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Sustained release of neurotrophin-3 and chondroitinase ABC from electrospun collagen nanofiber scaffold for spinal cord injury repair

Ting Liu,[1] Jinye Xu,[2] Barbara P. Chan,[2] Sing Yian Chew[1]

[1]School of Chemical and Biomedical Engineering, Nanyang Technological University, 62 Nanyang Drive, 637459 Singapore
[2]Medical Engineering, Department of Mechanical Engineering, The University of Hong Kong, Hong Kong Special Administrative Region, China

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Abstract: Nerve regeneration after spinal cord injuries (SCI) remains suboptimal despite recent advances in the field. One major hurdle is the rapid clearance of drugs from the injury site, which greatly limits therapeutic outcomes. Nanofiber scaffolds represent a potential class of materials for enhancing nerve regeneration because of its biomimicking architecture. In this study, we investigated the feasibility of incorporating neurotrophin-3 (NT-3) and chondroitinase ABC (ChABC) onto electrospun collagen nanofibers for SCI treatment. By using microbial transglutaminase (mTG) mediated crosslinking, proteins were loaded onto electrospun collagen nanofibers at an efficiency of ~45–48%. By combining NT-3 with heparin during the protein incorporation process, a sustained release of NT-3 was obtained (~96% by day 28). As indicated by dorsal root ganglion outgrowth assay, NT-3 incorporated collagen scaffolds supported neuronal culture and neurite outgrowth for a longer time period than bolus delivery of NT-3. The presence of heparin also protected ChABC from degradation. Specifically, as evaluated by dimethylmethylene blue assay, bioactive ChABC was detected from collagen scaffolds for at least 32 days *in vitro* in the presence of heparin (~32% of bioactivity retained). In contrast, ChABC bioactivity was only ~1.9% by day 22 in the absence of heparin. Taken together, these results clearly demonstrated the feasibility of incorporating NT-3 and ChABC via mTG immobilization to produce protein-incorporated collagen nanofibers. Such biofunctional nanofiber constructs may find useful applications in SCI treatment by providing topographical signals and multiple biochemical cues that can promote nerve regeneration while antagonizing axonal growth inhibition for CNS regeneration. J Biomed Mater Res Part A: 100A: 236–242, 2012.

Key Words: electrospinning, nerve regeneration, neural tissue engineering, neurite outgrowth, glial scar

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INTRODUCTION

Nerve regeneration following spinal cord injuries (SCI) is often restricted by many growth inhibitory factors that prevent neurons and support cells from infiltrating the injury site. Chief among which, is the formation of glial scar following injuries.[1] One of the major components of the glial scar is chondroitin sulfate proteoglycans (CSPGs).[2–4] Correspondingly, the delivery of chondroitinase ABC (ChABC) to digest the neuronal growth inhibitory glycosaminoglycan (GAG) side chains of CSPGs has been attempted with some success.[5,6] At present, ChABC treatment for SCI is mainly achieved by intrathecal injection with frequencies ranging from days to weeks and the typical duration lasting 2–6 weeks.[7,8] Unfortunately, ChABC is thermal sensitive and easily degraded by the host. Furthermore, the diffusion of ChABC into the injury site is often limited because of overflow beyond the intrathecal space.[6,9] Therefore, in order to achieve desirable therapeutic outcomes, a slow-release system that delivers ChABC locally is needed to maintain sufficient levels of bioactive enzyme at the injury site over prolonged time periods.

Therapeutic delivery of neurotrophins, such as neurotrophin-3 (NT-3), has also been attempted to promote the sprouting, survival, and regrowth of corticospinal tracts.[10,11] In most cases, direct injection of the drugs[12] or controlled delivery using hydrogels was used.[13] The combinatory treatment with ChABC and NT-3 in a sustained release manner from hydrogels has also showed significantly improved locomotor function and enhanced growth of sensory axons in rats.[14] Unfortunately, hydrogels are typically isotropic in nature and lack topographical features that may provide contact guidance to enhance nerve regeneration.

Nanofibers scaffolds serve as ideal biomimetic substrates because of their unique architecture that closely mimics the

Correspondence to: S. Y. Chew; e-mail: sychew@ntu.edu.sg
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structure of the natural extracellular matrix (ECM).[15] Nanofiber topography enhanced the maturation of human Schwann cells.[15] Endowed with glial-cell derived neurotrophic factor, sciatic nerve regeneration was enhanced.[16] Applied to neural tissue engineering in the central nervous system (CNS), electrospun collagen nanofibers decreased astrocyte proliferation and enhanced apoptosis without altering cellular activation as compared to two-dimensional flat surfaces. In addition, when implanted into a rat hemisection SCI model as spiral nerve conduits, $GFAP^+$ glial cell penetration was reduced and neurofilament sprouting was observed within the collagen scaffolds as early as 10 days postimplantation.[17]

In this study, we introduced a method to incorporate NT-3 and ChABC onto electrospun collagen nanofibers for sustained protein delivery to treat SCI. The electrospinning of collagen has been reported extensively.[18–20] However, the incorporation of drugs, particularly proteins, into electrospun collagen constructs remains unexplored. We hypothesized that the synergistic effects of topographical cues and multiple biochemical signals from a single construct would provide a potential therapy for SCI.

MATERIALS AND METHODS

Materials

Type I collagen was extracted from rat tails for electrospinning.[21] Ninety-nine percent acetic acid was purchased from MERCK. Dulbecco's modified eagle medium (DMEM), minimum essential medium (MEM), Neurobasal medium, B27, L-Glutamine, penicillin-streptomycin, anti-Tuj1 primary antibody, secondary antibodies, bovine serum albumin Alexa fluor 647 conjugates (BSA-647), and type I collagenase were purchased from Invitrogen. Fetal bovine serum (FBS) was purchased from Hyclone. Microbial transglutaminase (mTG) was kindly provided by Ajinomoto. Neurotrophin-3 (NT-3) and Duoset Enzyme-linked immunosorbent assay (ELISA) kits were ordered from R&D system. All other chemicals and reagents were purchased from Sigma-Aldrich.

Fabrication and crosslinking of electrospun collagen scaffolds

Collagen nanofibers were electrospun as described previously.[18] Randomly oriented collagen fibers were collected on aluminum foils that were mounted on a grounded rotating target (150 rpm). Thereafter, all samples were photochemically crosslinked as described previously.[18] Stabilized collagen scaffolds were then soaked in deionized water at 37°C overnight to remove chemical residues. Following that, the scaffolds were freeze-dried and UV sterilized for 15 min on each side prior to further analyses.

Optimization of mTG mediated protein immobilization

BSA-647 was used as the model protein for optimization. To elucidate the optimal initial loading amount of proteins, 1, 5, and 20 μg of BSA-647 were used. The protein samples were each dissolved in 200 μL of PBS. Thereafter, each sample was added onto a collagen scaffold (10 mg/scaffold, $n = 3$ for each group), and placed on a shaker to ensure uniform protein distribution. After 1 h, 300 μL of mTG Tris-buffer solution (2 wt %) was added to each scaffold and incubated at 37°C for 2 h. Subsequently, the scaffolds were retrieved and washed with PBS for three times before analysis.

To elucidate the optimal incubation time required for mTG crosslinking, scaffolds comprising of an initial theoretical loading of 5 μg of BSA-647 were used. The BSA-647 loaded scaffolds were soaked in mTG solution for 2, 4, or 20 h ($n = 3$ for each group). Thereafter, all samples were retrieved and washed with PBS for three times prior to further analyses.

To quantify protein loading efficiency, all BSA-647 incorporated scaffolds were digested by 1 mL of type I collagenase (10 mg/mL) at 37°C. After 15 min, all scaffolds were digested and 100 μL of sample was retrieved for fluorescence intensity measurement at 620 nm using a microplate reader (TECAN Infinite 200). The amount of BSA-647 in each sample was calculated based on a standard curve that was generated through serial protein dilutions. The protein loading efficiency was then calculated as:

$$\text{Protein loading efficiency}\,(\%) = \frac{\text{The amount of incorporated BSA} - 647}{\text{The amount of initially loaded BSA} - 647} \times 100\%$$

NT-3 incorporation and *in vitro* release kinetics

Three electrospun collagen scaffolds (10 mg each) were prepared for NT-3 incorporation studies. Firstly, 5 μg of NT-3 was reconstituted in 100 μL of BSA solution (0.1 wt %). The resulting solution was then added into 100 μL of heparin (5 mg/mL in PBS) to result in a final NT-3:heparin molar ratio of 800:1. Heparin was used to protect the bioactivity of NT-3[22,23] and the heparin to NT-3 ratio was chosen based on previous studies.[24] Thereafter, NT-3/heparin solution was added onto the collagen scaffolds and incubated on a shaker for 1 h at room temperature to allow thorough protein distribution. Following that, 300 μL of 2 wt% mTG solution was added and incubated at 37°C for 2 h. The NT-3 loaded collagen scaffolds were then washed with PBS for three times and soaked in 1.5 mL of serum-free neurobasal medium. Antibiotic-antimycotic solution was added at a dilution of 1:100 and the fibers were incubated under static conditions at 37°C. At various time points, 1.2 mL of supernatant was retrieved from each sample and an equal volume of fresh medium was replaced. The concentration of NT-3 in the supernatant was determined by ELISA assay and 1.0 mL of the supernatant was used for bioactivity studies. At day 60, the fibers were digested in 1.0 mL of type I collagenase (10 mg/mL) and any residual NT-3 was quantified by ELISA.

NT-3 bioactivity assay

Dorsal root ganglions (DRGs) were dissected from E15 old embryos of Sprague Dawley rats under current Institutional Animal Care and Use Committee (IACUC) guidelines. All explants were then cultured in a humidified incubator at 37°C with 5% CO_2.

Supernatant assay. Isolated DRGs were seeded onto glass cover slips coated with 1 mL of 0.1% poly-lysine in a

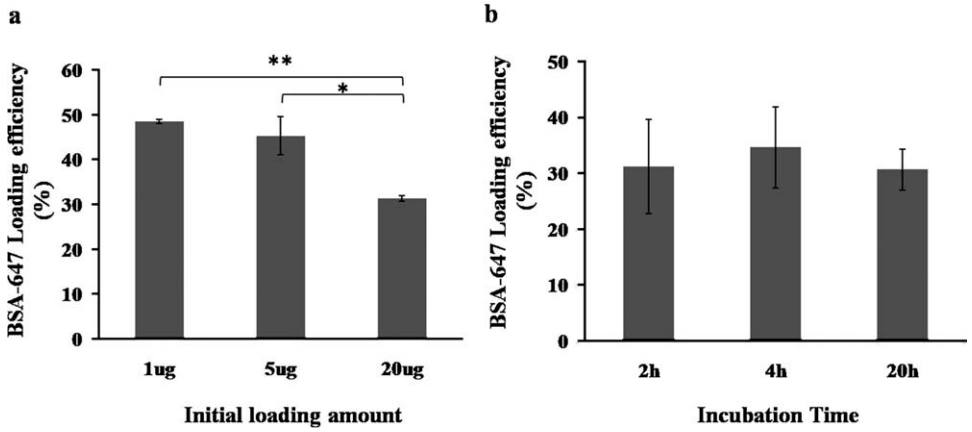

FIGURE 1. Variation of BSA-647 loading efficiency with respect to (a) initial protein loading mass and (b) mTG incubation time at 37°C. $^{**}p < 0.001$, $^{*}p < 0.01$, ANOVA.

24-well plate ($n = 3$). Thereafter, 1.0 mL of NT-3 supernatant was added. On the basis of our initial studies, at least 40 ng/mL of NT-3 was required for observable neurite outgrowth. Therefore, the positive control comprised of DRGs cultured in the presence of 40 ng/mL of NT-3 while the negative control comprised of DRGs cultured in plain neurobasal medium only. For all samples, 0.5 m*M* of L-glutamine and 1% B-27 were added as supplements. After two days of culture, the samples were examined under a bright-field microscope (Olympus IX 71) at 10× magnification.

Scaffold-based assay. After washing NT-3 loaded scaffolds for three times in PBS, 4–6 DRG explants were seeded directly onto each scaffold ($n = 3$) in a 24-well plate. The DRGs were cultured in 1.0 mL of neurobasal medium supplemented with 0.5 m*M* L-glutamine, 1% B-27, and 1% penicillin-streptomycin. The positive control comprised of DRGs that were seeded on plain electrospun collagen scaffolds in the presence of 40 ng/mL of fresh NT-3. A negative control comprised of DRGs cultured on plain collagen scaffolds in the absence of NT-3. After 2 days of culture, all samples were fixed with 10% formalin and permeabilized with 0.1% Triton X-100 for 20 min. After thorough washes with PBS, the scaffolds were blocked with 3% BSA. Primary antibody against Tuj-1 (1:500) was added and incubated for 1 h. Thereafter, secondary antibody (Alexa Fluor 633, 1:500) was added and incubated for 30 min. All incubation was carried out at room temperature and all samples were washed three times with PBS in between each step. The samples were then imaged under a confocal microscope (Zeiss, LSM 710) and analyzed using the Image J software (NIH). The average length of each neurite extension was defined as the linear distance from the point of exit to the end of the longest branch of that neurite. Length measurements of 18 neurites were taken from each explant and 9 explants were measured for each group following previous protocol.[25]

ChABC sustained release and bioactivity assay

Collagen scaffolds (10 mg/scaffold, $n = 3$ for each group) were either soaked in ChABC only (denoted as Incorporated ChABC) or ChABC/heparin solution (denoted as Incorporated ChABC/heparin). The former comprised of scaffolds being incubated with 200 μL of ChABC solution (200 mU of ChABC in PBS) while the latter comprised of 200 μL of ChABC/heparin solution (200 mU of ChABC and 150 μg of heparin in PBS to result in a heparin: ChABC ratio of 60 μmol:1U, which was chosen based on previous study[26]). The mTG-mediated protein incorporation protocol described earlier was utilized. Thereafter, all scaffolds were washed with PBS for three times prior to incubation in 1 mL of PBS. As a control, 200 mU of fresh ChABC was added directly into 1 mL of PBS (denoted as Soluble ChABC). Finally, all samples were incubated at 37°C.

At various time points, 10 μL of supernatant was collected from each sample to react with 10 μL of decorin (0.5 mg/mL, a small molecular CSPG) at 37°C. The enzymatic digestion of decorin was then allowed to proceed for 2 h. A DMMB assay was then used to quantify the sulfated glycosaminoglycan (CS-GAGs) that have been digested by ChABC. According to previously reported protocol,[27] 180 μL of DMMB reagent was added into 20 μL of reaction sample and the absorbance was recorded at 530 nm. The absorbance of the ChABC supernatant without decorin was used as the background control. The absorbance of 5 μg of intact decorin was considered as standard, 100% intact decorin. Finally, the percentage of sample absorbance relative to the standard was calculated, and the graphs of the percentage of decorin digested by ChABC samples were plotted.

Statistic analysis

All data presented in this study are expressed as mean ± standard error of mean (SE). For the analyses of BSA-647 loading efficiency, one-way ANOVA was used. Independent-samples *t*-test was used for analyzing the difference of DRG neurite length in different groups.

RESULTS

Optimized parameters for mTG mediated immobilization

Figure 1 shows the changes in protein loading efficiency with respect to variations in the initial mass of protein used and the mTG incubation time. When 1 and 5 μg of BSA-647

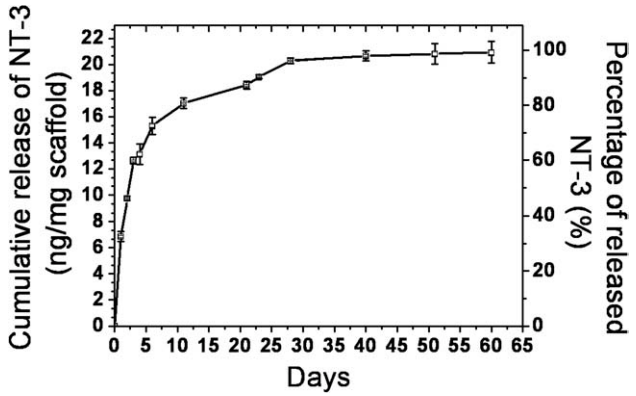

FIGURE 2. Cumulative release profile of NT-3 from electrospun collagen nanofiber scaffolds.

were used, the loading efficiencies were 48.4 ± 0.47% and 45.32 ± 2.27%, respectively with no significant difference [Fig. 1(a)]. However, a significant decrease in loading efficiency was observed when 20 μg of proteins were used. In the case of mTG incubation time, no significant difference in protein loading efficiency was observed [Fig. 1(b)]. Taken together, 5 μg of initial protein mass and 2 h of incubation in mTG were taken as the optimal parameters for subsequent NT-3 and ChABC incorporation studies.

NT-3 *in vitro* release kinetics

As shown in Figure 2, after an initial burst release of 35.9 ± 1.7% in day 1, a sustained release of NT-3 was obtained for up to 28 days. A total of 72.5 ± 3.1% of NT-3 was released during the first 6 days. Thereafter, the rate of protein release decreased. At day 60, a total of 99.2 ± 3.91% NT-3 was released relative to the total amount of NT-3 incorporated.

NT-3 bioactivity assay

Supernatant assay. As shown in Figure 3, significant neurite outgrowth was observed in the positive control and from DRGs that were exposed to day 4 supernatant, indicating the retention of NT-3 bioactivity. In contrast, neurite outgrowth was very limited in the negative control. Comparatively, DRGs exposed to NT-3 supernatant possessed neurites that extended over shorter distances than the positive control [Fig. 3(d), $p < 0.01$]. DRGs exposed to supernatants collected after day 4 did not show obvious neurite outgrowth.

Scaffold-based assay. As indicated in Figure 4(a,b), DRG neurites emanated radially on NT-3 loaded collagen scaffolds and the positive control. Quantitative measurements showed that the lengths of the neurites were comparable in both samples [Fig. 4(d)]. In contrast, no neurite outgrowth was observed in the negative control [Fig. 4(c)]. At day 4, neurite extensions remained prominent in DRGs that were seeded on NT-3 loaded collagen scaffolds. However, such neurite extension was absent in positive control [Fig. 4(d,e)].

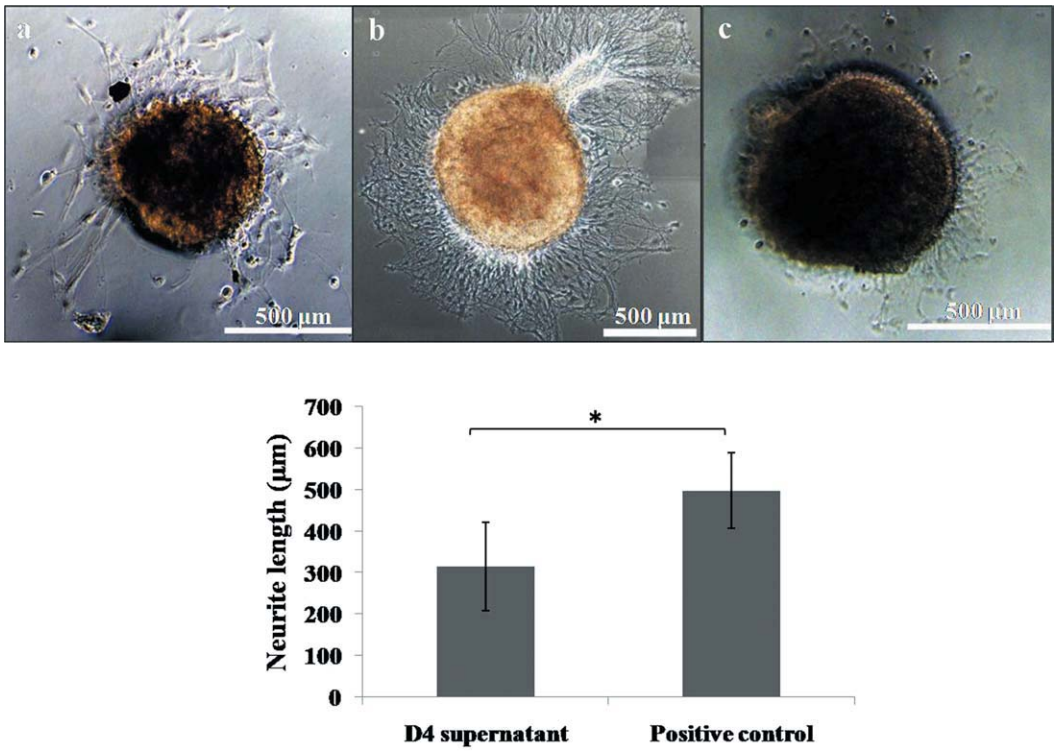

FIGURE 3. Supernatant bioactivity assay for NT-3. (a–c) Light microscopy images of DRGs cultured in (a) day 4 supernatant; (b) positive control comprising of 40 ng/mL NT-3; (c) negative control; and (d) Length of neurite extensions from DRGs cultured in day 4 supernatant and positive control. *$p < 0.01$, independent *t*-test. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

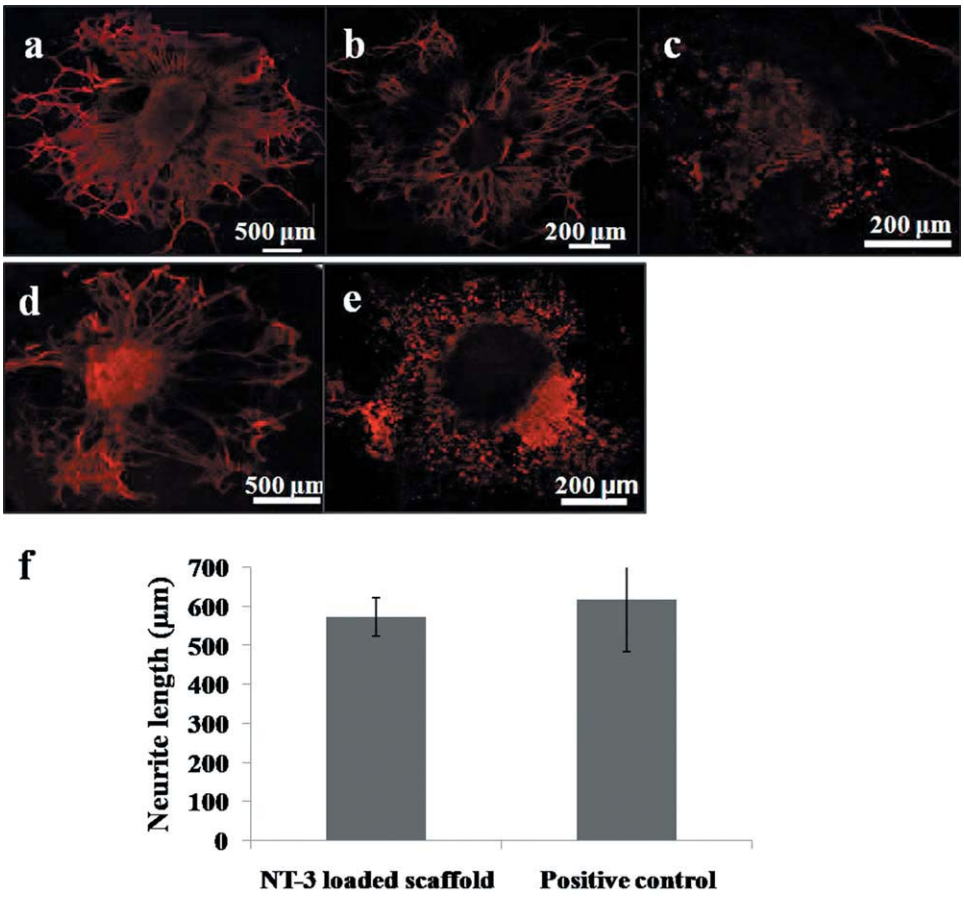

FIGURE 4. Scaffold-based bioactivity assay for NT-3. (a–e) Tuj-1 immunostaining of DRGs cultured on (a–d) NT-3 incorporated collagen scaffolds; (b–e) positive control; (c) negative control for (a–c) 2 days and (d and e) 4 days. (f) Length of neurite extensions from DRGs. Positive control: DRGs on plain collagen scaffolds subjected to bolus delivery of 40 ng/mL of NT-3. Negative control: DRGs on plain collagen scaffolds only. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ChABC bioactivity assay

As shown in Figure 5, sustained release of bioactive ChABC with a gradual decrease in enzymatic activity was obtained for at least 32 days in Incorporated ChABC/heparin samples. At day 32, the enzymatic activity of ChABC was 32.34 ± 2.61% as compared day 1 (66.5 ± 0.91%). In comparison, the enzymatic activities were 1.92 ± 0.95% and 3.5 ± 3.85% for Incorporated ChABC and Soluble ChABC groups by day 22 and 15, respectively.

DISCUSSION

The rapid clearance of drugs from the spinal cord remains a major hurdle to achieving effective therapeutic outcomes in SCI treatment.[14] As a result, various drug-delivery systems, particularly hydrogels, have been developed to facilitate the sustained and localized delivery of biomolecules such as NT-3 and ChABC.[14,24,28–30] Unfortunately, most of these platforms are isotropic and lack nanoscaled topographical cues[14,24,26] that may be helpful in manipulating the inhibitory microenvironment postSCI and directing regenerated axons towards their appropriate targets. Our previous results demonstrated the potential of plain electrospun collagen nanofibers for SCI treatment.[17] Therefore, in this study, we chose to enhance the functionality of these constructs by protein incorporation to provide synergistic topographical and biochemical cues to enhance nerve regeneration in the CNS.

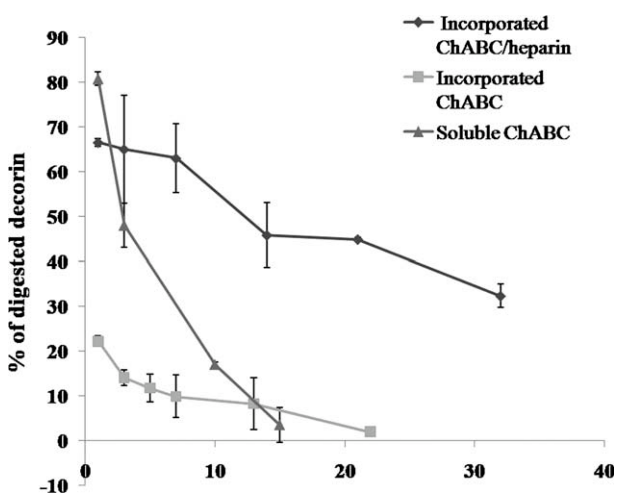

FIGURE 5. ChABC bioactivity assay. Percentage of decorin digested by ChABC supernatants.

Transglutaminases (TGase) are a group of structurally and functionally related enzymes that are distributed in various organisms.[31,32] TGase mediated crosslinking is nontoxic, and is widely adopted in commercial food processes to improve the texture of protein-rich products.[33,34] Gelatin and collagen are good substrates of TGase and mTG has been used to modify the physical properties of these materials.[35,36] Theoretically, proteins can be covalently immobilized onto collagen via mTG mediated acyl-transfer reaction that occurs between the γ-carboxamide groups of glutamine residues in collagen and the ε-amino groups of lysine residues in proteins at temperatures $\geq$37°C.[37]

The protein-incorporation optimization study indicated a decrease in loading efficiency when 20 μg of protein was used. This is likely due to the saturation of available binding sites on collagen. When mTG crosslinking duration was altered, the insignificant change in loading efficiency was likely attributed to the rapid mTG crosslinking kinetics. The fast rate of collagen-mTG crosslinking, occurring within 10 min, has been reported.[37] Therefore, in order to ensure that sufficient proteins were incorporated within reasonable loading efficiency limits, 5 μg of NT-3 and ChABC with a mTG incubation period of 2 h were chosen as the optimal parameters.

As indicated in the release kinetics study, ~72% of NT-3 was released within the first week. Thereafter, the release rate decreased and resulted in ~96% NT-3 release by day 28. Such a release profile is likely attributed to the degradation of the collagen scaffolds since previous *in vitro* studies indicated ~50% and 70% scaffold mass loss by days 7 and 15, respectively.[18] The nonspecific adsorption and crosslinking of heparin and NT-3 within our system may be another contributing factor. Covalently immobilizing heparin onto the nanofibers prior to absorbing NT-3 might provide better control over protein release rate[23] and will be explored in future studies.

Compared to heparin-incorporated hydrogels (~60% NT-3 released by day 14 with 800:1 heparin to NT-3 ratio[13,24]), our collagen scaffolds enabled a quicker and more efficient release of NT-3. Although, an initial burst availability of NT-3 may help reduce the apoptosis of neurons post-SCI and create a chemoattractive environment to guide axons towards the scaffolds,[24] the exact physiological implications of our constructs versus the current hydrogel-based platforms awaits further verification with detailed *in vivo* studies.

Corresponding to the drop in protein release rate after the first week, we observed a lack of neurite outgrowth in DRGs that were subjected to supernatants beyond day 4. Neurite length may be dependent on the dose of NT-3 when the dosage is low (~0–20 ng/mL).[24,25,38] However, at higher concentrations (>~20–50 ng/mL), neurite length appears to be independent of NT-3 concentration.[24,25] Correspondingly, we saw similar outcomes in our bioactivity assays. On the basis of ELISA assay, ~20 ng/mL of NT-3 remained in day 4 supernatant. Compared to the positive control of 40 ng/mL, we saw a significant decrease in neurite outgrowth length. In the scaffold-based assay, we estimated a total of ~97.2 ng of NT-3 being released from the scaffolds at day 2. This resulted in the insignificant difference in neurite length between the samples and the positive control. One possible reason may be the down-regulation of neurotrophin receptors on DRG neurons.[39,40] Regardless, both assays clearly demonstrated the retention of NT-3 bioactivity within the nanofiber constructs. Furthermore, NT-3-incorporated collagen scaffolds supported neurite outgrowth for at least 4 days. In contrast, neurite extension was not observed in DRGs in the positive control by the fourth day of culture. Although, we were unable to prolong DRG culture beyond 4 days (likely due to limited nutrient diffusion under static culture), our results suggested that the collagen-based drug release system protected and presented NT-3 in a sustained manner to support neuronal culture and neurite extensions for a longer time period as compared to a bolus delivery of NT-3.

Bioactive ChABC was released in a sustained manner from collagen nanofibers for at least 32 days. This appears to match the *in vivo* CSPG expression kinetics, where a significant increase followed by a peak at 2–4 weeks after SCI is frequently observed.[41,42] Comparing with currently available hydrogel platforms, the duration of ChABC release from collagen nanofibers appears similar (hydrogels provided 4 weeks sustained release *in vitro*[14]). In the soluble ChABC group, ChABC showed the highest bioactivity at day 1 because of the existence of higher amounts of soluble ChABC. However, such advantage was quickly nullified by day 2. Although, heparin was initially added to protect the bioactivity of NT-3,[24] we found that heparin was also able to prevent the degradation of ChABC. Further studies to evaluate the efficacy of these drug incorporated collagen scaffolds in providing synergistic nanofiber topography and multiple biochemical cues to enhance nerve regeneration in SCI treatment will be evaluated by detailed *in vivo* studies.

CONCLUSION

In this study, we demonstrated the feasibility of incorporating proteins onto electrospun collagen nanofiber scaffolds. While the electrospinning of collagen has been reported extensively, endowing such scaffolds with drugs for control over cell fate remains unexplored. Our results demonstrate the sustained release of NT-3 and ChABC for at least 28 days. Such biofunctional scaffolds may find useful applications in SCI treatment by providing topographical and multiple biochemical cues to manipulate the growth inhibitory environment and promote axonal regeneration.

REFERENCES

1. Fawcett JW, Asher RA. The glial scar and central nervous system repair. Brain Res Bull 1999;49:377–391.
2. Asher RA, Morgenstern DA, Fidler PS, Adcock KH, Oohira A, Braistead JE, Levine JM, Margolis RU, Rogers JH, Fawcett JW. Neurocan is upregulated in injured brain and in cytokine-treated astrocytes. J Neusci 2000;20:2427–2438.
3. McKeon RJ, Hoke A, Silver J. Injury-induced proteoglycans inhibit the potential for laminin-mediated axon growth on astrocytic scars. Exp Neurol 1995;136:32–43.

4. Smith GM, Strunz C. Growth factor and cytokine regulation of chondroitin sulfate proteoglycans by astrocytes. Glia 2005;52: 209–218.
5. Barritt AW, Davies M, Marchand F, Hartley R, Grist J, Yip P, McMahon SB, Bradbury EJ. Chondroitinase ABC promotes sprouting of intact and injured spinal systems after spinal cord injury. J Neusci 2006;26:10856–10867.
6. Garcia-Alias G, Barkhuysen S, Buckle M, Fawcett JW. Chondroitinase ABC treatment opens a window of opportunity for task-specific rehabilitation. Nat Neurosci 2009;12:1145–1151.
7. Caggiano AO, Zimber MP, Ganguly A, Blight AR, Gruskin EA. Chondroitinase ABCI improves locomotion and bladder function following contusion injury of the rat spinal cord. J Neurotraum 2005;22:226–239.
8. Houle JD, Tom VJ, Mayes D, Wagoner G, Phillips N, Silver J. Combining an autologous peripheral nervous system "bridge" and matrix modification by chondroitinase allows robust, functional regeneration beyond a hemisection lesion of the adult rat spinal cord. J Neurosci 2006;26:7405–7415.
9. Tester NJ, Plaas AH, Howland DR. Effect of body temperature on chondroitinase ABC's ability to cleave chondroitin sulfate glycosaminoglycans. J Neurosci Res 2007;85:1110–1118.
10. Giehl KM, Tetzlaff W. BDNF and NT-3, but not NGF, prevent axotomy-induced death of rat corticospinal neurons in vivo. Eur J Neurosci 1996;8:1167–1175.
11. Ramer MS, Bishop T, Dockery P, Mobarak MS, O'Leary D, Fraher JP, Priestley JV, McMahon SB. Neurotrophin-3-mediated regeneration and recovery of proprioception following dorsal rhizotomy. Mol Cell Neurosci 2002;19:239–249.
12. Coumans JV, Lin TTS, Hai Ning D, MacArthur L, McAtee M, Nash C, Bregman BS. Axonal regeneration and functional recovery after complete spinal cord transection in rats by delayed treatment with transplants and neurotrophins. J Neurosci 2001;21:9334–9344.
13. Taylor SJ, Sakiyama-Elbert SE. Effect of controlled delivery of neurotrophin-3 from fibrin on spinal cord injury in a long term model. J Control Release 2006;116:204–210.
14. Lee H, McKeon RJ, Bellamkonda RV. Sustained delivery of thermostabilized ChABC enhances axonal sprouting and functional recovery after spinal cord injury. Proc Natl Acad Sci U S A 2010; 107:3340–3345.
15. Chew SY, Mi R, Hoke A, Leong KW. The effect of the alignment of electrospun fibrous scaffolds on Schwann cell maturation. Biomaterials 2008;29:653–661.
16. Chew SY, Mi R, Hoke A, Leong KW. Aligned protein–polymer composite fibers enhance nerve regeneration: a potential tissue-engineering platform. Adv Funct Mater 2007;17:1288–1296.
17. Liu T, Xu J, Chan BP, Chew SY. Nanofibrous collagen nerve conduits for spinal cord repair. Tissue Eng. 2011. Submitted for publication.
18. Liu T, Teng WK, Chan BP, Chew SY. Photochemical crosslinked electrospun collagen nanofibers: Synthesis, characterization and neural stem cell interactions. J Biome Mate Res A 2010;95A: 276–282.
19. Jha BS, Ayres CE, Bowman JR, Telemeco TA, Sell SA, Bowlin GL, Simpson DG. Electrospun collagen: A tissue engineering scaffold with unique functional properties in a wide variety of applications. J Nanomate. 2011.
20. Zhong S, Teo WE, Zhu X, Beuerman RW, Ramakrishna S, Yung LYL. An aligned nanofibrous collagen scaffold by electrospinning and its effects on in vitro fibroblast culture. J Biomed Mate Res A 2006;79A:456–463.
21. Rajan N, Habermehl J, Cote M-F, Doillon CJ and Mantovani D. Preparation of ready-to-use, storable and reconstituted type I collagen from rat tail tendon for tissue engineering applications. Nat Protocols 2007;1:2753–2758.
22. Noushi F, Richardson RT, Hardman J, Clark G, O'Leary S. Delivery of neurotrophin-3 to the cochlea using alginate beads. Oto Neurotol 2005;26:528–533.
23. Wissink MJB, Beernink R, Poot AA, Engbers GHM, Beugeling T, van Aken WG, Feijen J. Improved endothelialization of vascular grafts by local release of growth factor from heparinized collagen matrices. J Control Release 2000;64:103–114.
24. Taylor SJ, McDonald JW, Sakiyama-Elbert SE. Controlled release of neurotrophin-3 from fibrin gels for spinal cord injury. J Control Release 2004;98:281–294.
25. Richardson RT, Thompson B, Moulton S, Newbold C, Lum MG, Cameron A, Wallace G, Kapsa R, Clark G, O'Leary S. The effect of polypyrrole with incorporated neurotrophin-3 on the promotion of neurite outgrowth from auditory neurons. Biomaterials 2007; 28:513–523.
26. Hyatt AJT, Wang D, Kwok JC, Fawcett JW, Martin KR. Controlled release of chondroitinase ABC from fibrin gel reduces the level of inhibitory glycosaminoglycan chains in lesioned spinal cord. J Controll Release 2010;147:24–29.
27. Cope AP, Mort JS, Roughley PJ. Measurement of glycosaminoglycan release from cartilage explants. Arthritis Research. New Jersey: Humana Press; 2007. p 201–209.
28. Massey JM, Amps J, Viapiano MS, Matthews RT, Wagoner MR, Whitaker CM, Alilain W, Yonkof AL, Khalyfa A, Cooper NGF, Silver J, Onifer SM. Increased chondroitin sulfate proteoglycan expression in denervated brainstem targets following spinal cord injury creates a barrier to axonal regeneration overcome by chondroitinase ABC and neurotrophin-3. Expe Neurol 2008;209:426–445.
29. Lee H, McKeon RJ, Bellamkonda RV. Sustained delivery of thermostabilized chABC enhances axonal sprouting and functional recovery after spinal cord injury. Proc Natl Acad Sci U S A 2010; 107:3340–3345.
30. Hyatt AJT, Wang D, Kwok JC, Fawcett JW, Martin KR. Controlled release of chondroitinase ABC from fibrin gel reduces the level of inhibitory glycosaminoglycan chains in lesioned spinal cord. J Controll Release 2010;147:24–29.
31. Mauro P, Grazia Farrace M, Piredda L, Matarrese P, Ciccosanti F, Falasca L, Rodolfo C, Giammarioli AM, Verderio E, Griffin M, Malorni W. Transglutaminase overexpression sensitizes neuronal cell lines to apoptosis by increasing mitochondrial membrane potential and cellular oxidative stress. J Neurochem 2002;81: 1061–1072.
32. Griffin M, Casadio R, Bergamini CM. Transglutaminases: Nature's biological glues. J Biochem 2002;368:377–396.
33. Jaros D, Partschefeld C, Henle T, Rohm H. Transglutaminase in dairy products: Chemistry, physics, applications. J Texture Stud 2006;37:113–155.
34. Cortez J, Anghieri A, Bonner PLR, Griffin M, Freddi G. Transglutaminase mediated grafting of silk proteins onto wool fabrics leading to improved physical and mechanical properties. Enzyme Microb Tech 2007;40:1698–1704.
35. Chau DYS, Collighan RJ, Verderio EAM, Addy VL, Griffin M. The cellular response to transglutaminase-cross-linked collagen. Biomaterials 2005;26:6518–6529.
36. Paguirigan AL, Beebe DJ. Protocol for the fabrication of enzymatically crosslinked gelatin microchannels for microfluidic cell culture. Nat Protocols 2007;2:1782–1788.
37. Ines Stachel US, Henle T, Meyer M. Cross-linking of type I collagen with microbial transglutaminase: Identification of cross-linking sites. Biomacromolecules 2010;11:698–705.
38. Morfini G, Ditella MC, Feiguin F, Carri N, Cáceres A. Neurotrophin-3 enhances neurite outgrowth in cultured hippocampal pyramidal neurons. J Neurosci Res 1994;39:219–232.
39. Zigmond SH. Consequences of chemotactic peptide receptor modulation for leukocyte orientation. J Cell Biol 1981;88:644–647.
40. Cao X, Shoichet MS. Investigating the synergistic effect of combined neurotrophic factor concentration gradients to guide axonal growth. Neurosci 2003;122:381–389.
41. Jones LL, Margolis RU, Tuszynski MH. The chondroitin sulfate proteoglycans neurocan, brevican, phosphacan, and versican are differentially regulated following spinal cord injury. Exp Neurol 2003;182:399–411.
42. Iseda T, Okuda T, Kane-Goldsmith N, Mathew M, Ahmed S, Chang Y-W, Young W, Grumet M. Single, high-dose intraspinal injection of chondroitinase reduces glycosaminoglycans in injured spinal cord and promotes corticospinal axonal regrowth after hemisection but not contusion. J Neurotraum 2008;25: 334–349.