Combining Human Induced Pluripotent Stem Cell-Derived Neuronal Grafts with Local Delivery of Chondroitinase ABC for Treatment of Spinal Cord Injury

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Statement of Purpose: Functional recovery following spinal cord injury (SCI) is limited due to the secondary injury: a cascade of cellular and biomolecular events that lead to cell loss and an inhibitory environment for regeneration. Because of the diversity of these events, it is likely that any regenerative strategy will be multifaceted including neuroprotective and neuroregenerative molecules, endogenous cell stimulation and/or exogenous cell transplants, and enzymes to break down the glial scar. This project focuses on combining the pro-survival effect of an injectable hydrogel on pre-differentiated human induced pluripotent stem cell-derived neuroepithelial cells (hNECs) with the local delivery of chondroitinase ABC (ChABC). Importantly, both systems are injectable and *in situ* gelling for minimally invasive delivery to the injured spinal cord. ChABC degrades chondroitin sulphate proteoglycans (CSPGs), which are part of the inhibitory environment after injury. ChABC can also degrade perineuronal nets, potentially promoting synapse formation between grafted and endogenous neurons.
Methods: hNECs were differentiated into immature neuronal precursor cells and sorted for PSA-NCAM using magnetic assisted cell sorting (MACS) prior to grafting. Cells were transplanted in a HAMC hydrogel, a physical blend of hyaluronan (HA) and methyl cellulose (MC). HA is shear thinning and can be delivered through a fine needle, whereas MC is inverse thermal gelling, enabling it to form a gel at 37°C to provide localized cell delivery. Recombinant ChABC with an N-terminal His tag and a C-terminal FLAG tag was expressed as a fusion protein with Src homology domain 3 (SH3) in *E. coli* (ChABC-SH3)[1]. MC-thiol and MC-peptide were formed by chemical modification of methylcellulose (MC), as previously described[2]. ChABC-SH3 was combined with MC-peptide, and MC-thiol in artificial cerebrospinal fluid (aCSF). MC-thiol was crosslinked with poly(ethylene glycol)-bismaleimide ($PEGMI_2$, 3000 Da, 0.75 maleimide: 1 thiol mol ratio).

One week following a moderate clip compression injury (26g) at level T2, female rats received cell transplants in HAMC (0.75% / 0.75% w/w) at 4 sites rostral and caudal to the lesion (20,000 cells/µl, 8µl in total injected). ChABC was injected intrathecally (5µl) at the time of injury and at one week post injury. Animals were tested for motor (BBB, ladder walk) and sensory function (tail flick) for up to 9 weeks. Transplanted cells were indentified with antibodies against human nuclei (hNUC) and human cytoplasm.
Results: hNECs were successfully differentiated into immature neurons, as indicated by the down-regulation of neural stem cell marker (SOX2, Nestin) and the up-regulation of neuronal marker (βIII-tubulin, doublecortin).

ChABC-SH3 was successfully expressed and purified from *E. coli*. Active ChABC-SH3 was released from MC-peptide for a period of at least 7 days. This release was tunable, either by choosing peptides with different dissociation constants or by varying the ratio of protein to peptide within the gel[1].

Neither the individual treatments (cells, ChABC) nor their combination (cells+ChABC) promoted robust functional recovery over the controls (average BBB of 11.5 to 12.5). Surviving cells were found with and without co-delivery of ChABC 9 weeks after injury (Figure 1). The majority of the cells was associated with the neuronal marker βIII-tubulin, and only some cells retained the ability to proliferate (Ki67). Cell transplantation led to a reduction in lesion site compared to lesion only controls. ChABC decreased CSPG levels at 2, but not at 9, weeks following injury.

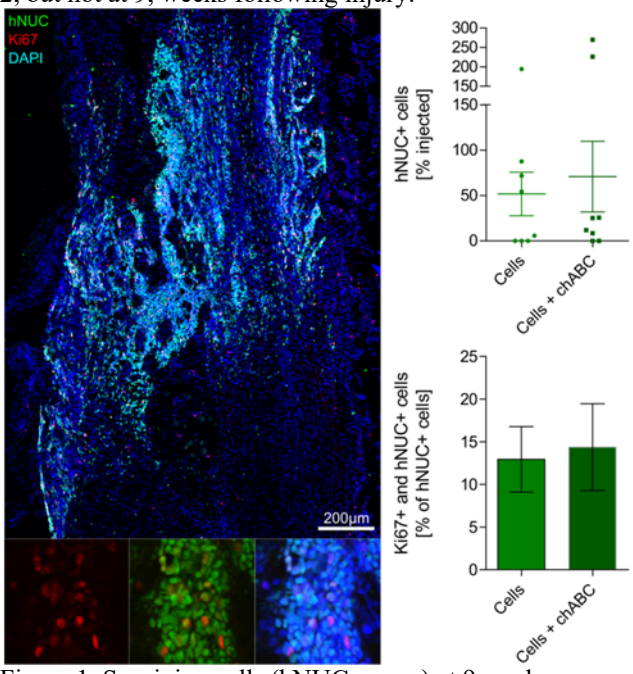

Figure 1. Surviving cells (hNUC, green) at 9 weeks following injury. Some cells were still proliferating (Ki67, red).

Conclusions: The combined therapy did not have any deleterious effects on motor or sensory function, demonstrating that the neuronal cells, ChABC and delivery vehicles are safe. hNECs survived and integrated into the host spinal cord. ChABC reduced early CSPG levels.

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References: [1]Pakulska M. et al. J. Contr. Rel. 2013;171(1):11-16; [2]Vulic et. al., JACS 2011;134:882-885.