Carbohydrate Polymers 84 (2011) 788–793

Contents lists available at ScienceDirect

Carbohydrate Polymers

journal homepage: www.elsevier.com/locate/carbpol

Controlled release of chondroitinase ABC in chitosan-based scaffolds and PDLLA microspheres

Yi-Cheng Huang[a], Sung-Hao Hsu[c], Meng-Tze Chen[b], Chin-Hsiung Hsieh[b], Wen-Chun Kuo[d], Henrich Cheng[d,e], Yi-You Huang[b,*]

[a] College of Life Sciences, Department of Food Science, National Taiwan Ocean University, Keelung City, 202, Taiwan
[b] Institute of Biomedical Engineering, College of Engineering, College of Medicine, National Taiwan University, Taipei, 100, Taiwan
[c] Institute of Zoology, College of Life Science, National Taiwan University, Taipei, 100, Taiwan
[d] Division of Neural Regeneration, Department of Neurosurgery, Taipei Veterans General Hospital, Taipei, Taiwan
[e] Department and Institute of Pharmacology, National Yang-Ming University, Taipei, Taiwan

ARTICLE INFO

Article history:
Received 27 September 2009
Received in revised form 10 February 2010
Accepted 19 March 2010
Available online 2 April 2010

Keywords:
Spinal cord injury
Chondroitin sulfate proteoglycans
Chondroitinase ABC
Chitosan
Nerve conduits
Poly(D,L-lactic acid)
Microspheres

ABSTRACT

Spinal cord injury (SCI) can trigger inhibitory signal cascades that promote the expression of chondroitin sulfate proteoglycans (CSPGs), which are the main structures in scar tissue. The digestion of CSPGs by chondroitinase ABC (ChABC) can promote axonal re-growth after SCI. However, ChABC cannot effectively digest CSPGs because it is unstable; therefore, stable ChABC must be released in a controlled manner in the repair of SCIs. Two methods of maintaining ChABC stability and bioactivity were examined. They were the immobilization of ChABC on nerve conduits (NCs) and the encapsulation of ChABC in poly(D,L-lactic acid) (PDLLA) microspheres.

Nerve conduits with variously sized pores were fabricated from chitosan and gelatin. The pore diameters of chitosan NCs were 100–160 μm, and those of chitosan/gelatin NCs were 20–40 μm. The ChABC in NCs was immobilized by ionic or covalent bonding. The experimental results reveal that immobilizing ChABC in NCs markedly improved its stability. The activity of ChABC that was immobilized in chitosan NCs by ionic bonding was 0.07 U/mg; 48% of this activity was retained at 48 h after immobilization. PDLLA microspheres, fabricated by the double emulsion method, were applied as carriers in the controlled release of ChABC. Stabilizers, including nanogold (10 nm), polylysine (Mw: 500–2000) and polylysine (Mw: 20,000–30,000), were added to microspheres to maintain the activity of ChABC. Polylysine (Mw: 500–2000) stabilized ChABC most effectively. The ChABC activity was 0.0162 U/ml after seven days of release. Experimental results indicate that ChABC activity can be preserved during release by immobilizing ChABC in chitosan NCs and encapsulating ChABC in PDLLA microspheres using an appropriate stabilizer.

1. Introduction

Injury to a spinal cord can induce inhibitory signal cascades that promote the formation of scar tissue. Such scars comprise a dense collagen IV meshwork, which functions as a binding matrix for numerous extracellular matrix components, inhibitory molecules, including proteoglycans and semaphorins, and growth-promoting factors (Klapka & Muller, 2006). Chondroitin sulfate proteoglycans (CSPGs), the principal inhibitors of neurite outgrowth, are up-regulated by spinal cord injury (SCI) (Busch & Silver, 2007). The CSPGs consist of a core protein with several covalently linked glycosaminoglycan (GAG) side chains. The GAGs is a key limited factor to block axon growth. Additionally, chondroitinase ABC (ChABC) digests GAG side chains, promoting functional recovery following SCI (Bradbury et al., 2002; Huang et al., 2006). However, a significant challenge to be met is that ChABC is too unstable to maintain its activity *in vivo* when ChABC is employed in SCI.

In 2002, Bradbury et al. injected Wistar rats with ChABC following SCI (Bradbury et al., 2002). In their experiments, a silastic tube was inserted intrathecally, such that it was just rostral to the lesion site, and externalized to deliver bolus injections of highly pure, protease-free ChABC. However, ChABC must be injected every other day because it is unstable. Such treatment is both inconvenient and painful. To control the continuous release of ChABC, Ikegami et al. utilized an osmotic pump for ChABC infusion to digest the CSPG in the injured spinal cord at one to two weeks after SCI (Ikegami et al., 2005). Huang et al. inserted a catheter for ChABC infusion following T8 completely spinal cord transaction (Huang et al., 2006). This

* Corresponding author at: Institute of Biomedical Engineering, College of Medicine, National Taiwan University, No. 1, Sec. 1, Jen-Ai Road, Taipei, Taiwan. Tel.: +886 2 23222499; fax: +886 2 23940049.
E-mail addresses: yyhuang@ha.mc.ntu.edu.tw, yyhuang@ntu.edu.tw (Y.-Y. Huang).

0144-8617/$ – see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.carbpol.2010.03.034

study aims to immobilize ChABC on NCs and encapsulate ChABC in microspheres to control the release of ChABC.

Chitosan and PDLLA were adopted to fabricate NCs and microspheres because of their biocompatibility and biodegradability (Huang, Khor, & Lim, 2004; Tsuji, 2005). Furthermore, chitosan has excellent nerve repair potential (Gong et al., 2000). A porous chitosan NC was previously formed by a lyophilizing and wire-heating process (Huang, Huang, Huang, & Liu, 2005). PDLLA has been fabricated in various forms for biomedical applications. For example, electrospun fiber mats are used as carriers for topical and/or transdermal delivery, and nanocomposites are used to release drugs (Chuysinuan, Chimnoi, Techasakul, & Supaphol, 2009; Dagnon et al., 2009). In this work, chitosan NCs and PDLLA microspheres are applied as carriers to release ChABC over a defined period to promote nerve regeneration. The effects of stabilizers, including nanogold and polylysine with various molecular weights, were also examined.

2. Materials and methods

All reagents were purchased from Sigma Aldrich (Oakville, ON, CA), unless otherwise stated. Poly(D,L-lactic acid) (PDLLA) was purchased from Bio Invigor (Taiwan). Poly (vinyl alcohol) (PVA) that was 80% hydrolyzed with a molecular weight of 6000 D was purchased from Wako (Japan). The MicroBCA Protein Assay Kit was purchased from Pierce (Rockford, IL, USA). Deionized water was obtained from Milli-RO 10 Plus and Milli-Q UF Plus (Bedford, MA, USA) water purification units at 18 MV resistance.

2.1. Preparation of chitosan or chitosan/gelatin scaffold

For preparing chitosan scaffold, the chitosan (DDA: 82.5 ± 1.15%, Mw 645,000)/acetic acid solution (2%, w/v) was injected into a mold and then frozen at −20, for 24 h. The acetic acid was then sublimated using temperature-controlled lyophilizers (VirTis, NY, USA). After lyophilization, the scaffolds were washed by 1:1 0.1 M NaOH–MeOH and 1:1 MeOH–water in order to neutralize the acid, and then dried again by lyophilizers. For preparing chitosan/gelatin scaffold, the chitosan scaffold already fabricated was soaked in gelatin solution (5%) for 1 min, cross-linked by glutaraldehyde (1%) for 30 s and then dried again by lyophilizers. Finally, the scaffolds were stored in a dry environment until use.

2.2. Immobilization of ChABC on chitosan-based scaffolds

The ChABC in the scaffolds were immobilized using two methods. The first is as follows. Prior to immobilization, the chitosan or chitosan/gelatin scaffold was washed with ChABC buffer solution (250 mM Tris HCl and 300 mM sodium acetate, at pH 8.0). Then 1 mg of washed scaffold was soaked in 100 μl ChABC solution (10 U/ml) at 4 °C overnight in a closed container to prevent evaporation. The scaffold was then washed again using ChABC buffer solution to remove un-immobilized ChABC. Next, 0.2 ml ChABC buffer solution that contained glutaraldehyde (GTA) (0.1–0.2%) was added to the scaffold at 4 °C for one hour, and the excess GTA was then removed.

In the second method, 1 ml 1%(v/v) GTA solution was added to 1 mg scaffold for three hours at room temperature, and then 100 μl ChABC (10 U/ml) was added. Changing the order in which these two chemicals, ChABC and GTA are added, changes the chemical bonds.

2.3. Preparation of microspheres

The PDLLA microspheres were prepared by standard double emulsion/solvent evaporation. Briefly, 0.1 g PDLLA was dissolved in 2 ml dichloromethane (CH_2Cl_2), and 200 μl of aqueous bovine serum albumin (BSA) or ChABC (20 U/ml) was then added. The mixture was stirred for 1.0 min at 4000 rpm and then added to 20 ml of a previously prepared aqueous 1 wt.% PVA solution, which was stirred for another 1 min at 2000 rpm. Finally, the entire volume was poured into a flask with ice and stirred for three hours (Caframo Stirrer Type RZR, speed setting 3.0) to evaporate CH_2Cl_2. Following all of the solvent had evaporated, the microspheres were washed three times with centrifugation (12,000 rpm) in distilled water. After the final centrifugation, the wet microspheres were freeze-dried and stored in a dry environment under aseptic conditions prior to use.

To fabricate microspheres that contained stabilizers, including nanogold (10 nm) and polylysine (Mw: 500–2000 or 20,000–30,000), to maintain ChABC activity, a ChABC/stabilizer solution was used instead of ChABC solution alone in the preparation process. In total, 10 U ChABC was added to 200 μl polylysine (5 mg/ml) or nanogold to prepare the ChABC/stabilizer solution.

2.4. Bioactivity of ChABC

The bioactivity of ChABC that was released from chitosan-based scaffolds or PDLLA microspheres was evaluated *in vitro* by determining its capacity to digest chondroitine sulfate (CS) to produce unsaturated disaccharide. The amount of unsaturated disaccharide was determined by ultraviolet (UV) absorbance at 232 nm using a Hitachi U-3010 UV–Vis photometer using a calibration curve for describing the absorbance–concentration relationship (Pojasek, Shriver, Kiley, Venkataraman, & Sasisekharan, 2001). The ChABC bioactivity was calculated as

$$\text{Units/ml} = \frac{(\Delta A_{232\,\text{nm}}/\text{ min test})(1.0)(\text{df})}{(E_{\text{mM}})(0.10)(0.8)}$$

df: factor of dilution; E_{mM}: mini-molar absorption coefficient (Ernst et al., 1996; Pojasek, Shriver, Hu, & Sasisekharan, 2000); $\Delta A_{232\,\text{nm}}$/min test: (absorption difference at 232 nm after reaction)/reaction time.

2.5. Determination of amount of ChABC released in vitro

The efficiency of the release of ChABC was determined from its bioactivity. The chitosan-based scaffold (0.001 g) was put into buffer solution containing CS (2%, 500 μl) for 5 min at 37 °C. Then, 100 μl buffer was mixed with 900 μl KCl (pH 1.8) solution at 90 °C to terminate the reaction. The bioactivity of ChABC was calculated using the absorption of supernatants at 232 nm. To release ChABC from PDLLA microspheres, 1.00 ml Tris–HCl sodium acetate solution was added to 0.05 g ChABC/PDLLA microspheres. At various times, 80 μl supernatant was mixed with 20 μl CS solution at 37 °C for 5 min; the reaction was then stopped at 90 °C. The absorption of the supernatant was quantified using ultraviolet (UV) absorbance at 232 nm.

The efficiency of release from PDLLA microspheres *in vitro* was determined using BSA instead of ChABC. The experimental processes are the same as those described above, although the detection method is different. The amount of BSA that was released in the supernatant was determined using BCA protein assay kits.

3. Results

3.1. Chitosan-based scaffolds

Fig. 1 presents the morphological changes of the chitosan scaffold when NaOH/H_2O or NaOH/methanol was used as a neutralizer. The scaffold that was neutralized by NaOH/H_2O shrank more by volume (50%) than did that neutralized by NaOH/methanol (30%). A comparison of scaffolds reveals that the pore diameter was greater in the NaOH/methanol group. These experimental results

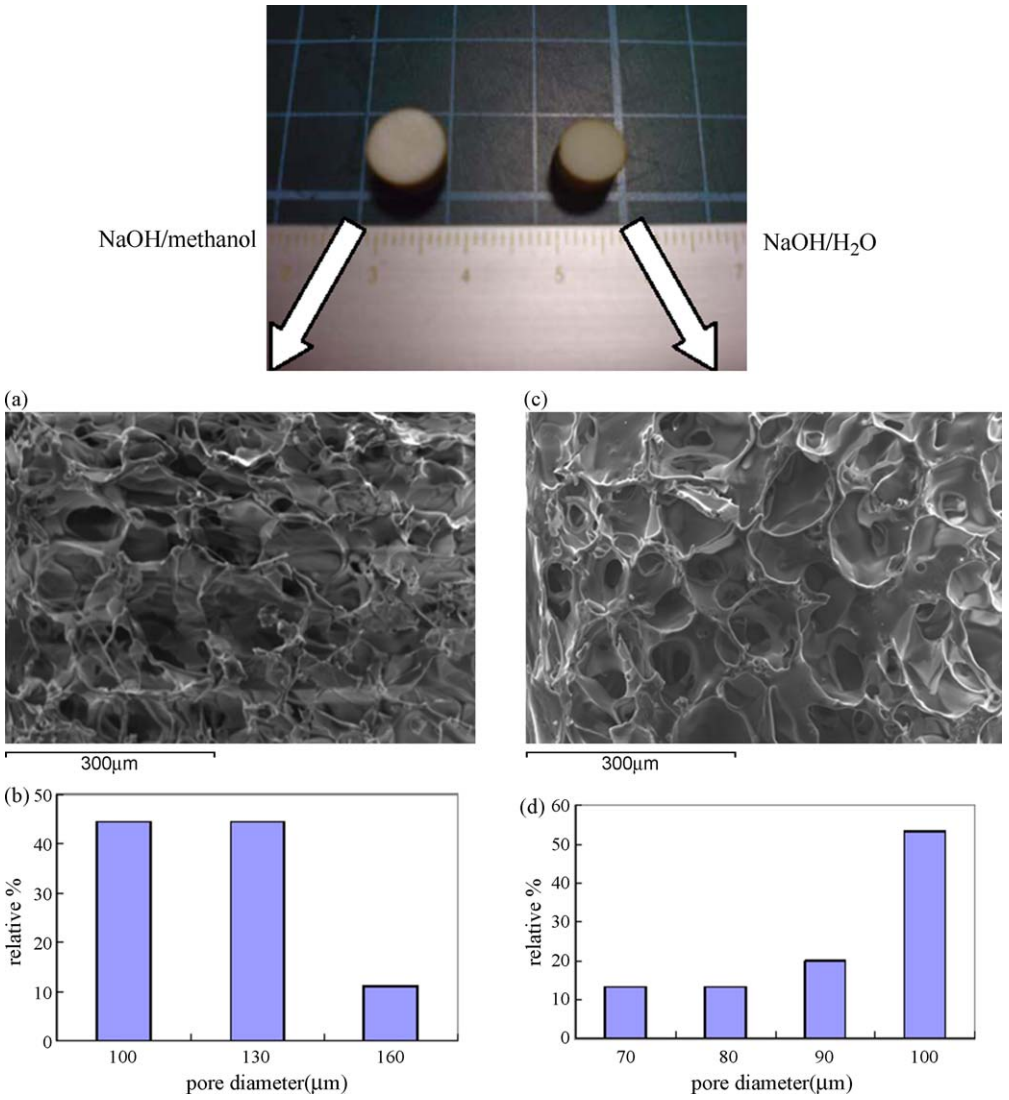

Fig. 1. Chitosan based scaffold was neutralized by (a,b) NaOH/methanol or (c,d) NaOH/H_2O. (a,c) SEM 200× (b,d) pore diameter distribution.

may follow from the fact that acetic acid remains in the chitosan scaffolds after lyophilization. Unlike H_2O, the used methanol can be esterified with acetic acid to reduce the acidity of the environment. Hence, the use of NaOH/methanol as the neutralizer prevents severe shrinkage.

Earlier work established that NCs with small pores are very effective for repairing nerve injuries (Vleggeert-Lankamp et al., 2007). Therefore, in this study two parameters, the concentrations of the chitosan solution with and without gelatin, were adjusted to optimize the pore sizes in NCs. Fig. 2 presents the experimental results. Pore sizes were reduced to 20–40 μm by adding gelatin. Although the pore size also declined as the concentration of the chitosan solution increased (data not shown), the chitosan solubility decreased markedly. Accordingly, in this investigation, NCs with small pores were fabricated by mixing 2% chitosan and 5% gelatin solutions.

3.2. Immobilization of enzymes in chitosan-based scaffolds

Fig. 3 displays the experimental results concerning the immobilization of ChABC in chitosan and chitosan/gelatin scaffolds. The approaches that were applied to immobilize ChABC fall into two groups—scaffold/ChABC/GTA and scaffold/GTA/ChABC. In the scaffold/ChABC/GTA group, ChABC is firstly added to the scaffold, and then cross-linking is performed using GTA; in the scaffold/GTA/ChABC group, the scaffold is cross-linked by GTA before ChABC is added. Experimental findings suggest that the activity of ChABC in the chitosan/gelatin scaffold was significantly lower than that in the chitosan scaffold, in both groups. A reasonable interpretation is that the smaller pores of the chitosan/gelatin scaffold (20–40 μm) are associated with lower ChABC fluidity. Therefore, the fall in the ChABC activity may have been caused by the absorption of a small percentage of the ChABC by the chitosan/gelatin scaffold. Furthermore, a comparison of the two groups indicates that enzyme activity was significantly higher in the scaffold/ChABC/GTA group—especially in the chitosan scaffold. This analytical result can be explained by considering the interactions that occur during ChABC immobilization. The interaction associated with the scaffold/ChABC/GTA group is ionic because of the polyelectrolyte complexation between negatively charged ChABC and positively charged chitosan before cross-linking by GTA. The bonding for the scaffold/GTA/ChABC group is covalent because of the cross-linking between the aldehyde groups of GTA and amino groups of chitosan and ChABC. The formation of amide between GTA and the competitive reactants, chitosan and ChABC, reduced the amount of ChABC that was immobilized in the scaffold.

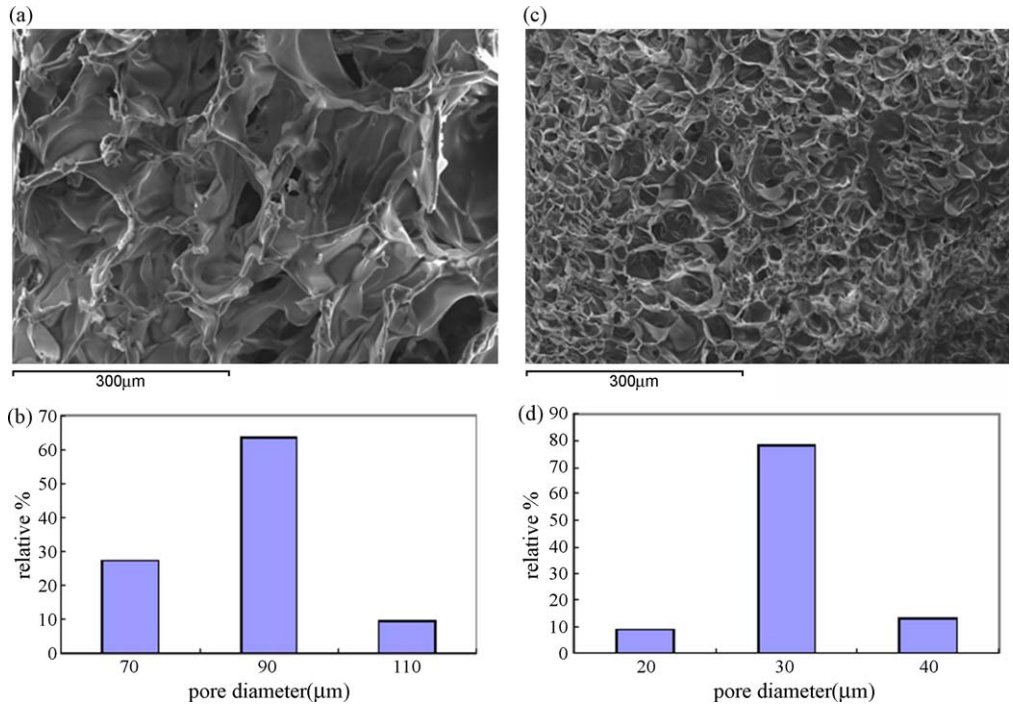

Fig. 2. The scaffold was made by (a,b) 4% chitosan or (b,d) 2% chitosan and 5% gelatin. (a,c) SEM 200× (b,d) pore diameter distribution.

3.3. PDLLA microspheres

The PDLLA microspheres (Fig. 4) that were prepared using the double emulsion/solvent evaporation technique were adopted as a model to evaluate the efficiency of the controlled release of ChABC. The measurements concerning the release of BSA (model drug) are presented as a cumulative molar percentage of factor released over time (Fig. 5). The BSA in microspheres was released continuously over 14 days. To maintain the bioactivity of ChABC that was encapsulated in the microspheres, various stabilizers, including nanogold (10 nm), polylysine (Mw: 500–2000) and polylysine (Mw: 20,000–300,000), were added to determine the release profiles. The cumulative release of ChABC from microspheres was assessed by detecting the absorption of UV by unsaturated disaccharides that were digested by ChABC (Fig. 6). Without a stabilizer, the activity of ChABC was 0.0026 U/ml two hours after release. ChABC activity declined over the study period. Conversely, adding stabilizers preserved ChABC activity. The highest activity level of the released ChABC was 0.0162 U/ml, seven days after release when

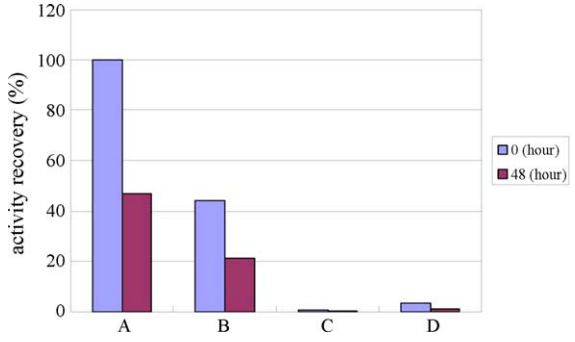

Fig. 3. Enzyme was immobilized in a chitosan based scaffold by two processes: (A,C) scaffold/ChABC/GTA and (B,D) scaffold/GTA/ChABC. (A,B) Chitosan scaffold; (C,D) chitosan/gelatin scaffold. Scaffold/ChABC/GTA means ChABC was added to the scaffold first, and then cross-linked by GTA; Scaffold/GTA/ChABC means the scaffold was first cross-linked by GTA, and then ChABC was added. ($n = 3$).

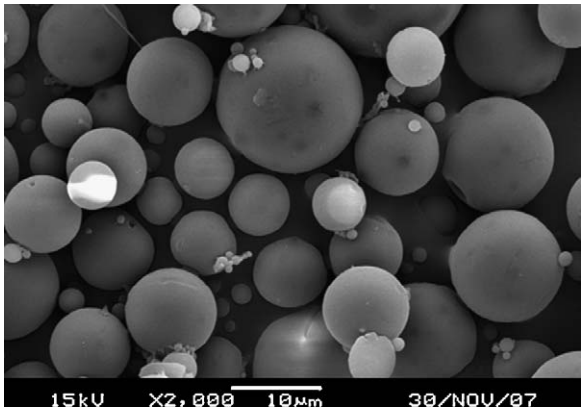

Fig. 4. PDLLA microspheres which were prepared by standard double emulsion/solvent evaporation method.

polylysine (Mw: 500–2000) was used as the stabilizer. Experimental data demonstrate that changes in activity decreased over the study period, regardless of which stabilizer was added. The initial high activity may be a response to ChABC at the surface of micro-

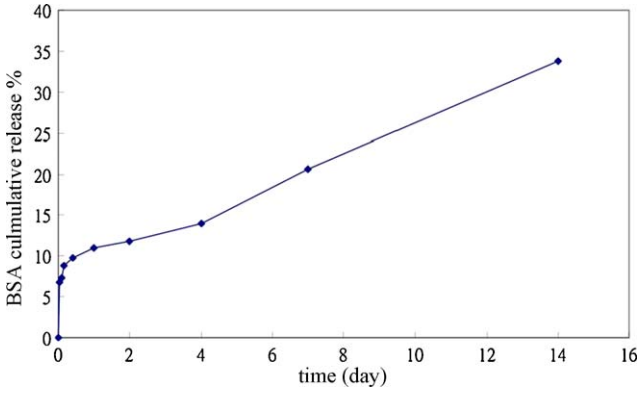

Fig. 5. BSA release from PDLLA microspheres ($n = 3$).

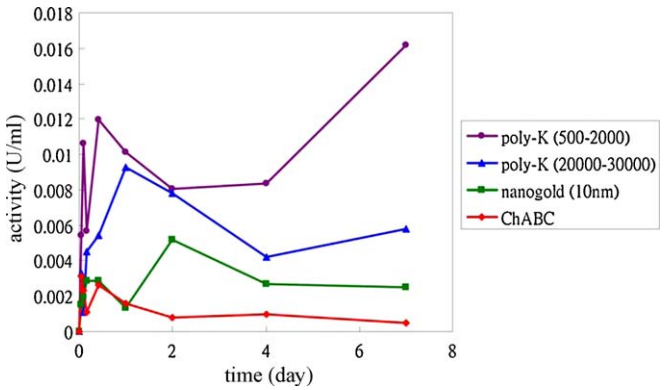

Fig. 6. The release curve of ChABC by adding different stabilizers ($n = 3$).

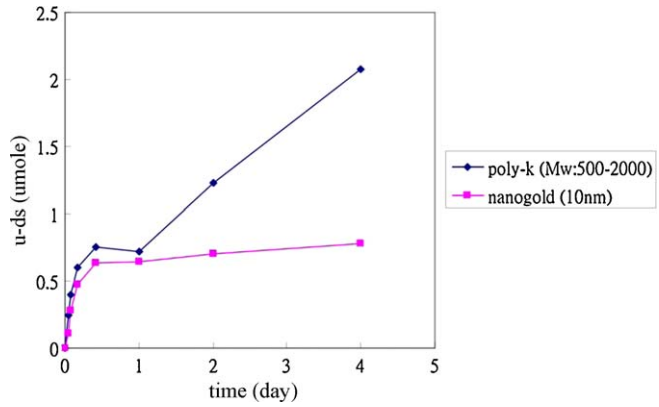

Fig. 7. The release curve of ChABC from PDLLA microspheres with nanogold and polylysine as stabilizers. The longitudinal axis means the unsaturated disaccharide (u-ds) formed after adding ChABC in chondroitin sulfate solution ($n = 3$).

spheres, whereas PDLLA degradation may be responsible for the increase in ChABC activity seven days after release.

The analytical results concerning unsaturated disaccharide that remained in solution (Fig. 7) suggest that polylysine (Mw: 500–2000) is a better stabilizer than nanogold. A tentative interpretation is that electrostatic interaction between positively charged polylysine and negatively charged ChABC protects ChABC against decomposition.

4. Discussion

Spinal cord repair is difficult because ChABC activity declined rapidly at body temperature. Hence, clinical treatment depends on strategies that stabilize ChABC. In this work, ChABC was immobilized in a chitosan-based scaffold by ionic bonding and covalent bonding, and 48% and 33% of its activity, respectively, was maintained 48 h after releasing. Experimental results reveal that the activity of ChABC immobilized in a chitosan-based scaffold was maintained more effectively than that of ChABC in the free-state.

In this study, another strategy was adopted to fabricate microspheres to encapsulate ChABC. Such microspheres protect enzymes from decomposition and control the rate at which enzyme are released. To preserve ChABC activity efficiently, numerous stabilizers were added to the microspheres. Fig. 7 summarizes the experimental results concerning polylysines and nanogold. Notably, polylysines were better stabilizers than nanogold, and those with low molecular weights were the most effective. We hypothesize that polylysine (Mw: 500–2000) surrounds ChABC, whereas polylysine (Mw: 20,000–30,000) can complex with it. Polylysine (Mw: 20,000–30,000) is too large to surround ChABC or be encapsulated by microspheres.

As a dissolved enzyme, ChABC digests the GAG side-chain of CSPGs to improve the rate of spinal cord repair (Bradbury et al., 2002; Ikegami et al., 2005). Bradbury et al. demonstrated that ChABC infusion via mini-pumps (10 U/ml, 6 μl) can promote the functional recovery of SCI in a rat model. Henrich, Cheng et al. injected ChABC (1 U/ml, 6 μl) through a catheter every other day for two weeks to reduce the inhibitory effects of limiting axonal re-growth following SCI (Huang et al., 2006). However, a second surgery is required for removing the catheter or the mini-pump. Accordingly, clinical treatment depends on encapsulation of ChABC in a controlled drug release system. In this work, ChABC was released by a controlled system, a chitosan-based scaffold and PDLLA microspheres, and stabilized by polylysine. To achieve the same working activity as that achieved in earlier studies (Bradbury et al., 2002; Huang et al., 2006; Ikegami et al., 2005), the amount of ChABC required herein is reduced dramatically to 5×10^{-3} U for 1 g of microspheres or 7×10^{-5} U for 1 g of chitosan-based scaffold.

In the preparation of microspheres using a standard double/emulsion evaporation approach, homogenization is commonly conducted in the second emulsion process. However, in this study, the mixture was stirred gently, and no homogenizer was used, to prevent ChABC deactivation. Accordingly, the mean particle diameter was 90.51 μm and 90% of particles had diameters of 20–280 μm, as determined using a laser diffraction particle size analyzer. As expected, the release profile was biphasic with a predominantly degradation-controlled mechanism (Fig. 5). Some of the BSA may have been lost owing to microsphere adsorption or the apparent loss may reflect inaccuracies in the analytical detection assay. After the initial burst of BSA on day 1 when 10% of the BSA was released, the rate of release slowed, as reflected by the decline in the gradient of the plot of the cumulative release of BSA as a function of release time.

5. Conclusions

Nerve regeneration depends on the controlled release of ChABC with sufficient bioactivity. Therefore, in this work, ChABC was immobilized in chitosan-based NCs and ChABC was encapsulated in PDLLA microspheres. Ionic bonding was better than covalent bonding in the immobilization of ChABC in chitosan-based NCs. The activity of ChABC that was immobilized by ionic bonding was 0.07 U/mg, double that achieved by covalent bonding. Furthermore, the pore size in chitosan NCs was reduced from 100–160 μm to 20–40 μm when gelatin was used as the modifier. The experimental results show that immobilizing ChABC in chitosan-based NCs preserved ChABC activity. The ChABC retained 48% of its activity after 48 h of release.

When ChABC was encapsulated in PDLLA microspheres without stabilizers, ChABC activity was <0.0026 U/ml after two hours of release. The ChABC activity increased when a stabilizer was added, and especially when low-molecular-weight polylysine (Mw: 500–2000) was added. It was maintained at 0.0162 U/ml after seven days of release. These experimental results suggest that the controlled release of ChABC encapsulated in PDLLA microspheres is an effective scheme for repairing SCI and, may represent a general model that may be useful as part of other multidisciplinary methods for treating complex neurological injuries.

Acknowledgments

The authors would like to thank the National Science Council of the Republic of China, Taiwan, for financially supporting this research under Contract No. NSC97-2314-B-019-001. Ted Knoy is appreciated for his editorial assistance.

References

Bradbury, E. J., Moon, L. D. F., Popat, R. J., King, V. R., Bennett, G. S., Patel, P. N., et al. (2002). Chondroitinase ABC promotes functional recovery after spinal cord injury. *Nature*, *416*(6881), 636–640.

Busch, S. A., & Silver, J. (2007). The role of extracellular matrix in CNS regeneration. *Current Opinion in Neurobiology*, *17*(1), 120–127.

Chuysinuan, P., Chimnoi, N., Techasakul, S., & Supaphol, P. (2009). Gallic acid-loaded electrospun poly(L-lactic acid) fiber mats and their release characteristic. *Macromolecular Chemistry and Physics*, *210*(10), 814–822.

Dagnon, K. L., Ambadapadi, S., Shaito, A., Ogbomo, S. M., DeLeon, V., Golden, T. D., et al. (2009). Poly(L-lactic acid) nanocomposites with layered double hydroxides functionalized with ibuprofen. *Journal of Applied Polymer Science*, *113*(3), 1905–1915.

Ernst, S., Venkataraman, G., Winkler, S., Godavarti, R., Langer, R., Cooney, C. L., et al. (1996). Expression in *Escherichia coli*, purification and characterization of heparinase I from Flavobacterium heparinum. *The Biochemical Journal*, *315*(Pt 2), 589–597.

Gong, H. P., Zhong, Y. H., Li, J. C., Gong, Y. D., Zhao, N. M., & Zhang, X. F. (2000). Studies on nerve cell affinity of chitosan-derived materials. *Journal of Biomedical Materials Research*, *52*(2), 285–295.

Huang, Y. C., Huang, Y. Y., Huang, C. C., & Liu, H. C. (2005). Manufacture of porous polymer nerve conduits through a lyophilizing and wire-heating process. *Journal of Biomedical Materials Research Part B-Applied Biomaterials*, *74B*(1), 659–664.

Huang, M., Khor, E., & Lim, L. Y. (2004). Uptake and cytotoxicity of chitosan molecules and nanoparticles: Effects of molecular weight and degree of deacetylation. *Pharmaceutical Research*, *21*(2), 344–353.

Huang, W. C., Kuo, W. C., Cherng, J. H., Hsu, S. H., Chen, P. R., Huang, S. H., et al. (2006). Chondroitinase ABC promotes axonal re-growth and behavior recovery in spinal cord injury. *Biochemical and Biophysical Research Communications*, *349*(3), 963–968.

Ikegami, T., Nakamura, M., Yamane, J., Katoh, H., Okada, S., Iwanami, A., et al. (2005). Chondroitinase ABC combined with neural stem progenitor cell transplantation enhances graft cell migration and outgrowth of growth-associated protein-43-positive fibers after rat spinal cord injury. *European Journal of Neuroscience*, *22*(12), 3036–3046.

Klapka, N., & Muller, H. W. (2006). Collagen matrix in spinal cord. *Journal of Neurotrauma*, *23*(3–4), 422–435.

Pojasek, K., Shriver, Z., Hu, Y., & Sasisekharan, R. (2000). Histidine 295 and histidine 510 are crucial for the enzymatic degradation of heparan sulfate by heparinase III. *Biochemistry*, *39*(14), 4012–4019.

Pojasek, K., Shriver, Z., Kiley, P., Venkataraman, G., & Sasisekharan, R. (2001). Recombinant expression, purification, and kinetic characterization of chondroitinase AC and chondroitinase B from Flavobacterium heparinum. *Biochemical and Biophysical Research Communications*, *286*(2), 343–351.

Tsuji, H. (2005). Poly(lactide) stereocomplexes: Formation, structure, properties, degradation, and applications. *Macromolecular Bioscience*, *5*(7), 569–597.

Vleggeert-Lankamp, C., de Ruiter, G. C. W., Wolfs, J. F. C., Pego, A. P., van den Berg, R. J., Feirabend, H. K. P., et al. (2007). Pores in synthetic nerve conduits are beneficial to regeneration. *Journal of Biomedical Materials Research Part A*, *80A*(4), 965–982.