Title:

Transplantation of neural stem/progenitor cells in a chemically-modified methyl cellulose-based hydrogel promotes repair of the injured spinal cord

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Introduction:

Spinal cord injury (SCI) is debilitating injury and functional recovery is restricted by the limited ability of cells in the spinal cord to regenerate. Transplantation of exogenous neural stem/progenitor cells (NSPCs) into the spinal cord offers a potential therapeutic strategy to repair damaged tissue; however, the low viability and integration of transplanted stem cells and their progeny are significant hurdles that must be overcome to enable the clinical use of this strategy.

To address these issues, our lab has developed an injectable cell delivery vehicle comprised of hyaluronan (HA) and methyl cellulose (MC) conjugated with bioactive cell adhesive peptides and growth factors that increases cell viability and promotes differentiation of NSPCs into oligodendrocytes *in vitro* compared to cells cultured in media alone. The *in vitro* bioactivity of this chemically-modified biomaterial, and beneficial effects as a cell delivery vehicle into the injured rat spinal cord will be presented.

Materials and Methods:

HAMC was chemically modified with the cell adhesive peptide RGD and platelet-derived growth factor (PDGF) using thiol-maleimide and biotin-streptavidin conjugation chemistry, respectively. NSPCs were cultured *in vitro* in chemically-modified hydrogels, unmodified hydrogels or media alone, and assessed for cell viability and differentiation. Cells were encapsulated in HAMC modified with PDGF (HAMC-PDGF) and transplanted into the injured rat spinal cord (n=9). Cells were also transplanted in artificial cerebral spinal fluid (aCSF) alone as a control (n=8). Behavioral assessment (ladder walk) was performed 9 weeks following spinal cord injury, and analyzed by blinded examiners. Immunohistochemistry was also performed. 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. Data was analyzed using one-way ANOVA, followed by pairwise multiple comparisons using the Bonferroni test.

Results:

Chemical conjugation of HAMC hydrogels with RGD peptide and PDGF resulted in greater differentiation of NSPCs into oligodendrocytes (53 ± 10%) compared to unmodified hydrogels (23 ± 2%). NSPCs encapsulated in HAMC-PDGF were transplanted into the injured rat spinal cord, and resulted in improved functional recovery (ladder walk) and tissue repair (reduced lesion size and

increased sparing of perilesional host neurons and oligodendrocytes) compared to rats injected with NSPCs in aCSF.

Discussion:

Cell transplantation therapy is a promising strategy for promoting tissue repair following injury to the CNS, but is limited by low cell viability and integration upon transplantation. We show that HAMC modified with PDGF increases differentiation of NSPCs into oligodendrocytes, which are cells responsible for producing myelin that surrounds neurons, *in vitro*. As demyelination is a major pathological consequence following spinal cord injury, we transplanted NSPCs in HAMC-PDGF into the injured rat spinal cord. We show enhanced tissue benefit and functional recovery when NSPCs are delivered in HAMC-PDGF compared to NSPCs delivered in aCSF. These data suggest the therapeutic potential of HAMC as a cell delivery system for SCI repair.