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 allostynia [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].
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, Shizen Metolose SM-4000, Japan]) was functionalized to its carboxylated derivative using an excess of bromoacetic acid and 1.5M 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(propiolic dihydrazide), followed by disulfide bond reduction with dithiothreitol (DTT). Maleimide-streptavidin (9.1 mol maleimide: mol streptavidin, MW 58 kDa, Sigma Aldrich) was conjugated to sulphydryl-MC in 0.1 μM phosphate buffer (pH 7.4) overnight at 4 °C, followed by the addition of N-ethylhydroxy maleimide to quench
unreacted free sulphhydryl groups. Unbound maleimide-containing reagents were removed by dialysis. MC-streptavadin (2) was then lyophilized to an amorphous white solid.

A recombinant rat PDGF-A was designed to incorporate a hexahistidine tag, a BirA recognition sequence (GLNDFIEAQKKHEWIE) [31] and a flexible spacer (EFPKRSTPFGSGSSGAP) [32] at the N-terminus, and the rat PDGF-A sequence at the C-terminus to enable protein purification and conjugation to MC-streptavadin (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 arginine buffer pH 8.5 at 4°C. Reombinant biotinylated-rPDGF-A was conjugated to MC-streptavadin (2) in 0.05 M Tris, 0.1 M NaCl buffer, pH 8.5 at 4°C: purification was achieved by dialysis (100 kDa, and 10 kDa) 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-streptavadin was quantified by performing a parallel reaction with biotinylated-rPDGF-A that was fluorescently labeled with N-hydroxysuccinimide-Alexa Fluor 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% glucose (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 recipients dissolved hydrogel was then mixed in a speedmexer 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-Tg(N-CAG-GFP)1B4yys) (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% glucose (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 HA 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 (Sigma–Aldrich), and 10 µg/ml penicillin and streptomycin. Cells were cultured 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 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 Cryomatrim (VWR Laboratories, Mississauga, ON, Canada) and cryosectioned parasagittally to 20 µm serial sections.

2.6. Transplantation

All rats underwent a second operative procedure 9 days after SCI. Injured rats were divided into two groups based on their Baso-Beattie-Bresnahan (BBB) open field locomotor score (see below, functional analysis) to ensure equivalent deficits across groups before starting treatment. Rats were anesthetized 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/C14). The HAMC-rPDGF-A formulation was pre-mixed with the cell suspension resulting in a final cell concentration of 1 × 10^6 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 mm rostral and 2 mm caudal to 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 × 10^6 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 for 2 min 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 transplant, 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 uninjured locomotion as observed in normal uninjured rats. We also performed ladder walk analysis utilizing apparatus previously described [38] to assess deficits in five 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 and trained to cross it. Behavioral assessment. Rats were deeply anesthetized 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 to 20 µm serial sections.

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 = 8, NSPC/C14 media) were used to detect oligodendrocytes, and mouse anti-NeuN (1:200; Chemicon) for neurons and ED-1 (1:2000, Serotec, Raleigh, North Carolina) for activated macrophages/microglia. Following incubation with mouse anti-NeuN (1:500; Chemicon) and omission of primary antibody was used as negative controls. 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 (Vectorstain Elite ABC Kit

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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 (Areacav) and the total spinal cord area (Areatotal) were measured. The total cavity volume (Volcav) and total spinal cord volume (Voltotal) 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 (%Volcav) was determined according to the following equation: 

%Volcav = Volcav/Voltotal × 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 sections. 140 μm 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 × 105 μm2 area) were counted rostrally and caudally within 600 μm of 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 × 105 μm2 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 in every section 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).

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...
coordination (Fig. 2). While rats transplanted with NSPC/HAMC- rPDGF-A showed only slightly improved (but not statistically sig- niﬁcant) 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. 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 (mm³) and (C) a lower % cavitation relative to rats injected with NSPC/media (n = 7). (Data are mean ± SEM; * p < 0.05, ** p < 0.01).

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.)
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 mm³ in rats injected with NSPC/HAMC-rPDGF-A which was significantly less than that of 4.3 ± 0.9 mm³ 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. 4B,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...
References to colour in this NSPC/media. (Data are mean ± SEM; n = 6/group; *p < 0.05). 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

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**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.
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 hyaluronic-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


