Controlled Release of Chondroitinase ABC to the Injured Spinal Cord

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Statement of Purpose: Each year, there are approximately 1000 traumatic spinal cord injuries (SCI) in Canada and 12,000 new cases of paraplegia and quadriplegia in the US. SCI is particularly devastating because functional recovery is very limited. Recently, application of the bacterial enzyme chondroitinase ABC (ChABC) has been shown to have a positive effect on restoring function to the injured spinal cord by degrading the chondroitin sulphate proteoglycans (CSPG) that are part of the glial scar[1]. However, beneficial effects require sustained delivery of ChABC for a period of at least 7 days. This is complicated by the thermal instability of ChABC and its fragile nature as a protein therapeutic. Proteins often lose activity during formulation due to any number of factors such as heat, shear stress, or solvent interactions. Popular sustained delivery approaches such as encapsulation within poly(lactic-co-glycolic acid) (PLGA) nanoparticles use all of these destructive forces during formulation.

Herein we show that encapsulation within PLGA nanoparticles has an extremely detrimental effect on ChABC activity. We then demonstrate the tunable, sustained release of bioactive ChABC for a period of at least 7 days from a modified methylcellulose hydrogel (MC-peptide) using an affinity-based approach.

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*, and hereafter referred to as ChABC-SH3. ChABC was encapsulated in PLGA nanoparticles using a standard water/oil/water double emulsion, solvent evaporation method. Encapsulation efficiency was measured by dissolving the particles and assaying for protein content using the micro bicinchoninic acid (BCA) assay. MC-peptide was formed by chemical modification of methylcellulose (MC) with an SH3-biding peptide[2]. *In vitro* release of ChABC-SH3 from 5% w/v MC-peptide hydrogels was monitored using a His/FLAG ELISA developed in our lab, where the His tag is bound to a nickel coated plate and an antibody against the FLAG tag is used for detection. Activity of release samples was measured using a dimethyl methylene blue (DMMB) assay for sulfated glycosaminoglycans using decorin as a substrate.

Results: We have designed an affinity release system for protein therapeutics. 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 (Figure 1).

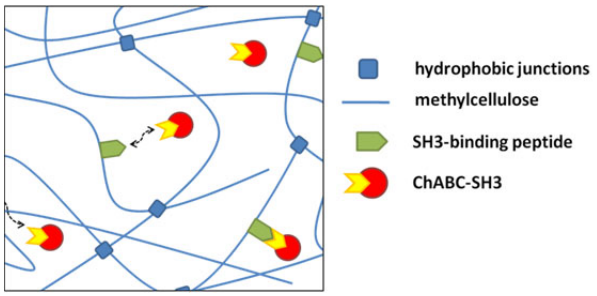

Figure 1: Schematic diagram of the affinity release system

ChABC-SH3 was successfully expressed and purified from *E. coli*. Encapsulation of ChABC-SH3 in PLGA nanoparticles yielded an encapsulation efficiency of 57 ± 4%, however none of the encapsulated protein was active; sonication and contact with dichloromethane completely destroyed ChABC-SH3 activity. Conversely, active ChABC-SH3 was release from MC-peptide for a period of at least 7 days (Figure 2). Importantly, this release was tunable, either by choosing peptides with different dissociation constants (K_d, weak binder vs. strong binder $p<0.01$) or by varying the ratio of protein to peptide within the gel (100X or 300X molar excess of peptide to protein, $p<0.001$).

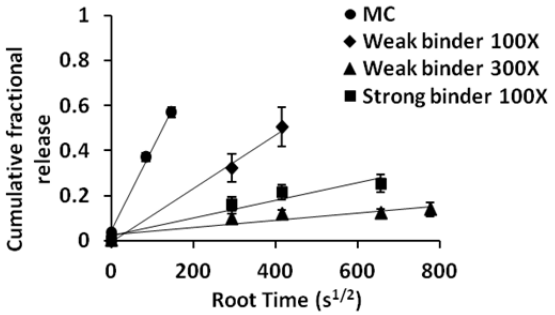

Figure 2: Cumulative release of ChABC-SH3 from MC-peptide fit to a short time approximation for unidirectional diffusion from a plane sheet.

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Conclusions: We have demonstrated that encapsulation within PLGA nanoparticles is unsuitable for the release of ChABC. Further, we have shown the tunable, sustained release of active ChABC from a modified methylcellulose hydrogel for a period of at least 7 days. Importantly, this affinity release system can be used a platform technology for other recombinant protein therapeutics.

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References: **1**. Bradbury et. al., *Nature* **416**, 636-640 (2002). **2.** Vulic et. al., *JACS*, **134**, 882-885 (2011).