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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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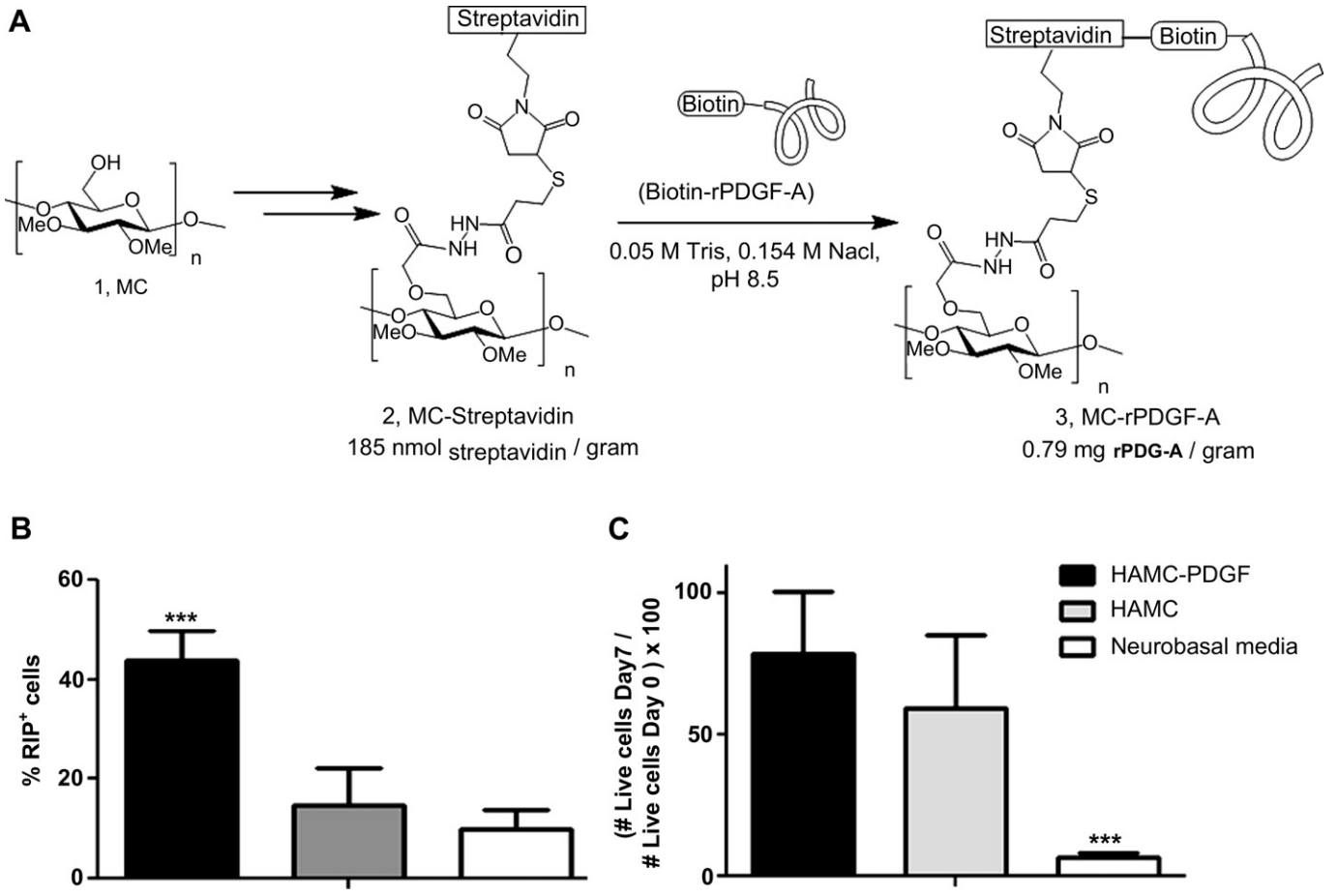

Fig. 1. A) Chemical conjugation of biotin-rPDGF-A to MC. MC (**1**) was chemically modified to covalently conjugate maleimide-streptavidin (**2**, 185 $nmol_{streptavidin}/g$). Conjugation of biotin-rPDGF-A to MC-streptavidin (**2**) results in MC-rPDGF-A (**3**, 0.79 $mg_{r\text{-}PDGF\text{-}A}/g$) [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