A Composite Methylcellulose Hydrogel for Controlled Release of Multiple Therapeutics to the Injured Spinal Cord

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Statement of Purpose: Functional recovery following spinal cord injury (SCI) is very limited due to the secondary injury: a cascade of cellular and biomolecular events that leads to an inhibitory environment for regeneration[1]. Because of the diversity of these events, it is likely that any regenerative strategy will be multifaceted including neuroprotective and neuroregenerative molecules, endogenous stem cell stimulation and/or exogenous stem cell transplants, and enzymes to break down the glial scar. Herein we present a drug delivery system that is able to simultaneously deliver at least two different therapeutic molecules with independently controlled release rates. This is achieved by combining affinity-based release from a methylcellulose hydrogel with poly(lactic-co-glycolic) acid nanoparticles (PLGA np). Importantly, this system is injectable and *in situ* gelling for minimally invasive delivery of these therapeutics to the injured spinal cord.

Methods: 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*, hereafter referred to as ChABC-SH3[2]. Therapeutic proteins were encapsulated in PLGA np using a standard water/oil/water double emulsion, solvent evaporation method. MC-thiol and MC-peptide were formed by chemical modification of methylcellulose (MC), as previously described[3]. ChABC-SH3 and/or protein-loaded PLGA np were combined with MC-peptide, and MC-thiol in artificial cerebrospinal fluid (aCSF). Release of proteins from this drug delivery system was monitored by ELISA. Activity of ChABC-SH3 in release samples was measured using a dimethyl methylene blue (DMMB) assay for sulfated glycosaminoglycans with decorin as a substrate.

Results: We have designed a composite methylcellulose hydrogel drug delivery system that allows for sustained release of protein therapeutics in two different ways. One release is governed by affinity interaction between SH3 and its binding peptides. The protein of choice is expressed as a fusion with SH3, while the methylcellulose is chemically modified with an SH3 binding peptide (MC-peptide). The reversible binding of the protein/peptide pair slows release from the hydrogel. The second release is governed by diffusion from PLGA nanoparticles (Figure 1).

We have used this delivery system to simultaneously control the release of ChABC and other therapeutic molecules. 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[2].

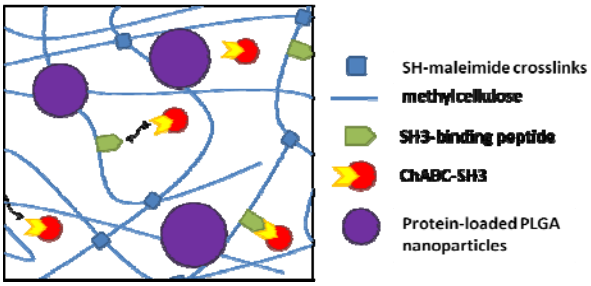

Figure 1: Schematic diagram of the release system

By combining this affinity release system with protein-loaded PLGA np, we can achieve simultaneous, yet independent, release of ChABC-SH3 and other therapeutic molecules (Figure 2). This drug delivery system is injectable through a 30 gauge needle, and gels upon heating to 37°C due to the inverse thermogelling properties of MC, making it a minimally invasive method for localized release of therapeutics to the injured spinal cord.

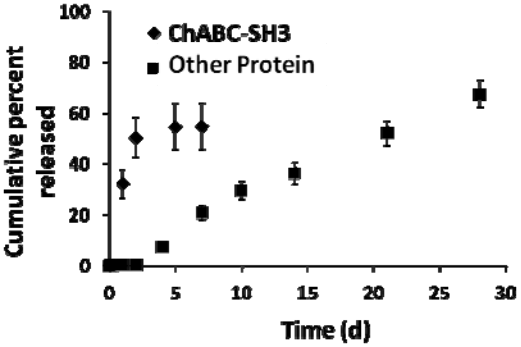

Figure 2: Simultaneous yet independent release of ChABC-SH3 and another therapeutic molecule from the MC/PLGA np composite.

Conclusions: We have designed a composite methylcellulose/PLGA np drug delivery system that is able to simultaneously, yet independently, control the release of at least two therapeutic molecules to the injured spinal cord. We have demonstrated this by releasing active ChABC-SH3 for 7 days and a second therapeutic molecule for 28 days. *In vivo* testing of this drug delivery system in a rat model of SCI is ongoing.

Acknowledgments: We are grateful to funding from CIHR and NSERC (Canada).

References: 1. Sekhon, L. H. S. *et al.*, *Spine* **26** S2-S12 (2001). **2.** Pakulska *et. al.*, *J. Contr. Rel.* **171**(1) 11-16 (2013). **3.** Vulic *et. al.*, *JACS*, **134**, 882-885 (2011).