluronan and methyl cellulose (HAMC) into a subacute, clinically relevant model of rat SCI. Prior to use, HAMC was covalently modified with recombinant rat platelet-derived growth factor-A (rPDGF-A) to promote oligodendrocytic differentiation. SCI rats transplanted with NSPCs in HAMC-rPDGF-A showed improved behavioral recovery compared to rats transplanted with NSPCs in media. Rats with NSPC/HAMC-rPDGF-A transplants had a significant reduction in cavitation, improved graft survival, increased oligodendrocytic differentiation, and sparing of perilesional host oligodendrocytes and neurons. These data suggest that HAMC-rPDGF-A is a promising vehicle for cell delivery to the injured spinal cord.

1. Introduction

Spinal cord injury (SCI) is a devastating condition with sudden loss of function caudal to the level of trauma resulting in paralysis and associated complications. A cascade of secondary events follows the initial traumatic injury, characterized by ischemia, cell death, hemorrhage, inflammation, edema and further tissue damage resulting in demyelination, axonal degeneration, and cavitation at the site of injury [1]. The most frequent type of traumatic SCI is acute impact/compression of the spinal cord [2]. Treatment is limited due, in part, to the complexity of the pathophysiology of the injured spinal cord [3]. Interestingly, there is often some tissue preservation in the subpial region containing damaged, demyelinated axons [4,5], which provides a potential target for therapeutic approaches.

A promising treatment strategy for SCI is cell transplantation to replace dead or damaged cells and provide trophic support for repair [6,7]. In particular, adult neural stem/progenitor cells (NSPCs) are attractive as they self-renew and are committed to the neural lineage, effectively differentiating into oligodendrocytes, astrocytes and neurons [8]. NSPC transplantation into the injured rat spinal cord has improved recovery on the basis of both neuroprotective and neuroregenerative effects including axonal ensheathment and remyelination by oligodendrocytic progeny [9–14]. However, poor cell survival and uncontrolled differentiation of transplanted stem cells are current limitations to this approach [15]. For example, astrocytic differentiation of grafted NSPCs was reported to be associated with allodynia [14,16]. Specific factors, such as platelet-derived growth factor, can be used to promote differentiation of NSPCs to oligodendrocytes [17–19]. Concomitant growth factor infusion using osmotic mini-pumps with intrathecal catheters has improved transplant survival and promoted oligodendrocytic differentiation of brain-derived NSPCs [9,20]. However issues with catheter patency, scarring, compression, infection, and formation of proliferative meningeal lesions around the catheter insertion site are undesirable potential complications [21,22].

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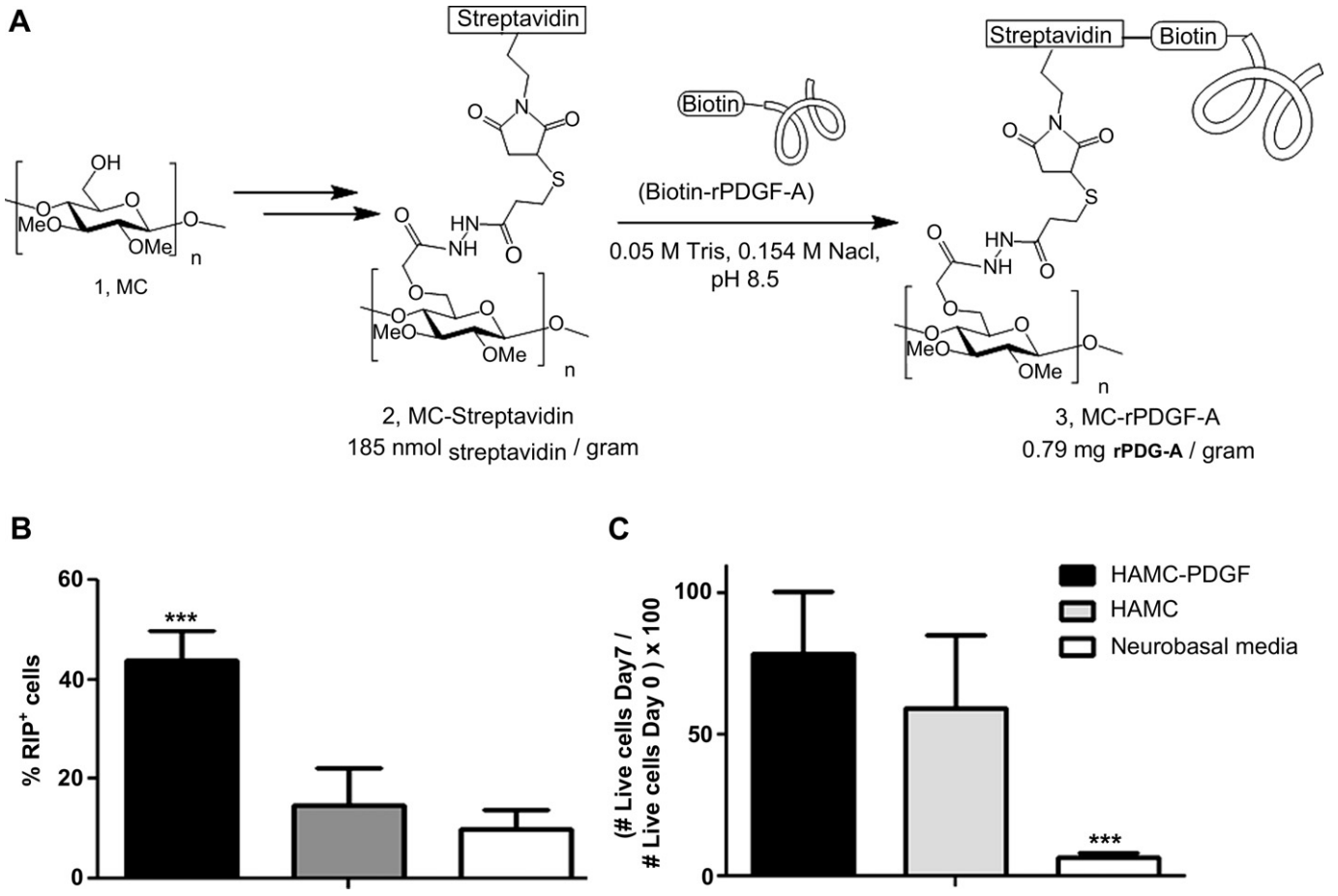

Fig. 1. A) Chemical conjugation of biotin-rPDGF-A to MC. MC (**1**) was chemically modified to covalently conjugate maleimide-streptavidin (**2**, 185 $nmol_{streptavidin}/g$). Conjugation of biotin-rPDGF-A to MC-streptavidin (**2**) results in MC-rPDGF-A (**3**, 0.79 $mg_{r\text{-}PDGF\text{-}A}/g$) [30]. (B,C) *In vitro* characterization of NSPCs in various culture conditions. Cells were pre-mixed into the HAMC hydrogel or in neurobasal media and then plated at 4×10^4 cells/well into an 8-well chamber slide for 7 d. Values are shown as mean ± standard deviation ($n = 6$). One way ANOVA was performed for all samples. (B) Percentage of RIP^+ oligodendrocytes, as determined by immunocytochemistry. (■) HAMC-rPDGF-A promotes oligodendrocyte differentiation and shows a higher percentage of RIP^+ cells compared to cells cultured in (▩) HAMC alone or in (□) neurobasal media alone (***$p < 0.001$). (C) Cell viability of NSPCs, as determined by live/dead assay. Viability of NSPCs cultured in (□) neurobasal media was significantly lower than cells cultured in (■) HAMC-rPDGF-A or (▩) HAMC alone (***$p < 0.001$).

Biomaterials used for stem cell delivery, such as solid scaffolds, have improved viability of transplanted cells in full transection and hemisection models of SCI [13,20,23]. An injectable biomaterial containing cells would be advantageous because the mixture could be transplanted into clinically relevant models of impact/compression SCI. Physical or chemical cues could also be incorporated into these injectable biomaterials to influence cell viability and fate [24,25]. Hyaluronan-based hydrogels, such as the physical blend of hyaluronan (HA) and methyl cellulose (MC), are particularly interesting because they are biodegradable, non-cytotoxic, and injectable into the injured spinal cord [26]. HAMC is a rapidly inverse-gelling polymer that will gel at physiological temperatures and has been used as a drug delivery vehicle in the central nervous system [27,28]. Interestingly, when HAMC was used to deliver retinal stem/progenitor cells to the sub-retinal space of the mouse eye, greater cell survival and distribution was observed relative to controls that received the cells by conventional methods in saline [29].

In the present study, we used a chemically modified HAMC formulation wherein recombinant platelet-derived growth factor-A (rPDGF-A) was covalently conjugated to MC. We first examined whether this modified HAMC (HAMC-rPDGF-A) promoted the survival and differentiation of NSPCs into oligodendrocytes *in vitro*. Then, we investigated the efficacy of HAMC-rPDGF-A as a cell delivery vehicle for adult rat brain-derived NSPCs in an experimental model of SCI in terms of functional recovery and tissue response. We examined the survival and oligodendrocytic differentiation of transplanted NSPCs, and the host tissue in terms of lesion size, inflammatory response and sparing of host oligodendrocytes and neurons.

2. Materials and methods

2.1. Chemical conjugation of recombinant rPDGF-A to MC

Recombinant rPDGF-A was expressed and modified with biotin and then immobilized to MC-streptavidin as shown in Fig. 1A and as previously described [30]. Briefly, MC (**1**, MW 310 kg mol^{-1}, degree of substitution (or number of hydroxyl groups that were methylated): 1.8 of 3 hydroxyl groups per glucoside unit, ShinEtsuMetolose SM-4000, Japan)) was functionalized to its carboxylated derivative using an excess of bromoacetic acid and 1.5 M sodium hydroxide. Following purification by dialysis, reactive sulfhydryl groups were incorporated into the carboxylated-MC polymer backbone upon reaction with 4-(4,6-dimethoxy-1,3,5-triazin-2-yl)-4-methylmorpholinium chloride (DMT-MM) and 3,3′-dithiobis(propionic dihydrazide), followed by disulfide bond reduction with dithiothreitol (DTT). Maleimide-streptavidin (9:1 mol maleimide: mol streptavidin, M_W 58 kDa, Sigma Aldrich) was conjugated to sulfhydryl-MC in 0.1 M phosphate buffer (pH 7.4) overnight at 4 °C, followed by the addition of N-ethylhydroxy maleimide to quench

unreacted free sulfhydryl groups. Unbound maleimide-containing reagents were removed by dialysis. MC-streptavidin (**2**) was then lyophilized to an amorphous white solid.

Recombinant rat PDGF-A was designed to incorporate a hexahistidine tag, a BirA recognition sequence (GLNDIFEAQKIEWHE) [31] and a flexible spacer (EFPKPSTPPGSSGGAP) [32] at the N-terminus, and the rat PDGF-A sequence at the C-terminus to enable protein purification and conjugation to MC-streptavidin (**2**). Biotinylation of rPDGF-A was performed using a biotin-protein ligase kit (EC 6.3.4.15, Avidity) as previously described [30], and purified by dialysis in 0.05 M Tris, 0.1 M NaCl, 0.1 M arginine buffer pH 8.5 at 4 °C.

Recombinant biotinylated-rPDGF-A was conjugated to MC-streptavidin (**2**) in 0.05 M Tris, 0.1 M NaCl buffer, pH 8.5 at 4 °C; purification was achieved by dialysis (100 kDa MWCO) in 0.05 M Tris, 0.1 M NaCl, 0.1 M arginine buffer, pH 8.5, to remove unbound rPDGF-A, then in distilled deionized (DD) water to remove excess salts. The final product was sterile-filtered and lyophilized to afford MC-rPDGF-A (**3**) as an amorphous white solid. The amount of biotinylated-rPDGF-A that was immobilized to MC-streptavidin was quantified by performing a parallel reaction with biotinylated-rPDGF-A that was fluorescently labeled with N-hydroxysuccinimide-AlexaFluor 633, as previously described [33]. Fluorescent-rPDGF-A was also incubated with unmodified MC as an adsorption control reaction. After dialysis, the fluorescence was measured and the amount of protein immobilized quantified.

2.2. Preparation of HAMC-rPDGF-A

HA and MC or MC-rPDGF-A (**3**) were dissolved in DD water, sterile filtered, lyophilized, and then weighed on an analytical balance in a biosafety cabinet. Neurobasal-A medium (Gibco-Invitrogen, Burlington, Ontario) supplemented with 2% B27 (Gibco-Invitrogen), 1% L-glutamine (Gibco-Invitrogen), 1% penicillin/streptomycin (Gibco-Invitrogen) was added to HA and MC or MC-rPDGF-A, and gently agitated overnight at 4 °C. The dissolved hydrogel was then mixed in a speedmixer for 20 s at 3500 RPM, followed by centrifugation at 14,400 RPM for 45 s and cooled on ice until cells were added.

2.3. Neural stem/progenitor cell culture

NSPCs were isolated from the subventricular zone of the brain of adult transgenic Wistar rats expressing green fluorescent protein (GFP) (Wistar-TgN(CAG-GFP) 184ys) (YS Institute Inc., Utsunomiya, Tochigi, Japan) [34], as previously described [35]. Briefly, the tissue was enzymatically and mechanically dissociated and seeded in free-floating culture in neurobasal-A medium (Gibco-Invitrogen) supplemented with 2% B27 (Gibco-Invitrogen), 1% L-glutamine (Gibco-Invitrogen), 1% penicillin/streptomycin (Gibco-Invitrogen), 20 ng/ml epidermal growth factor (Sigma–Aldrich, Oakville, Ontario), 20 ng/ml fibroblast growth factor-2 (Sigma–Aldrich), and 2 μg/ml heparin (Sigma–Aldrich). The neurospheres generated were passaged via mechanical dissociation weekly in the serum-free medium described above.

2.4. In vitro characterization of NSPCs

A suspension of NSPCs was mixed with equal volumes of either neurobasal-A media, or HAMC hydrogels for a final polymer concentration of 0.5/0.5 wt%, and supplemented with 3 ng/ml each of epidermal growth factor and fibroblast growth factor-2, and 0.3 ng/ml of heparin. Cells were incubated for 7 d (37 °C, 5% CO_2) in the same media without further supplementation. NSPCs were characterized for cell viability and differentiation into RIP^+ cells as previously described [30]. Images were taken with an Olympus FV1000 confocal microscope. For each sample, three images at 10x (for cell viability assay) and 20x (for immunocytochemistry) magnifications were taken at random locations in each well and counted using ImageJ. Six replicates were performed for each sample.

2.5. Animals and spinal cord impact/compression injury

Twenty-five adult female wild-type Wistar rats (Charles River, St. Constant, QC, 250-300g) were the recipients in this study. All animal procedures were approved by the Animal Care Committee of the Research Institute of the University Health Network in accordance with policies established by the Canadian Council on Animal Care. Pre-operatively, rats were acclimatized and trained for baseline behavioral assessment. Rats were anaesthetized by inhalation of 2% isofluorane in combination with a mixture of nitrous oxide and oxygen (1:2, v/v). The spinal cord was exposed by laminectomy and a clip compression injury was made at spinal cord level T2 with a 26 g force for 1 min according to the method of Rivlin and Tator [36]. This is a clinically relevant model of SCI reflecting human pathology. The overlying muscle and skin were sutured with 3-0 vicryl sutures (Johnson and Johnson, Peterborough ON, Canada). All animals received buprenorphine subcutaneously before awakening and then every 12 h for 3 d after surgery. Bladders were evacuated manually 3 times daily until spontaneous voiding was established, and hematuria or urinary tract infection was treated with Clavomax (62.5 mg PO BID for 7 d). The rats were housed singly in a temperature-controlled room at 26 °C with a 12 h light/dark cycle. Water and food were provided ad libitum.

2.6. Transplantation

All rats underwent a second operative procedure 9 days after SCI. Injured rats were divided into two groups based on their Basso-Beattie-Bresnahan (BBB) open field locomotor score (see below, functional analysis) to ensure equivalent deficits across groups before starting treatment. Rats were anaesthetized as described above, and the previous operative site was re-exposed. Rats were transplanted with either NSPCs mixed in HAMC-rPDGF-A ($n = 13$, NSPC/HAMC-rPDGF-A) or NSPCs in culture medium alone ($n = 12$, NSPC/media). The HAMC-rPDGF-A formulation was pre-mixed with the cell suspension resulting in a final cell concentration of 1×10^4 cells/μl in a 0.5/0.5 wt% HA/MC-rPDGF-A. The cell suspension/HAMC-rPDGF-A mix was then carefully loaded into a 10 μl Hamilton syringe with a customized 32 gauge needle for injection, avoiding the formation of bubbles. A total of 4 injections were made: 2 injections 1 mm rostral and 2 injections 1 mm caudal from the site of the clip injury, and on each side of the cord. Each injection was made 1 mm from the midline and contained 2 μl of cells at a concentration of 1×10^4 cells/μl. Injections were made stereotactically with the aid of an operating microscope using a motorized microinjector at a rate of 1 μl/min, as described previously [12]. The needle was left in place for an additional 2 min after injection to prevent cell leakage. To aid transplant survival all animals received cyclosporine (15 mg/kg, Sandimmune, Novartis Pharma, Canada Inc, Dorval, Quebec, Canada) injected subcutaneously daily from the day of transplantation until sacrifice.

2.7. Functional analysis

Functional tests were performed and analyzed by at least two examiners blinded to treatment groups. Functional tests were performed before the injury and transplantation, and then weekly for 9 weeks after transplantation. Locomotor function was evaluated using the BBB locomotor rating scale [37]. Rats were placed individually in an open field with a non-slippery surface and hind-limb movements were video recorded for 4 min to assess the animal's motor function including joint movements, stepping ability, coordination, paw placement, and toe clearance [37]. A score of 0 indicates no hind-limb movement whereas a score of 21 indicates unimpaired locomotion as observed in normal uninjured rats.

We also performed ladder-walk analysis utilizing apparatus previously described [38] to assess deficits in fine motor functions. Rats were trained for 1 week prior to injury to cross a horizontal ladder. Starting 1 week after SCI and weekly thereafter, rats who were able to perform weight supported stepping (BBB score greater than 10) were placed on the horizontal ladder-walk apparatus and three crossings were recorded. Recordings were analyzed in slow motion and the number of footfalls for each hind-limb was recorded and the average was calculated for each rat every week. Injured rats with dragging hind-limbs were scored the maximum footfalls of 9, as we have previously described [11]. Normal uninjured rats had between 0 and 1 footfall per crossing.

2.8. Tissue preparation

Rats were sacrificed at 1 week post-transplantation (or 2 weeks post-SCI, $n = 4$/group) and 8 weeks post-transplantation (or 9 weeks post-SCI, NSPC/HAMC-rPDGF-A, $n = 9$; NSPC/media, $n = 8$) following weekly behavioral assessment. Rats were deeply anaesthetized with intraperitoneal sodium pentobarbitol and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffered saline (PBS), pH 7.4. A 1.5 cm segment of tissue encompassing the lesion site at T2 was excised and cryoprotected in 30% sucrose in 0.1 M PBS for at least 24 h. Tissue was embedded in Shandon Cryomatrix (VWR Laboratories, Mississauga, ON, Canada) and cryosectioned parasagittally into 20 μm serial sections.

2.9. Histology and immunohistochemistry

Every eighth section was stained with Luxol Fast Blue and hematoxylin and eosin (LFB/H&E) for general morphology. Immunohistochemistry was performed as described previously [39,40]. Tissue sections for fluorescence immunohistochemistry were rehydrated in 0.1 M PBS, blocked for 1 h, and incubated with primary antibodies overnight at 4oC. Mouse anti-CC1/APC monoclonal antibody (CC1; 1:1000; Calbiochem, San Diego, CA) was used to detect oligodendrocytes, and mouse anti-GFP (1:200; Chemicon, Temecula, CA) was used to detect the transplanted cells. Double-labeling was performed as described previously [12]. Tissue sections were washed with 0.1 M PBS and incubated with fluorescent-conjugated secondary antibodies for 1 h at room temperature, washed with PBS and then coverslipped with Vectashield mounting medium containing 4′, 6-diamidino-2-phenyl-indole (DAPI) (Vector Laboratories, Burlington, ON, Canada) nuclear counterstain. Species-specific non-immune IgG and omission of primary antibody was used as negative controls. Immunofluorescent staining was examined using a Nikon Eclipse TE 300 microscope and a Zeiss LSM 510 confocal microscope.

For immunoperoxidase staining, sections were blocked as above and incubated with mouse anti-NeuN (1:500; Chemicon) for neurons and ED-1 (1:2000, Serotec, Raleigh, North Carolina) for activated macrophages/microglia. Following incubation in block and primary antibody, tissue sections were incubated for 1 h with biotinylated anti-mouse secondary antibody (Vector Laboratories), washed in PBS, and incubated with avidin-biotin-peroxidase complex (Vectastain Elite ABC Kit

Standard, Vector Laboratories) for 1 h. Diaminobenzidine (DAB) (Vectastain Elite ABC Kit Standard, Vector Laboratories) was applied as the chromogen.

2.10. Cavitation analysis

To analyze cavitation, every eighth section from the spinal cords of rats sacrificed at 9 weeks post-SCI was processed for LFB/H&E. One of the rats from the NSPC/media group was removed from the analysis due to poor sectioning of tissue. The sections were imaged with a Nikon Eclipse TE 300 microscope (Nikon, Mississauga, ON, Canada) and the area of cavitation of each section was traced using Nikon NIS Elements v.3.1 software. Any necrotic tissue within the cavities was counted as part of the lesion. The total spinal cord area of the segment was also measured, and included 5 mm rostral and caudal to the injury epicenter for a total of 1 cm length of spinal cord. Thus, the cavity area ($Area_{cav}$) and the total spinal cord area ($Area_{total}$) were measured. The total cavity volume (Vol_{cav}) and total spinal cord volume (Vol_{total}) were calculated using the Cavalieri method [41]. Briefly, this method is a summation of the measured area of each section multiplied by the inter-section distance. The percentage cavitation ($\%Vol_{cav}$) was determined according to the following equation: $\%Vol_{cav} = Vol_{cav}/Vol_{total} \times 100\%$.

2.11. Quantitative cell analyses

All quantitative cell analyses were performed in a blinded fashion. For quantitative analysis of the survival of transplanted cells, all GFP^+ cells were counted in parasagittal sections, 140 μm apart, through the entire thickness of each rat spinal cord as we have previously described [40]. The fluorescent signal was examined under multiple filter blocks to ensure quantitation of GFP^+ cells with a DAPI-labeled nucleus. As previously described [11,40], cell counts were adjusted to compensate for the sampling frequency to obtain an estimation of the total cell count for the entire cord thickness. To quantify the percentage of transplanted NSPCs that differentiated into oligodendrocytes, we used confocal microscopy to count the number of $CC1^+/GFP^+$ double-labeled cells in every section through the entire thickness of the cord as described above. The percentage of double-labeled cells was expressed as a proportion of GFP^+ transplanted cells.

Survival of host oligodendrocytes adjacent to the site of injury was also examined in rats sacrificed at 9 weeks post-SCI. Sections were immunostained with CC1 to identify oligodendrocytes, and using a Nikon Eclipse TE 300 microscope, 3 sections with maximal cavitation and similar dorsal-ventral distances were examined per animal. The number of $CC1^+$ cells in a field (2.9×10^5 μm^2 area) were counted rostrally and caudally within 600 μm from the edge of the lesion in each section. In determining the number of host oligodendrocytes, only $CC1^+/GFP^-$ were counted, to exclude transplanted cells.

ED-1 immunoreactivity was quantified at 1 week and 8 weeks post-transplantation. Using a Nikon Eclipse TE 300 microscope, 3 sections with maximal cavitation and similar dorsal-ventral distances were examined per rat. Using identical settings and exposure times for each section, the mean pixel density of the ED-1 DAB reaction product was determined using Nikon NIS Elements BR v.3.1 software. Four regions (2.9×10^5 μm^2 area) containing ED-1 staining were quantified per section at 0.45 mm and 1.8 mm rostral and caudal from the edge of the lesion. For each region, mean pixel density values from each animal were averaged per group.

To quantify spared host neurons, the number of $NeuN^+$ cells were counted. Every section, 140 μm apart, through the entire thickness of the cord was sampled to avoid double-counting of cells. All NeuN positive neurons were counted 1.8 mm rostral and caudal from the lesion epicentre using Nikon NIS Elements BR v.3.1 software. Data are presented as group means of total counts which have not been normalized for the entire cord thickness.

2.12. Statistical analysis

Data were analyzed using SigmaStat v.3.11 software (Systat, Point Richmond, CA). Functional tests were analyzed by two-way repeated-measures ANOVA comparing groups versus time points followed by post-hoc pairwise multiple comparisons using the Bonferroni method. Differences in percentage cavitation, graft cell survival, percentage of grafted cells that differentiated into oligodendrocytes, host neuronal and oligodendrocyte sparing, and mean pixel density measurements, were analyzed using one-way ANOVA, followed by pairwise multiple comparisons using the Bonferroni test. *In vitro* cell differentiation and viability data are presented as mean ± standard deviation. All other data are presented as mean ± standard error of the mean. For all studies, *p*-values of less than 0.05 were considered to be significantly different ($^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$).

3. Results

3.1. In vitro survival and differentiation of NSPCs in HAMC

Recombinant rat PDGF-A was conjugated to MC as shown (Fig. 1A) [30]. NSPCs were either encapsulated in a 0.5/0.5 wt% HAMC or HAMC-rPDGF-A gel, or in an equal volume of neurobasal media and cultured *in vitro* for 7 d. HAMC-rPDGF-A significantly increased the percentage of oligodendrocytes (44 ± 6% RIP^+ cells) compared to cells cultured in unmodified HAMC (15 ± 8% RIP^+ cells) or in neurobasal media alone (10 ± 4% RIP^+ cells) (Fig. 1B).

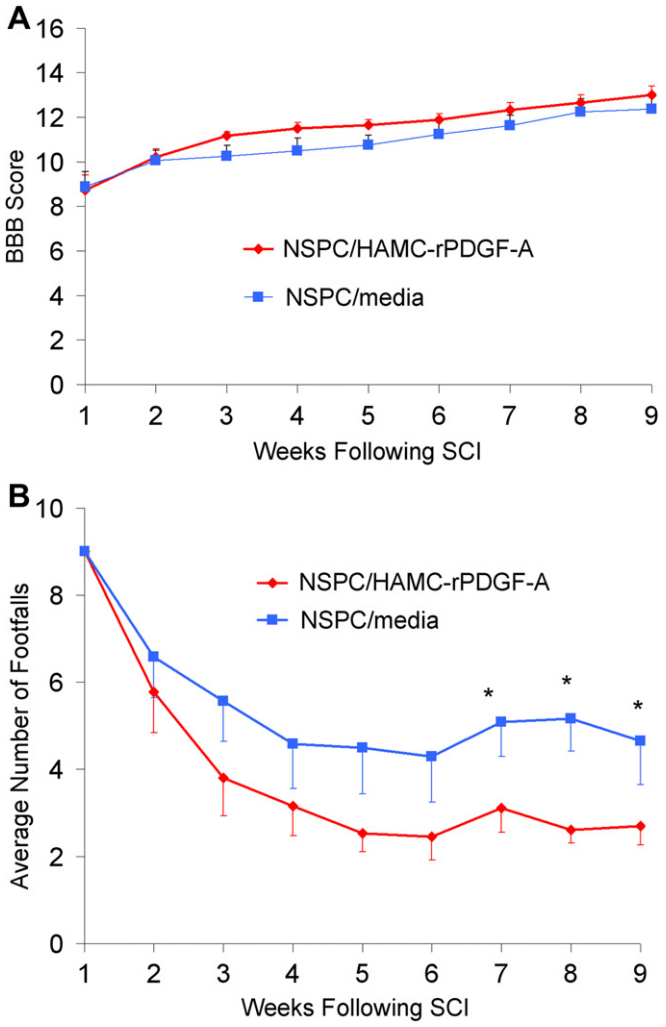

Fig. 2. Functional recovery. A) BBB locomotor score. The NSPC/HAMC-rPDGF-A group ($n = 9$) showed slightly improved recovery although this was not statistically significant. B) Ladder-walk score. The NSPC/HAMC-rPDGF-A group ($n = 9$) showed significantly fewer footfalls relative to the NSPC/media group ($n = 8$). (Data are mean ± SEM; $^*p < 0.05$).

Cell viability assays were performed to compare the effects of culture conditions (Fig. 1C). The percentage of live cells after 7 d of culture was significantly greater when cells were cultured either in HAMC alone (60 ± 26%) or HAMC-rPDGF-A (79 ± 22%) than when cells were cultured in the absence of the HAMC hydrogel (7 ± 2% live cells). Given the significantly greater percentage of RIP^+ cells (and similar percentage of viable cells) grown in HAMC-rPDGF-A relative to HAMC, further *in vivo* studies focused on HAMC-rPDGF-A hydrogels.

3.2. Functional analysis in vivo

NSPCs mixed in either HAMC-rPDGF-A or media were transplanted rostral and caudal to the lesion site 9 days post-SCI. Functional recovery was assessed weekly following SCI using the BBB locomotor rating scale and ladder-walk test for fine hind-limb

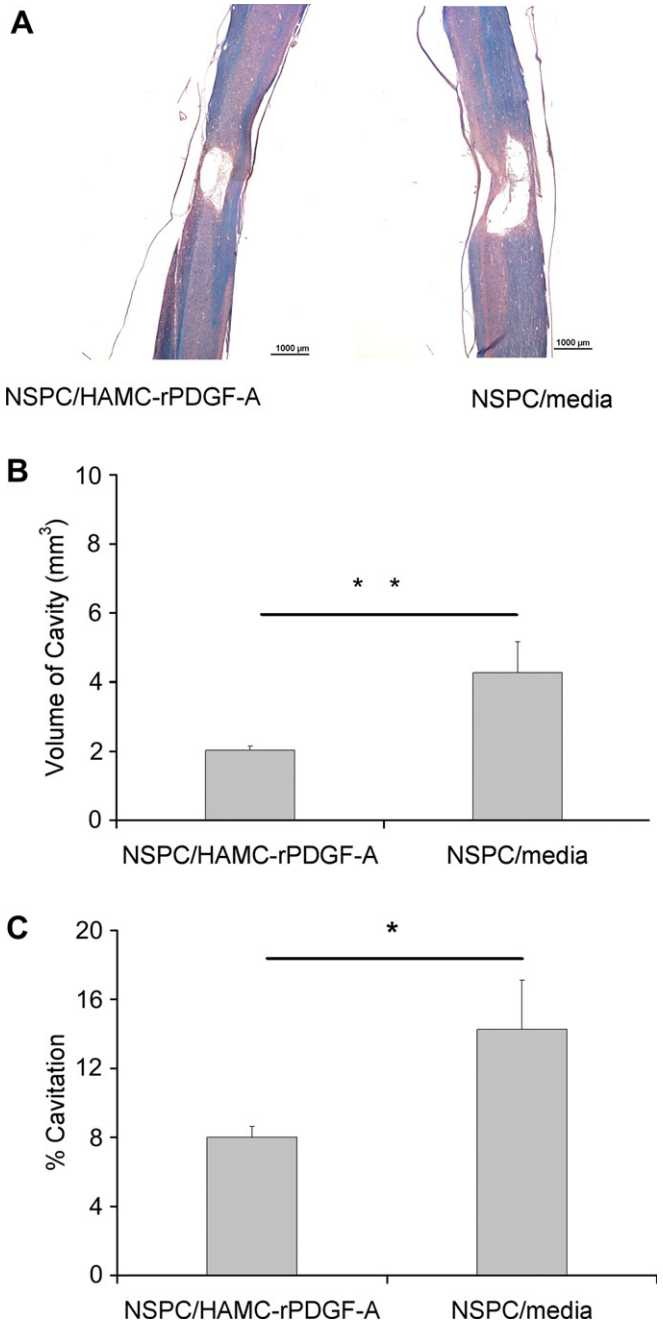

Fig. 3. HAMC-rPDGF-A reduced cavitation. A) Parasagittal sections of injured spinal cord stained with LFB/H&E showing cavitation at 9 weeks post-injury. Rats transplanted with NSPC/HAMC-rPDGF-A ($n = 9$) showed (B) reduced cavity volume (mm^3) and (C) a lower % cavitation relative to rats injected with NSPC/media ($n = 7$). (Data are mean ± SEM; $^{*}p < 0.05$, $^{**}p < 0.01$).

coordination (Fig. 2). While rats transplanted with NSPC/HAMC-rPDGF-A showed only slightly improved (but not statistically significant) BBB scores relative to NSPCs injected in media alone, rats injected with NSPCs in HAMC-rPDGF-A showed significantly fewer footfalls at weeks 7–9 post-SCI compared to rats injected with NSPC/media ($p < 0.05$). Since footfalls are considered errors, a lower score reflects better coordination and recovery. Thus, rats transplanted with NSPC/HAMC-rPDGF-A showed better functional repair in terms of fine motor control compared to rats transplanted with NSPCs in media.

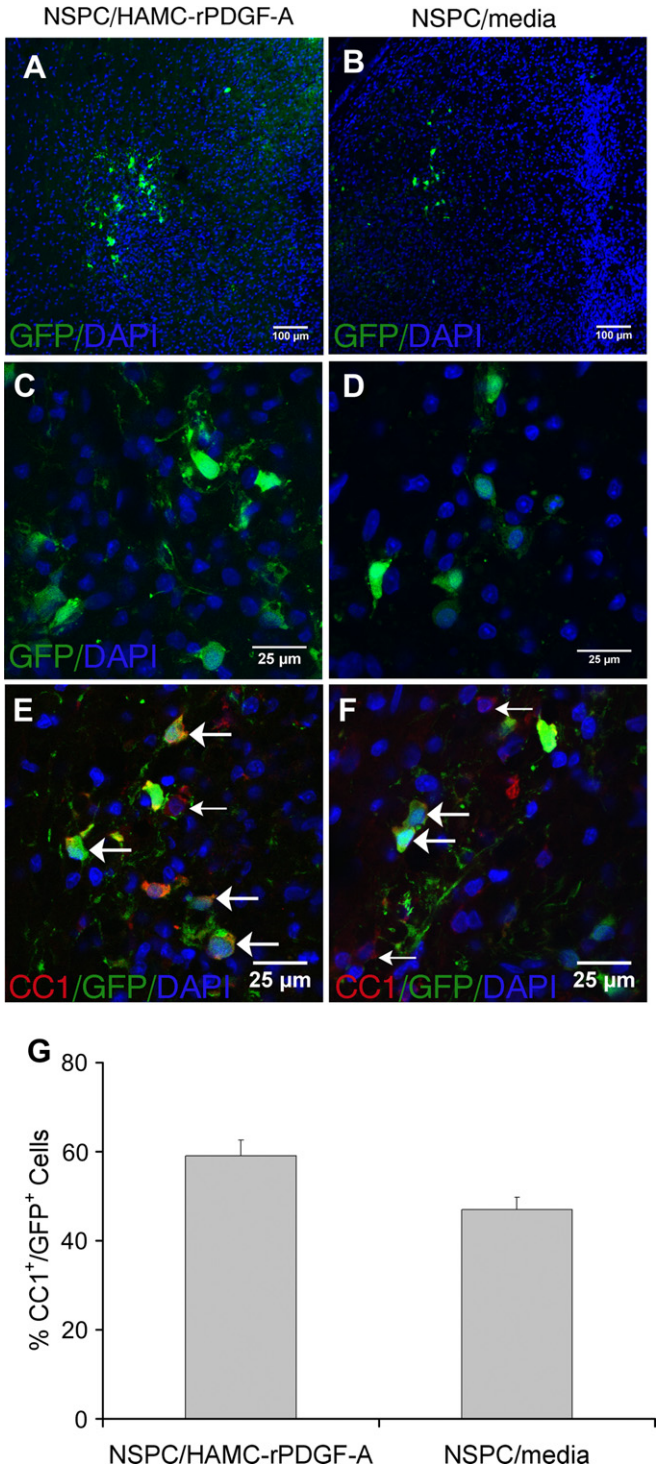

Fig. 4. HAMC-rPDGF-A improved NSPC survival and promoted oligodendrocyte differentiation. GFP^+ cells (green) are shown at 1 week post-transplantation at (A, B) low and (C, D) high magnification. Sections are stained with DAPI nuclear counterstain (blue). NSPC injections in HAMC-rPDGF-A (A, C) showed better survival with a higher proportion of $GFP^+/CC1^+$ double-labeled progeny (E, G), although this was not statistically significant ($CC1^+$ cells, red). $GFP^+/CC1^+$ double-labeled cells are denoted by large arrows, and $GFP^-/CC1^+$ cells are indicated with small arrows. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

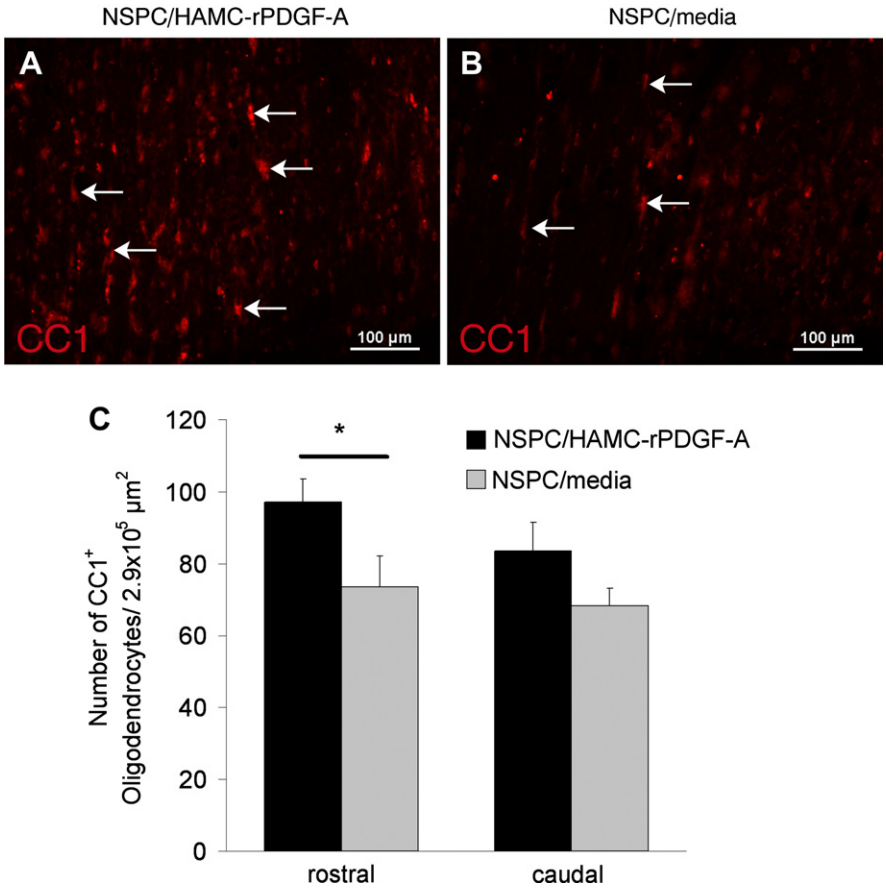

Fig. 5. Increased number of host oligodendrocytes with NSPC/HAMC-rPDGF-A transplants. (A, B) $CC1^+$ host oligodendrocytes (red, white arrows) in the rostral region adjacent to the lesion site. C) The density of $CC1^+$ oligodendrocytes was significantly higher in rostral regions adjacent to the lesion in rats transplanted with NSPC/HAMC-rPDGF-A than those transplanted with NSPCs in media. (Data are mean ± SEM; $n = 7$/group; $^*p < 0.05$). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

3.3. Lesion size and inflammatory response

Lesion volumes, calculated using LFB/H&E stained sections at 9 weeks post-SCI, represent tissue degeneration, thus a lower volumes represent less tissue loss (Fig. 3A). The total lesion volume was 2.0 ± 0.1 mm^3 in rats injected with NSPC/HAMC-rPDGF-A which was significantly less than that of 4.3 ± 0.9 mm^3 in rats injected with NSPC/media ($p < 0.01$, Fig. 3B). Thus, rats injected with NSPC/HAMC-rPDGF-A showed a 52% reduction in lesion size compared to rats injected with cells in media alone. Taking into account the total spinal cord volume, the mean percentage cavitation in rats injected with NSPC/HAMC-rPDGF-A was significantly reduced to 8.0 ± 0.6% versus 14.3 ± 2.8% in the NSPC/media group ($p < 0.05$, Fig. 3C). Thus injection of NSPCs in HAMC-rPDGF-A into the injured spinal cord resulted in reduced secondary damage and cavitation relative to injection of NSPCs in media alone.

To examine the inflammatory response, tissue was immunostained with ED-1 for activated macrophages and microglia. We found greater ED-1 immunofluorescence at 1 week post-transplantation (Supplementary Fig. 1A) compared to 8 weeks post-transplantation (Supplementary Fig. 1B), and more ED-1 staining in regions closer to the lesion cavity. However, there were no significant differences in ED-1 immunoreactivity between groups at either 1 or 8 weeks, indicating that NSPCs injected in HAMC-rPDGF-A neither exacerbated nor attenuated an inflammatory reaction relative to NSPCs injected in media.

3.4. Graft survival and differentiation

At 1 week post-transplantation, transplanted GFP^+ cells were present in all rats (Fig. 4). While rats receiving NSPC/HAMC-rPDGF-A showed better survival (Fig. 4A,C; 1.2 ± 0.3%) than rats injected with NSPC in media alone (Fig. 4 B,D; 0.4 ± 0.1%), the difference was only significant at 94% confidence ($p = 0.06$; $n = 4$/group). The percentage of transplanted GFP^+ cells expressing an oligodendrocyte phenotype was determined by immunostaining with CC1, a marker for mature oligodendrocytes. Rats transplanted with NSPC/HAMC-rPDGF-A showed a higher percentage of $GFP^+/CC1^+$ expression (59.0 ± 4.6%) than rats receiving NSPC/media (43.3 ± 3.9%); however, the difference was not significant ($p = 0.11$; $n = 3$/group) (Fig. 4E,F,G). At 8 weeks post-transplantation, very few transplanted NSPCs survived (<0.05%) in both groups, which is common in stem cell transplantation studies [11].

3.5. Oligodendrocyte sparing in host tissue

We examined the number of oligodendrocytes adjacent to the lesion site at 9 weeks post-SCI to assess the effect of NSPC/HAMC-rPDGF-A transplants on host oligodendrocyte survival. There was a greater number of spared host oligodendrocytes in rats receiving NSPC/HAMC-rPDGF-A compared to NSPC/media both rostral and caudal to the injury site, with the rostral tissue showing a statistically significant increase of 23% more host $CC1^+$ oligodendrocytes ($p < 0.05$, Fig. 5). Thus, while there were very few transplanted

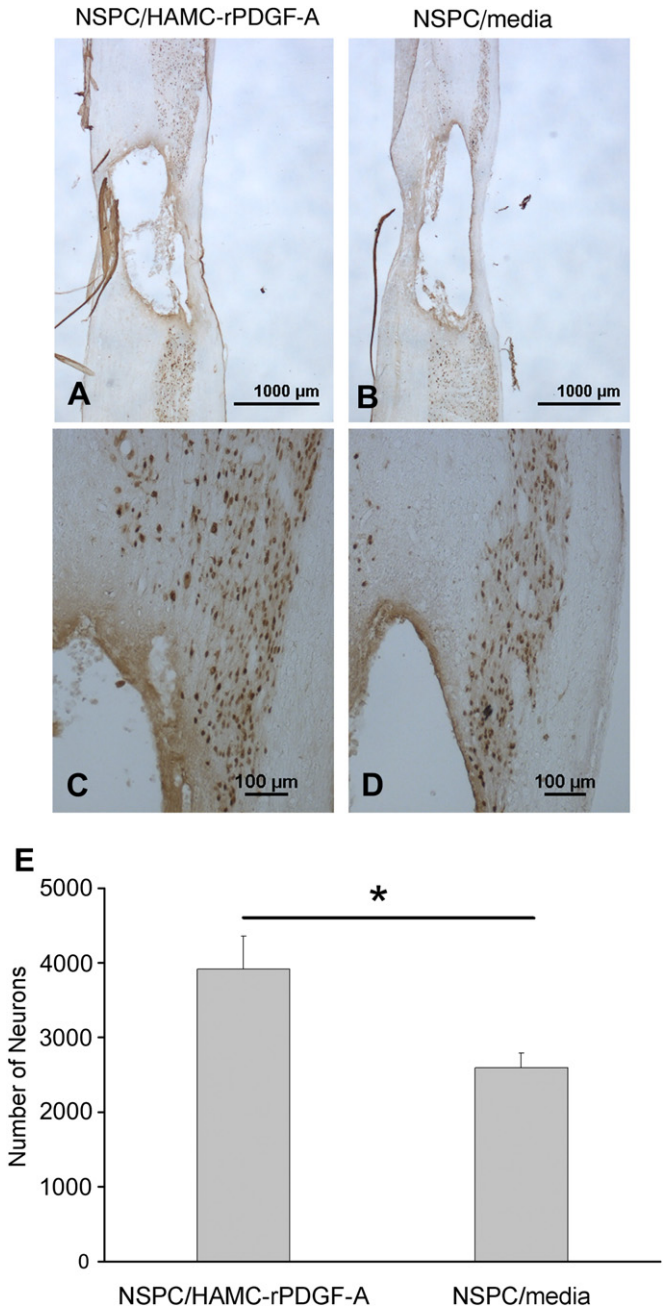

Fig. 6. HAMC-rPDGF-A promotes neuronal sparing. The absolute number of neurons within a defined region surrounding the lesion site both rostral and caudal was counted throughout the thickness of the spinal cord. (A,B) Low and (C,D) high magnification images show NeuN staining (brown). The total number of $NeuN^+$ neurons was significantly higher in rats transplanted with NSPC/HAMC-rPDGF-A relative to NSPC/media. (Data are mean ± SEM; $n = 6$/group; $^*p < 0.05$). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

GFP^+ NSPCs that could be accounted for at 9 weeks post-SCI, the longer term tissue benefit was evident in terms of greater numbers of host oligodendrocytes.

3.6. Host neuronal sparing

At 9 weeks post-SCI, host neurons were counted to assess the effect of NSPCs delivered in HAMC-rPDGF-A compared to media controls. We found significantly more $NeuN^+$ cells adjacent to the lesion site in rats receiving NSPC/HAMC-rPDGF-A than in rats receiving NSPC/media transplants ($p < 0.05$, Fig. 6). The NSPC/HAMC-rPDGF-A transplanted rats showed 33% more spared neurons compared to NSPC/media, further demonstrating the host tissue benefit achieved with NSPC transplantation in HAMC-rPDGF-A.

4. Discussion

HAMC-rPDGF-A was synthesized and evaluated first *in vitro* and then *in vivo* for the delivery of NSPCs into the injured rat spinal cord. Soluble rPDGF-A in HAMC has been shown to enhance differentiation of adult rat NSPCs to oligodendrocytes and this differentiation profile was maintained after immobilization to HAMC [30,42]. Given the improved *in vitro* differentiation profile to oligodendrocytes and similar survival of NSPCs in HAMC-rPDGF-A relative to HAMC, only HAMC-rPDGF-A was tested *in vivo* and compared to the conventional delivery vehicle of media alone. Unexpectedly, the greater *in vitro* oligodendrocytic differentiation profile of NSPCs was not maintained *in vivo* with statistical significance, suggesting that the complexity and diversity of signals present *in vivo* attenuated the signaling from the defined HAMC-rPDGF-A in terms of preferential differentiation to oligodendrocytes. These results are consistent with another study where NSPCs pre-differentiated *in vitro* along a neuronal lineage resulted in a higher percentage of neuronal progeny after transplantation than NSPCs exposed to neuronal differentiating factors *in vivo* [13]. This raises the interesting question of what type of cells are best to transplant - immature NSPCs, as was done in the present study, oligodendrocyte progenitor cells [43], or fully mature oligodendrocytes.

When tested *in vivo*, HAMC-rPDGF-A promotes greater cell survival than media controls at early time points, which is consistent with previous studies that compared HAMC to saline controls for the transplantation of retinal stem/progenitor cells [29]. The greater cell survival is likely due to the hyaluronan in the HAMC-rPDGF-A hydrogel. HA is a key component of the extracellular matrix and interacts with cells primarily through CD44 [44,45] and RHAMM receptors [46,47]. The HA-CD44/RHAMM interaction influences cell survival and migration [48] and may be responsible for the enhanced *in vivo* NSPC survival that we observed. Moreover, both HA and cellulose derivatives, such as MC, have antioxidant properties [49] which may reduce the flux of free radicals at the cell surface and thereby both reduce cell damage [50,51] and enhance NSPC survival.

Interestingly, while NSPC survival was only minimal at 8 weeks post-transplantation, NSPCs delivered in HAMC-rPDGF-A enhanced perilesional sparing of both host neurons and oligodendrocytes. The greater host tissue response may be attributed to the sustained presence of rPDGF-A co-delivered with the NSPCs in the HAMC matrix. For example, PDGF-AA has been shown to protect cultured hippocampal neurons against oxidative injury by reducing cellular peroxides and increasing the enzymatic activity of antioxidants such as catalase, glutathione peroxidase and superoxide dismutase [52]. Moreover, PDGF-AA has shown both enhanced survival of host oligodendrocytes and attenuated oligodendrocyte death following trauma [18]. Alternatively, the sparing of host neurons may be due to the effects of the HA component of HAMC as *in vitro* studies demonstrated that HA oligosaccharides blocked NMDA-induced neuronal cell death [53].

The ladder-walk test is sensitive to fine motor changes in locomotor function such as stepping and coordination [38]. Rats injected with NSPC/HAMC-rPDGF-A showed significantly fewer hind-limb footfall errors at later time points on the ladder-walk test compared to rats injected with NSPC/media. The trend for enhanced survival of grafted cells at early time points coupled with greater host tissue sparing and reduced cavitational volume of the

lesion with delivery of NSPCs in HAMC-rPDGF-A, relative to NSPCs in media, may account for the improved locomotor function observed by the ladder-walk test. These data demonstrate the tissue and functional benefits of NSPCs delivered in HAMC-rPDGF-A and its potential to reduce secondary damage following trauma.

Previous studies have suggested that HA reduces microglia/macrophage activation following SCI [53,54]. Decreased ED-1 immunoreactivity at the lesion site was observed with intrathecal injection of HAMC into the injured rat spinal cord [26]. However, intrathecal injection of HAMC into a rat model of post-traumatic syringomyelia did not affect microglia/macrophage activation as shown by Western blotting [55]. As expected, we observed greater ED-1 staining in regions closer to the lesion cavity and at 1 week post-transplantation compared to 8 weeks. However, we found no significant differences in ED-1 immunoreactivity between groups at either time point. It is possible that this method of analysis may not be sensitive enough to detect differences in the inflammatory response, or alternatively, other markers of inflammation may be more informative. Importantly, these data show that HAMC-rPDGF-A does not induce or exacerbate inflammation when mixed with NSPCs and injected into the injured spinal cord.

Collectively, these data show that HAMC-rPDGF-A hydrogel is a promising vehicle for cell delivery to the injured spinal cord where improved fine motor locomotion was observed, potentially due to greater sparing of host neurons and oligodendrocytes and reduced cavitation. Future studies will explore delivery of oligodendrocyte precursor cells in combination strategies to achieve even greater tissue and functional repair.

5. Conclusions

The use of biomaterials for stem cell transplantation is promising for enhanced functional recovery following spinal cord injury. Injection of NSPCs into the injured rat spinal cord shows enhanced tissue benefit and functional recovery when NSPCs are delivered in the hyaluronan-methyl cellulose hydrogel chemically-conjugated with the rPDGF-A growth factor relative to controls. Rats treated with HAMC-rPDGF-A showed reduced lesion size and increased sparing of perilesional host neurons and oligodendrocytes. These data suggest the therapeutic potential of HAMC as a cell delivery system for SCI repair.

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.biomaterials.2013.02.002.

References

[1] Tator CH, Fehlings MG. Review of the secondary injury theory of acute spinal cord trauma with emphasis on vascular mechanisms. J Neurosurg 1991;75:15–26.
[2] Tator CH. Epidemiology and general characteristics of the spinal cord injury patient. In: Benzel EC, editor. Neurosurgical topics “contemporary management of spinal cord injury”. IL: Park Ridge; 1995. p. 9–13.
[3] Tator CH. Review of treatment trials in human spinal cord injury: issues, difficulties, and recommendations. Neurosurgery 2006;59:957–82. discussion 82–7.
[4] Hulsebosch CE. Recent advances in pathophysiology and treatment of spinal cord injury. Adv Physiol Educ 2002;26:238–55.
[5] Tator CH. Update on the pathophysiology and pathology of acute spinal cord injury. Brain Pathol 1995;5:407–13.
[6] Mothe AJ, Tator CH. Advances in stem cell therapy for spinal cord injury. J Clin Invest 2012;122:3824–34.
[7] Tetzlaff W, Okon EB, Karimi-Abdolrezaee S, Hill CE, Sparling JS, Plemel JR, et al. A systematic review of cellular transplantation therapies for spinal cord injury. J Neurotrauma 2011;28:1611–82.
[8] Weiss S, Dunne C, Hewson J, Wohl C, Wheatley M, Peterson AC, et al. Multipotent CNS stem cells are present in the adult mammalian spinal cord and ventricular neuroaxis. J Neurosci 1996;16:7599–609.
[9] Karimi-Abdolrezaee S, Eftekharpour E, Wang J, Morshead CM, Fehlings MG. Delayed transplantation of adult neural precursor cells promotes remyelination and functional neurological recovery after spinal cord injury. J Neurosci 2006;26:3377–89.
[10] Moreno-Manzano V, Rodriguez-Jimenez FJ, Garcia-Rosello M, Lainez S, Erceg S, Calvo MT, et al. Activated spinal cord ependymal stem cells rescue neurological function. Stem Cells 2009;27:733–43.
[11] Parr AM, Kulbatski I, Zahir T, Wang X, Yue C, Keating A, et al. Transplanted adult spinal cord-derived neural stem/progenitor cells promote early functional recovery after rat spinal cord injury. Neuroscience 2008;155:760–70.
[12] Mothe AJ, Tator CH. Transplanted neural stem/progenitor cells generate myelinating oligodendrocytes and Schwann cells in spinal cord demyelination and dysmyelination. Exp Neurol 2008;213:176–90.
[13] Kim H, Zahir T, Tator CH, Shoichet MS. Effects of dibutyryl cyclic-AMP on survival and neuronal differentiation of neural stem/progenitor cells transplanted into spinal cord injured rats. PLoS ONE 2011;6:e21744.
[14] Hofstetter CP, Holmstrom NA, Lilja JA, Schweinhardt P, Hao J, Spenger C, et al. Allodynia limits the usefulness of intraspinal neural stem cell grafts; directed differentiation improves outcome. Nat Neurosci 2005;8:346–53.
[15] Nomura H, Zahir T, Kim H, Katayama Y, Kulbatski I, Morshead CM, et al. Extramedullary chitosan channels promote survival of transplanted neural stem and progenitor cells and create a tissue bridge after complete spinal cord transection. Tissue Eng Part A 2008;14:649–65.
[16] Macias MY, Syring MB, Pizzi MA, Crowe MJ, Alexanian AR, Kurpad SN. Pain with no gain: allodynia following neural stem cell transplantation in spinal cord injury. Exp Neurol 2006;201:335–48.
[17] Richardson WD, Pringle N, Mosley MJ, Westermark B, Dubois-Dalcq M. A role for platelet-derived growth factor in normal gliogenesis in the central nervous system. Cell 1988;53:309–19.
[18] Barres BA, Hart IK, Coles HS, Burne JF, Voyvodic JT, Richardson WD, et al. Cell death and control of cell survival in the oligodendrocyte lineage. Cell 1992;70:31–46.
[19] Hu JG, Fu SL, Wang YX, Li Y, Jiang XY, Wang XF, et al. Platelet-derived growth factor-AA mediates oligodendrocyte lineage differentiation through activation of extracellular signal-regulated kinase signaling pathway. Neuroscience 2008;151:138–47.
[20] Guo X, Zahir T, Mothe A, Shoichet MS, Morshead CM, Katayama Y, et al. The effect of growth factors and soluble Nogo66 receptor protein on transplanted neural stem/progenitor survival and axonal regeneration after complete transection of rat spinal cord. Cell Transplant 2012;21:1177–97.
[21] Jones LL, Tuszynski MH. Chronic intrathecal infusions after spinal cord injury cause scarring and compression. Microsc Res Tech 2001;54:317–24.
[22] Parr AM, Tator CH. Intrathecal epidermal growth factor and fibroblast growth factor-2 exacerbate meningeal proliferative lesions associated with intrathecal catheters. Neurosurgery 2007;60:926–33.
[23] Nomura H, Tator CH, Shoichet MS. Bioengineered strategies for spinal cord repair. J Neurotrauma 2006;23:496–507.
[24] Arany PR, Mooney DJ. At the edge of translation - materials to program cells for directed differentiation. Oral Dis 2011;17:241–51.
[25] Fisher OZ, Khademhosseini A, Langer R, Peppas NA. Bioinspired materials for controlling stem cell fate. Acc Chem Res 2010;43:419–28.
[26] Gupta D, Tator CH, Shoichet MS. Fast-gelling injectable blend of hyaluronan and methylcellulose for intrathecal, localized delivery to the injured spinal cord. Biomaterials 2006;27:2370–9.
[27] Kang CE, Poon PC, Tator CH, Shoichet MS. A new paradigm for local and sustained release of therapeutic molecules to the injured spinal cord for neuroprotection and tissue repair. Tissue Eng Part A 2009;15:595–604.
[28] Shoichet MS, Tator CH, Poon P, Kang C, Baumann MD. Intrathecal drug delivery strategy is safe and efficacious for localized delivery to the spinal cord. Prog Brain Res 2007;161:385–92.
[29] Ballios BG, Cooke MJ, van der Kooy D, Shoichet MS. A hydrogel-based stem cell delivery system to treat retinal degenerative diseases. Biomaterials 2010;31:2555–64.
[30] Tam RY, Cooke M, Shoichet MS. Covalently modified hydrogel blend of hyaluronan-methyl cellulose with peptides and growth factors influences neural stem/progenitor cell fate. J Mat Chem 2012;22:19402–11.
[31] Beckett D, Kovaleva E, Schatz PJ. A minimal peptide substrate in biotin holoenzyme synthetase-catalyzed biotinylation. Protein Sci 1999;8:921–9.
[32] Deyev SM, Waibel R, Lebedenko EN, Schubiger AP, Pluckthun A. Design of multivalent complexes using the barnase*barstar module. Nat Biotechnol 2003;21:1486–92.

[33] Wylie RG, Ahsan S, Aizawa Y, Maxwell KL, Morshead CM, Shoichet MS. Spatially controlled simultaneous patterning of multiple growth factors in three-dimensional hydrogels. Nat Mater 2011;10:799–806.
[34] Mothe AJ, Kulbatski I, van Bendegem RL, Lee L, Kobayashi E, Keating A, et al. Analysis of green fluorescent protein expression in transgenic rats for tracking transplanted neural stem/progenitor cells. J Histochem Cytochem 2005;53:1215–26.
[35] Morshead CM, Reynolds BA, Craig CG, McBurney MW, Staines WA, Morassutti D, et al. Neural stem cells in the adult mammalian forebrain: a relatively quiescent subpopulation of subependymal cells. Neuron 1994;13:1071–82.
[36] Rivlin AS, Tator CH. Effect of duration of acute spinal cord compression in a new acute cord injury model in the rat. Surg Neurol 1978;10:38–43.
[37] Basso DM, Beattie MS, Bresnahan JC. A sensitive and reliable locomotor rating scale for open field testing in rats. J Neurotrauma 1995;12:1–21.
[38] Metz GA, Whishaw IQ. Cortical and subcortical lesions impair skilled walking in the ladder rung walking test: a new task to evaluate fore- and hindlimb stepping, placing, and co-ordination. J Neurosci Methods 2002;115:169–79.
[39] Bozkurt G, Mothe AJ, Zahir T, Kim H, Shoichet MS, Tator CH. Chitosan channels containing spinal cord-derived stem/progenitor cells for repair of subacute spinal cord injury in the rat. Neurosurgery 2010;67:1733–44.
[40] Mothe AJ, Bozkurt G, Catapano J, Zabojova J, Wang X, Keating A, et al. Intrathecal transplantation of stem cells by lumbar puncture for thoracic spinal cord injury in the rat. Spinal Cord 2011;49:967–73.
[41] Hains BC, Saab CY, Lo AC, Waxman SG. Sodium channel blockade with phenytoin protects spinal cord axons, enhances axonal conduction, and improves functional motor recovery after contusion SCI. Exp Neurol 2004;188:365–77.
[42] Aizawa Y, Leipzig N, Zahir T, Shoichet M. The effect of immobilized platelet derived growth factor AA on neural stem/progenitor cell differentiation on cell-adhesive hydrogels. Biomaterials 2008;29:4676–83.
[43] Keirstead HS, Nistor G, Bernal G, Totoiu M, Cloutier F, Sharp K, et al. Human embryonic stem cell-derived oligodendrocyte progenitor cell transplants remyelinate and restore locomotion after spinal cord injury. J Neurosci 2005;25:4694–705.
[44] Aruffo A, Stamenkovic I, Melnick M, Underhill CB, Seed B. CD44 is the principal cell surface receptor for hyaluronate. Cell 1990;61:1303–13.
[45] Liu Y, Han SS, Wu Y, Tuohy TM, Xue H, Cai J, et al. CD44 expression identifies astrocyte-restricted precursor cells. Dev Biol 2004;276:31–46.
[46] Casini P, Nardi I, Ori M. RHAMM mRNA expression in proliferating and migrating cells of the developing central nervous system. Gene Expr Patterns 2010;10:93–7.
[47] Turley EA. Hyaluronan and cell locomotion. Cancer Metastasis Rev 1992;11:21–30.
[48] Martino G, Pluchino S. The therapeutic potential of neural stem cells. Nat Rev Neurosci 2006;7:395–406.
[49] Moseley R, Leaver M, Walker M, Waddington RJ, Parsons D, Chen WY, et al. Comparison of the antioxidant properties of HYAFF-11p75, AQUACEL and hyaluronan towards reactive oxygen species in vitro. Biomaterials 2002;23:2255–64.
[50] Presti D, Scott JE. Hyaluronan-mediated protective effect against cell damage caused by enzymatically produced hydroxyl (OH.) radicals is dependent on hyaluronan molecular mass. Cell Biochem Funct 1994;12:281–8.
[51] Cortivo R, Brun P, Cardarelli L, O'Regan M, Radice M, Abatangelo G. Antioxidant effects of hyaluronan and its alpha-methyl-prednisolone derivative in chondrocyte and cartilage cultures. Semin Arthritis Rheum 1996;26:492–501.
[52] Cheng B, Mattson MP. PDGFs protect hippocampal neurons against energy deprivation and oxidative injury: evidence for induction of antioxidant pathways. J Neurosci 1995;15:7095–104.
[53] Wakao N, Imagama S, Zhang H, Tauchi R, Muramoto A, Natori T, et al. Hyaluronan oligosaccharides promote functional recovery after spinal cord injury in rats. Neurosci Lett 2011;488:299–304.
[54] Khaing ZZ, Milman BD, Vanscoy JE, Seidlits SK, Grill RJ, Schmidt CE. High molecular weight hyaluronic acid limits astrocyte activation and scar formation after spinal cord injury. J Neural Eng 2011;8:046033.
[55] Austin JW, Kang CE, Baumann MD, DiDiodato L, Satkunendrarajah K, Wilson JR, et al. The effects of intrathecal injection of a hyaluronan-based hydrogel on inflammation, scarring and neurobehavioural outcomes in a rat model of severe spinal cord injury associated with arachnoiditis. Biomaterials 2012;33:4555–64.