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Affinity-based release of chondroitinase ABC from a modified methylcellulose hydrogel

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ABSTRACT

Chondroitinase ABC (ChABC) is a promising therapeutic for spinal cord injury as it can degrade the glial scar that is detrimental to regrowth and repair. However, the sustained delivery of bioactive ChABC is a challenge requiring highly invasive methods such as intra-spinal injections, insertion of intrathecal catheters, or implantation of delivery vehicles directly into the tissue. ChABC is thermally unstable, further complicating its delivery. Moreover, there are no commercial antibodies available for its detection. To achieve controlled release, we designed an affinity-based system that sustained the release of bioactive ChABC for at least 7 days. ChABC was recombinantly expressed as a fusion protein with Src homology domain 3 (SH3) with an N-terminal histidine (HIS) tag and a C-terminal FLAG tag (ChABC–SH3). Protein purification was achieved using a nickel affinity column and, for the first time, direct quantification of ChABC down to 0.1 nM was attained using an in-house HIS/FLAG double tag ELISA. The release of active ChABC–SH3 was sustained from a methylcellulose hydrogel covalently modified with an SH3 binding peptide. The rate of release was tunable by varying either the binding strength of the SH3-protein/SH3-peptide pair or the SH3-peptide to SH3-protein ratio. This innovative system has the potential to be used as a platform technology for the release and detection of other proteins that can be expressed using a similar construct.

1. Introduction

Chondroitinase ABC (ChABC) is a bacterial enzyme that degrades chondroitin sulfate glycosaminoglycans (CSPGs) — potent growth inhibitory molecules upregulated at the site of central nervous system injuries, such as traumatic spinal cord injury [1,2], stroke [3,4], retinal degeneration [5,6], and glioma extra-cellular matrix [7]. Treatment with ChABC after spinal cord injury has been shown to increase axon regrowth and plasticity [8,9], decrease axonal dieback, and aid in the survival and integration of transplanted or endogenous stem cells [10–12]. However, sustained delivery of ChABC to the spinal cord is currently highly invasive, requiring repeated intra-spinal injections, insertion of intrathecal catheters, or implantation of delivery systems directly into the tissue, all of which damage the very tissue requiring repair. Long-term delivery of ChABC is additionally complicated by its thermal instability [13,14].

Many of the current, less invasive methods for sustaining release, such as encapsulation within poly(lactic-co-glycolic acid) (PLGA) particles, were originally designed for small molecule drugs and are often inadequate for proteins. The protein can lose activity during formulation due to heat, shear stress, or organic solvents that result in a disruption of secondary, tertiary, and/or quaternary structures [15]. In order to achieve sustained release of proteins, existing formulation methods must be adapted on a case-by-case basis by varying formulation parameters such as solvent, excipients, or sonication, among others, often requiring a trial and error approach [16,17]. Controlled release systems based on affinity interactions are emerging as alternative strategies [18]. These systems offer mild formulation conditions and sustained release using the protein's natural affinity for specific ligands. However, current strategies are limited to the heparin-binding proteins [19].

An injectable, *in situ* gelling, hydrogel-based affinity release platform for proteins that does not expose proteins to either organic solvents or sonication was recently described [20]. The strategy consists of a physical blend of hyaluranon (HA) and methylcellulose (MC) that contribute shear thinning and inverse thermal gelling properties to the hydrogel, respectively [21]. Methylcellulose hydroxyl groups modified with Src homology domain 3 (SH3) binding peptides, hereafter referred to as MC-peptide, bind reversibly with the SH3 protein which, when expressed as a fusion with a protein, results in its sustained release. Thus the reversible binding interaction between the SH3 peptide and SH3 protein slows the release of the protein from the hydrogel [22]. The SH3 binding peptides are ideal for this affinity release system as they are well characterized, with a large range of affinities (K_d from 10^{-5} to 10^{-7} M) that while strong enough to

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appreciably bind SH3, are weak enough to allow diffusion over several days [20,22].

Testing new protein controlled release formulations is often limited by protein detection techniques. On the one hand, methods such as ELISA can detect picogram quantities of protein, yet require protein-specific antibodies that are expensive and not always readily available. On the other hand, generic protein detection assays such as the microBCA [23] or Bradford [24] are broadly applicable but not sufficiently sensitive (>1 μg/mL).

Here we present a recombinant ChABC construct (ChABC–SH3) that contains all the components necessary for purification, detection, and tunable controlled release from a hydrogel matrix (Fig. 1). A HIS tag enables purification by a nickel (Ni) affinity column while HIS and FLAG tags allow detection by ELISA. The SH3 protein binds orthogonally and reversibly with its binding peptides and is used to control release from a methylcellulose hydrogel covalently modified with an SH3 binding peptide. We show, for the first time, that we are able to sustain the release of active ChABC for 7 days *in vitro* and that we can directly quantify the concentration of released ChABC. This release is tunable by modifying the SH3 peptide to SH3 protein binding strength, or the SH3 peptide to SH3 protein ratio. Importantly, this method is broadly applicable to other recombinant proteins expressed as such a construct.

2. Materials and methods

2.1. Materials

pET28 vector was purchased from Invitrogen (Burlington, Canada). Restriction enzymes were purchased from New England Biolabs (Pickering, ON). Methyl cellulose of 300 kg/mol was purchased from Shin Etsu (Tokyo, Japan). Anti-FLAG antibody was purchased from Abcam (Cambridge, USA). Poly(lactic-co-glycolic acid) was purchased from Lactel (Birmingham, USA) (50:50 lactide:glycolide, ester terminated, 0.15–0.25 dL/g). All buffers were made with distilled and deionized water prepared using a Millipore Milli-RO 10 Plus and Milli-Q UF Plus at 18 MΩ resistance (Millipore, Bedford, USA). All other solvents and reagents, including chondroitinase ABC, were purchased from Sigma-Aldrich and used as received.

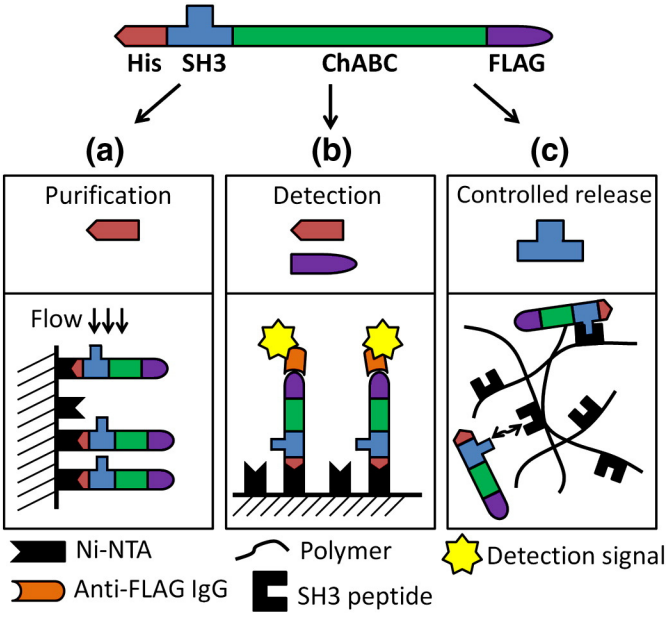

Fig. 1. Schematic diagram of the recombinant protein construct and all its applications. A HIS tag enables purification by a nickel (Ni) affinity column while HIS and FLAG tags allow detection by ELISA. The SH3 protein binds orthogonally and reversibly with its binding peptides and is used to control release from a polymer covalently modified with an SH3 binding peptide.

2.2. Chondroitinase ABC–SH3 fusion expression and purification

To obtain HIS–SH3–ChABC–FLAG DNA, a sequence coding for ChABC with a C-terminal FLAG tag was subcloned into a pET28b + vector already containing the sequence coding for HIS-SH3 followed by a flexible linker region [20]. The flexible linker region was added in order to ensure that the activity of ChABC would not be affected by the addition of this extra bulk at the N-terminus, as had been previously demonstrated with another construct [25]. ChABC-FLAG was inserted into this target vector using the restriction enzymes XhoI and EagI. The plasmid was transformed into chemically competent BL21(DE3) *Escherichia coli* cells, plated on Luria-Bertani (LB)-agar plates containing 50 μg/mL kanamycin and incubated overnight at 37 °C. Resulting clones were grown in starter cultures of 20 mL of Luria-Bertani (LB) broth containing 50 μg/mL kanamycin overnight at 37 °C. Starter cultures were inoculated into 1.8 L of Terrific Broth (TB) supplemented with 0.8% glycerol, 50 μg/mL kanamycin, and 5 drops of Anti-foam 204. Cells were grown with air sparging at 37 °C until an OD_{600} of 0.6–0.8 was reached. Cells were then induced with a final concentration of 0.8 mM IPTG and grown overnight at 22 °C. Cells were collected by centrifugation for 15 min at 6000 rpm and 4 °C (Beckman Coulter centrifuge Avanti J-26 with rotor JLA-8.1000), resuspended in 60 mL of buffer (50 mM Tris pH 7.5, 500 mM NaCl, 5 mM imidazole) and sonicated for 10 min at 30% amplitude with a pulse of 2 s (Misonix S-4000 Sonicator Ultrasonic Processor equipped with a Dual Horn probe). The slurry was centrifuged at 45,000 *g* for 15 min at 4 °C (Beckman Coulter centrifuge Avanti J-26 with rotor JA-25.50). The liquid fraction was incubated with 2 mL of Ni-NTA resin solution for 15 min at 4 °C. The resin was collected in a column with a glass frit and washed 10 × 10 mL with wash buffer (50 mM Tris, pH 7.5, 500 mM NaCl, 30 mM imidazole) and eluted with elution buffer (50 mM Tris, pH 7.5, 500 mM NaCl, 250 mM imidazole). Eluate was concentrated to ~1 mL using a Vivaspin 10,000 kDa cutoff centrifugal filter (Sartorius) and further purified by size-exclusion chromatography (SEC) in 10 mM phosphate buffer (pH 8.0, 50 mM sodium acetate) using fast protein liquid chromatography (FPLC, Hi-load 16/60 Superdex 200 prep grade column, AKTA Explorer 10, Amersham Pharmacia). Protein concentrations were determined by absorbance at 280 nm using an ND-1000 Nanodrop spectrophotometer.

2.3. Chondroitinase ABC kinetic activity assay

The kinetic ChABC activity assay tracks the absorbance at 232 nm of the double bond formed after ChABC digestion of chondroitin sulfate A (CS-A). 10 μL of recombinant ChABC–SH3 (1 μg) was placed into a Costar 96-well UV transparent plate (Corning Inc., Corning, USA). 90 μL of 10 mg/mL chondroitin sulfate A (CS-A) was then placed simultaneously into each well using a multi-pipette. The plate was immediately placed inside a TECAN Infinite M200 Pro spectrophotometer and measured every 20 s at 232 nm for 20 min at room temperature with 5 s of orbital shaking between each measurement. 100 μL of CS-A with no ChABC was used as a blank measurement and was subtracted from the measured absorbance at each time point.

2.4. Dimethylmethylene blue activity assay

The DMMB assay for sulfated glycosaminoglycans was performed as previously described [26,27]. 10 μL of 0.5 mg/mL decorin from bovine articular cartilage was incubated in a 96-well plate with 10 μL of ChABC–SH3 solution (released or positive control) at 37 °C with gentle shaking for 24 h. The plate was covered and water was placed in all unused wells to prevent evaporation of reagents. 180 μL of

1,9-dimethylmethylene blue working solution (16 mg of dye in 1 L of water containing 3.04 g glycine, 2.37 g NaCl and 95 mL, 0.1 M HCl) was added and to all wells and the plate was immediately read at 530 nm in a TECAN Infinite M200 Pro spectrophotometer. Decorin incubated with no ChABC–SH3 was used as a blank.

2.5. Double-tag ELISA for ChABC–SH3

200 μL of ChABC–SH3 protein sample or known standard was added to each well of a Hisorb Ni-NTA 96-well plate (Qiagen, Toronto, ON) and incubated for 2 h at 37 °C on an orbital shaker. The wells were then washed with 250 μL 1X PBS three times for 10 s each with vigorous tapping and blotted dry on paper towels. 200 μL of anti-FLAG antibody coupled with HRP diluted 1:5000 in 1X PBS was added to each well and incubated at 37 °C for 2 h on an orbital shaker. The wells were then washed as above and blotted dry. 100 μL of 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was then added to each well and incubated at room temperature for 25 min on an orbital shaker. The absorbance was measured at 405 nm with a wavelength correction at 650 nm. Protein concentration was calculated based on the linear range of a standard curve from the same plate. Protein samples were diluted to fit within this linear range.

2.6. Hydrogel preparation

MC-peptide was synthesized as described previously using either KPPVVKKPHYLS (weak binder, $K_d = 2.7 \times 10^{-5}$ M) or KKTKPTPP PKPSHLKPK (strong binder, $K_d = 2.7 \times 10^{-7}$ M) [20,22]. Briefly, Williamson ether synthesis was used to produce carboxylated MC. This was then coupled with 3,3′-dithiobis(propionic dihydrazide) using 1-ethyl-3-[3-(dimethylamino)propyl]-carbodiimide (EDC), followed by disulfide reduction with dithiothreitol (DTT) to yield thiolated MC. Thiolated MC was reacted with 3-maleimidopropionic-SH3-binding peptide *via* a Michael addition to afford MC–SH3-binding peptide (MC-peptide). 20 mg total of MC and MC-peptide were dissolved in artificial cerebrospinal fluid (aCSF: 350 mM NaCl, 3 mM KCl, 0.8 mM $MgCl_2$, 1.4 mM $CaCl_2$, 1.5 mM Na_2HPO_4, 0.2 mM NaH_2PO_4, pH 7.6). The relative amounts of modified and unmodified MC used depended on the desired peptide to protein ratio. The volume of aCSF used depended on the concentration of the ChABC–SH3 solution. For example, with a ChABC–SH3 stock solution at 5.56 mg/mL and MC-peptide at 216.3 μmol peptide/g MC the following amounts were used to make 400 μL of 5% w/v gel with a peptide:protein ratio of 100:1, 43 μL of ChABC solution (0.00182 μmol), 0.844 mg of MC-peptide (0.182 μmol), 19.16 mg unmodified MC, and 357 μL of aCSF. The MC solution was mixed using a SpeedMixer DAC 150 FV2 (FlackTek Inc., Landrum, USA) for 4 min at 35,000 rpm and centrifuged at 14,000 rpm in a microcentrifuge for 10 min at 4 °C. 60 μg of ChABC was then added to bring the total volume of gel to 400 μL (5% MC/MC-peptide). The protein was incorporated into the gel by speedmixing for 1 min followed by centrifugation at 10,000 rpm for 4 min. The resulting hydrogel was allowed to equilibrate overnight before being used.

2.7. Release studies

100 μL of the MC-peptide containing ChABC–SH3 was injected into each of three 2 mL tubes. The tubes were gently centrifuged to allow the gel to spread across the whole tube bottom. The tubes were then placed at 37 °C for 10 min to allow the gels to set. 400 μL of aCSF was then carefully placed on top of the gel. The gels were kept in a 37 °C incubator with gentle shaking. At designated timepoints (0, 1, 2, 5, 7 days) the supernatant was completely removed and replaced with fresh aCSF. MC-peptide gel without ChABC–SH3 was monitored in a similar manner and used as a blank for each timepoint. Release samples were stored at −80 °C until analysis by ELISA.

2.8. Statistical analysis

Each release study was performed in triplicate in three independent trials (3 times, 3 releases for each condition). Standard deviations from each of the three independent trials were pooled. Significant differences between release studies were determined using a one-way ANOVA analysis with a post-hoc Bonferroni multiple comparison test between each of the test groups. Analysis was done using GraphPad Prism software (San Diego, USA).

3. Results

3.1. Chondroitinase ABC expression and ELISA quantification

ChABC was recombinantly expressed as HIS-SH3-*linker*-ChABC-FLAG in *E. coli*, hereafter referred to as ChABC–SH3 (Fig. 2A). Purification was achieved using a Ni affinity column followed by size exclusion chromatography. The purified protein appeared as a single band on an SDS-PAGE gel at its expected molecular weight of 131 kDa (Fig. 2B). The activity was verified by measuring the rate at which it degraded chondroitin sulfate A (CS-A) which is equal to the slope of Fig. 2C. Based on this rate, the specific activity of ChABC–SH3 was calculated to be 104 U/mg, where one unit is defined as the quantity of the enzyme that catalyzes the formation of 1 μmol of unsatured disaccharide from CS-A in 1 min. Fig. 2C also shows that the addition of the SH3 protein at the N-terminus of recombinant ChABC did not affect is activity.

In order to detect the ChABC despite the lack of commercially available antibodies, we expressed the ChABC with HIS and FLAG tags at the N and C-termini respectively and designed a double tag ELISA detection system where the HIS tag binds to a Ni-NTA coated plate and the FLAG tag is detected using a commercial anti-FLAG antibody. The resulting standard curve had a lower limit of quantification (LOQ) of 0.1 nM, corresponding to a mass concentration of 13 ng/mL (Fig. 3).

3.2. Affinity release

To achieve affinity release, MC was modified with one of two SH3 binding peptides: KPPVVKKPHYLS (weak binder, $K_d = 2.7 \times 10^{-5}$ M) or KKTKPTPPPKPSHLKPK (strong binder, $K_d = 2.7 \times 10^{-7}$ M). The peptide substitution rate was 180–200 μmol peptide/g MC, as

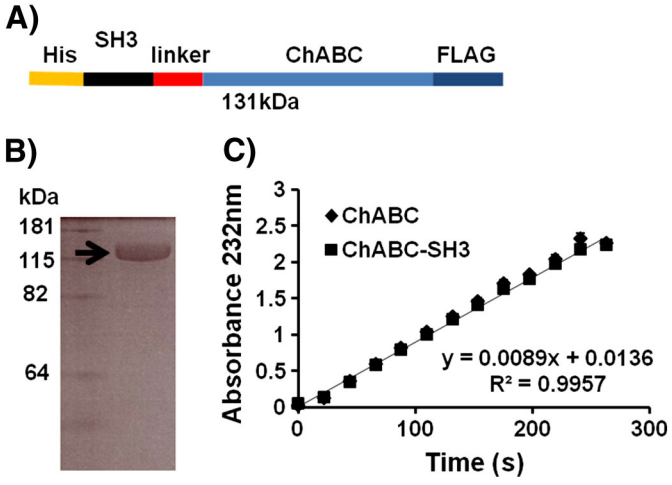

Fig. 2. Chondroitinase expression: A) Schematic diagram of ChABC–SH3 fusion with an N-terminal HIS tag and C-terminal FLAG tag. B) SDS-PAGE gel shows purified ChABC–SH3 at expected molar mass of 131 kDa. C) Kinetic activity assay shows that ChABC-SH3 fusion has similar activity to recombinant ChABC without the SH3 protein.

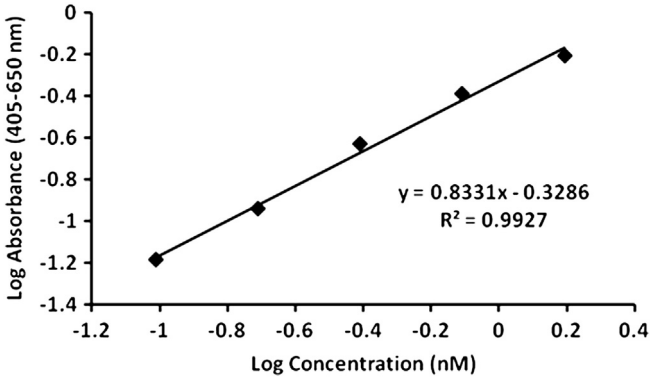

Fig. 3. Chondroitinase detection: Typical linear region of the double tag ELISA standard curve with a range between 0.1 and 2 nM ChABC–SH3 (n = 15, mean ± standard deviation plotted).

previously characterized by amino acid analysis [20]. ChABC was expressed as a fusion protein with SH3 as shown in Fig. 2A. MC and MC-peptide were dissolved in aCSF to a total of 5% by weight to create an inverse thermogelling affinity release gel. ChABC–SH3 was incorporated into the gel by mixing. The rate of release of ChABC–SH3 from the MC hydrogel was controlled using one of two strategies: (1) MC-peptide to ChABC–SH3 protein ratio or (2) dissociation constant (K_d) between the SH3 peptide–protein binding partners.

Fig. 4 shows the cumulative release profiles of ChABC–SH3 from the affinity release system. Release rates were tuned by varying the peptide to protein ratio, where 100X and 300X indicate a 100-fold and 300-fold molar excess of SH3 peptide to ChABC–SH3 respectively, or by modifying the MC with either the weak binding peptide or the strong binding peptide.

While not all of the protein was detected, there were still detectable quantities of protein being released at all timepoints corresponding on average to 0.01–2 U (0.1–19.3 μg, Supplementary Table 1) which is comparable with the doses delivered to the tissue in many spinal cord injury studies [8,9,14,29]. One explanation for the decrease in protein release over time is the instability of ChABC at 37 °C. ChABC is thermally unstable and may denature and aggregate in both the gel and supernatant between detection timepoints [13,14]. The denatured and aggregated protein may either be non-detectable by ELISA or not diffuse from the gel. Alternatively, the inability to detect all released protein may be explained by a theoretical model of the affinity-based release system itself. As the protein diffuses from the gel, the MC-peptide to protein ratio increases, thereby slowing the release of the remaining protein [30]. In reality, a combination of these two factors is most likely. The release likely slows due to the increased peptide to protein ratio and, because the release is slower, there is more time for the protein to denature and aggregate resulting in less of it being detected in total. No protein was detected by ELISA in the dissolved gel after the release assay, preventing a mass balance from being completed.

In order to better compare the release rates, the release data were fit to a short time approximation for unidirectional diffusion from a plane sheet [28].

$$\frac{M_t}{M_\infty} = kt^{1/2}$$

In this equation, k is proportional to the apparent diffusivity of the protein within the gel and is also the slope of a plot of cumulative fractional release *versus* square root time (Fig. 4B). Modification of the MC hydrogel with the SH3 weak binding peptide results in significantly smaller k values for MC (0.00365 $s^{-1/2}$) compared to all other gels ($p < 0.001$), extending the Fickian diffusion release segment from 6 h (147 $s^{1/2}$, MC) to up to 7 days (777 $s^{1/2}$, Weak binder 300X) (Fig. 4B). There is also a significant decrease in k values using the same weak binding peptide but increasing the peptide to protein excess from 100-fold (0.00119 $s^{-1/2}$) to 300-fold (0.00016 $s^{-1/2}$) ($p < 0.001$). Additionally, keeping peptide to protein excess constant at 100-fold, the k value is significantly decreased with the strong binding peptide (0.00042 $s^{-1/2}$) compared to weak binder peptide (0.00119 $s^{-1/2}$) ($p < 0.01$) (Fig. 5).

Importantly, the ChABC released from this affinity release system was shown to be active for the entire release period (Fig. 6). This is the first system to demonstrate and quantify sustained and tunable controlled release of bioactive ChABC.

4. Discussion

ChABC is a promising therapeutic molecule not only for the treatment of spinal cord injury, but also for the treatment of diseases of the eye [5,6], stroke [3], and cancer [7]. This diversity of therapeutic potential is due to the many different effects of CSPG degradation. By degrading CSPGs, ChABC can enhance synaptic plasticity, degrade scar tissue, break down chemical and physical barriers to axon regrowth, and enhance the permeability of the tumor extracellular matrix. Notwithstanding these positive and widespread attributes, ChABC is a challenging molecule to deliver in a controlled manner due to its thermal instability [13], and its detection has been plagued by the lack of commercially available antibodies.

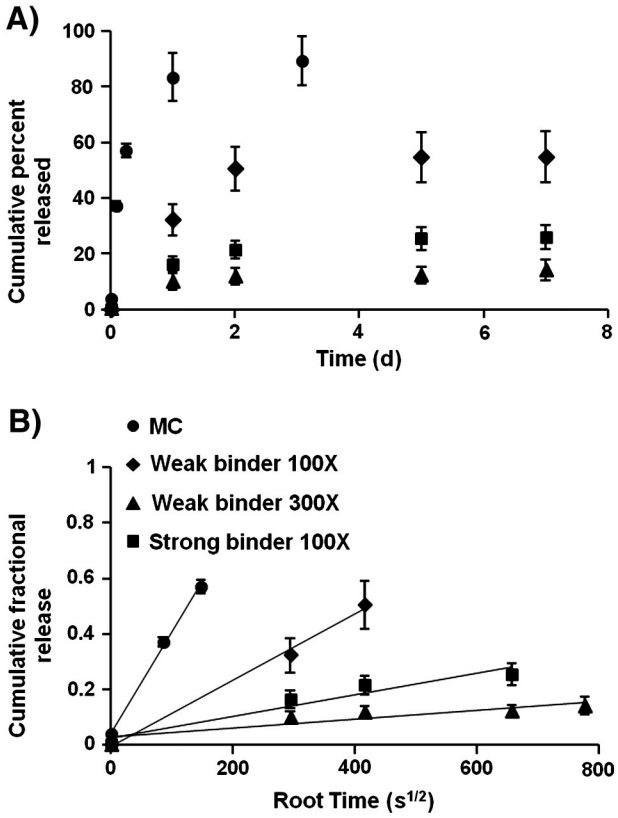

Fig. 4. Release of ChABC–SH3 from modified methylcellulose hydrogels over time. A) Cumulative release profiles of ChABC–SH3 from a series of gels: MC alone *vs.* MC-weak binding peptide 100X *vs.* MC-weak binding peptide 300X *vs.* MC-strong binding peptide 100X. 100X and 300X indicate 100-fold and 300-fold molar excess of peptide to protein within the gel, respectively. Weak binder $K_d = 2.7 \times 10^{-7}$ M; strong binder $K_d = 2.7 \times 10^{-5}$ M. B) Release profiles of (A) fit to a short time approximation for unidirectional diffusion from a plane sheet [28]. The slopes are proportional to apparent diffusivity of the protein through the gel. Release can be controlled by changing the ratio of peptide to protein (weak binder 100X *vs.* weak binder 300X *vs.* no peptide, MC alone) or changing the peptide–protein binding strength (weak binder 100X *vs.* strong binder 100X). (n = 3 independent studies for each condition, mean ± cumulative standard deviation are plotted).

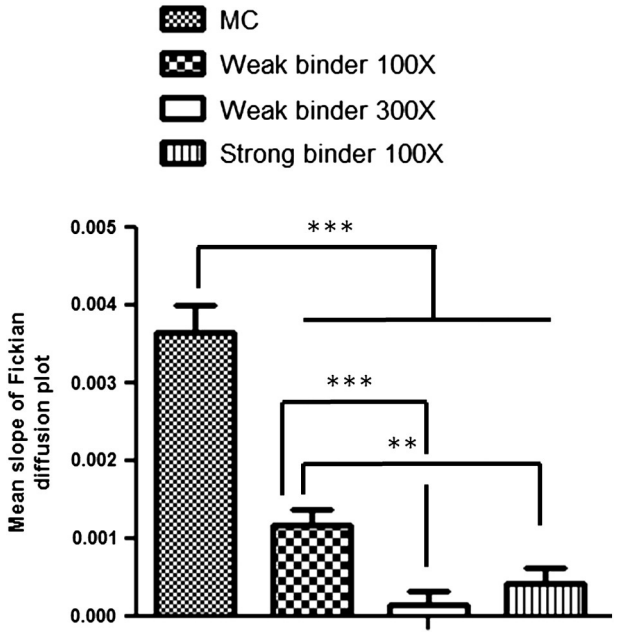

Fig. 5. Slope of release profiles (of Fig. 4B) fit to a short time approximation for unidirectional diffusion from a plane sheet [28]. The slope is proportional to the apparent diffusivity of the protein through the gel. 100X and 300X indicate 100-fold and 300-fold molar excess of MC-peptide to protein within the gel, respectively. Weak binder $K_d = 2.7 \times 10^{-7}$ M; strong binder $K_d = 2.7 \times 10^{-5}$ M. (n = 3 independent studies for each condition, mean ± standard deviation are plotted). ** indicates $p < 0.01$; *** indicates $p < 0.001$.

Sustained release of ChABC was previously investigated; however, release was not directly measured. Quantification was previously based on either activity assays alone, which do not reflect the amount of protein present [14,27,29], or model molecules such as BSA [31] or Texas red dextran [32], which only serve as proxies for ChABC. Model proteins are the last resort in the absence of direct measurement; however, there are many factors that affect protein release including size, shape, charge, and stability that prevent these model proteins from accurately reflecting the true ChABC release profile. In this study we have been able to quantify the amount of ChABC released in addition to measuring its bioactivity.

With the affinity release system, we achieved sustained release of bioactive ChABC and obviated the use of organic solvents and harsh formulation methods (sonication, homogenization) used in traditional double emulsion/solvent evaporation encapsulation strategies, such as those used with poly(lactide-co-glycolide) micro/nano-particles [33]. While Huang et al. report the encapsulation and release of ChABC from poly(lactic acid) (PLA) microspheres without any excipients [31], less than 1% of initial encapsulated ChABC was reported to be bioactive and only the release profile of BSA was shown. Moreover, only large particles with a high burst release were formed; these are unsuitable for injection and ultimately do not provide adequate controlled release.

By covalently modifying methylcellulose with the desired SH3 binding peptides and at the desired concentration, affinity release was achieved. While this strategy to control release may be applied to other polymers, methylcellulose is a particularly compelling hydrogel as it is injectable through a 30 gauge needle and is thermally-inverse gelling, allowing *in situ* gelation at 37 °C. This provides a minimally invasive, localized, and sustained release strategy for therapeutic protein delivery to the spinal cord [21,34].

Interestingly, in the absence of the methylcellulose hydrogel, ChABC–SH3 incubated at 37 °C resulted in a 95% loss in activity over 48 h (Supplementary Fig. 1), suggesting a protective effect of the hydrogel on the protein, at least over the first 48 h of release. Protein polymer interactions in hydrogels have previously been shown to retain native protein structure and maintain activity [35], and enzyme stabilization by immobilization is a well-known industrial technique. While the mechanism by which ChABC stabilization was achieved in the MC-peptide hydrogel has not been fully elucidated, by analogy to other systems [36], the hydrogel may reduce ChABC aggregation or proteolysis, thereby preserving more of its bioactivity.

By expressing and purifying ChABC from *E. coli* with HIS and FLAG tags at either terminus, we were able to quantitatively detect ChABC for the first time, using an in-house, HIS–FLAG ELISA. With an LOQ of 13 ng/mL, this ELISA is significantly more sensitive than other protein detection techniques when there is no commercially available antibody. It is one order of magnitude more sensitive than the microplate format of the NanoOrange protein assay (Invitrogen), two orders of magnitude more sensitive than the microplate format of the microBCA protein assay [23], three orders of magnitude more sensitive than absorbance at 280 nM, and four orders of magnitude more sensitive than the microplate format of the Bradford protein assay [24]. Moreover, because the two tags responsible for detection are on opposite termini of the ChABC protein, this method detects only intact protein and will not give false positives if protein fragments are present. Since this detection system does not rely on the identity of the protein in between the two tags, it can be used to detect other proteins that can be expressed with HIS and FLAG tags, providing a platform protein detection technology.

In this study, we demonstrated affinity-based release of active ChABC from a methylcellulose hydrogel. The release of active ChABC was sustained for at least 7 days, and the release profile was tunable by varying the peptide–protein binding constant or the ratio of peptide to protein. Additionally, for the first time, ChABC release was measured directly using a new double-tag ELISA system. Although we have demonstrated this system with ChABC, purification, detection, and controlled release were all independent of protein identity. This system provides a new paradigm for the combined purification, detection, and controlled release of proteins.

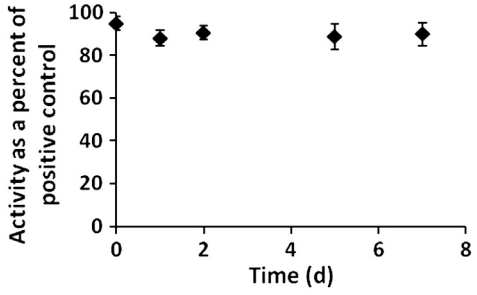

Fig. 6. Representative activity of released ChABC remains high throughout the 7 day release period as measured by degradation of decorin in a dimethylmethylene blue assay. Data shown are for strong binder 100X curve in Fig. 4A. All samples were compared to a positive control of fresh ChABC–SH3 (n = 3, mean ± standard deviation plotted).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.jconrel.2013.06.029.

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