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Research Report

Sustained delivery of chondroitinase ABC by poly (propylene carbonate)-chitosan micron fibers promotes axon regeneration and functional recovery after spinal cord hemisection

Shilei Ni[a,1], *Tongliang Xia*[a,1], *Xingang Li*[a], *Xiaodong Zhu*[a], *Hongxu Qi*[b], *Shanying Huang*[c], *Jiangang Wang*[a,*]

[a]*Department of Neurosurgery, Qilu Hospital, Shandong University, 107# Wenhua Xi Road, Jinan 250012, PR China*
[b]*Institute of Polymer Science & Engineering, Tsinghua University, 30# Shuangqing Road, Beijing 100084, PR China*
[c]*Key laboratory of Cardiovascular Remodeling and Function Research, Chinese Ministry of Health and Education, 107# Wenhua Xi Road, Jinan 250012, PR China*

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ABSTRACT

We describe the sustained delivery of chondroitinase ABC (ChABC) in the hemisected spinal cord using polypropylene carbonate (PPC) electrospun fibers with chitosan (CS) microspheres as a vehicle. PPC and ChABC-loaded CS microspheres were mixed with acetonitrile, and micron fibers were generated by electrospinning. ChABC release was assessed *in vitro* with high-performance liquid chromatography (HPLC) and revealed stabilized and prolonged release. Moreover, the released ChABC showed sustained activity. PPC-CS micron fibers with or without ChABC were then implanted into a hemisected thoracic spinal cord. In the following 4 weeks, we examined functional recovery and performed immunohistochemical analyses. We found that sustained delivery of ChABC promoted axon sprouting and functional recovery and reduced glial scarring; PPC-CS micron fibers without ChABC did not show these effects. The present findings suggest that PPC-CS micron fibers containing ChABC are a feasible option for spinal cord injury treatment. Furthermore, the system described here may be useful for local delivery of other therapeutic agents.

Abbreviations: PPC, polypropylene carbonate; dbcAMP, dibutyryl cyclic adenosine monophosphate; SCI, spinal cord injury; CO_2, carbon dioxide; SEM, scanning electron microscopy; GAP-43, growth-associated protein 43; NF-200, neurofilament-200; GFAP, glial fibrillary acidic protein; BBB, Basso, Beattie, and Bresnahan; ChABC, chondroitin sulfate ABC; CSPGs, chondroitin sulfate proteoglycans; CS, chitosan; PVP, polyvinylpyrrolidone; GAG, glycosaminoglycan; CS-56, anti-chondroitin sulfate.

*Corresponding author. Fax: +86 531 86927544.
E-mail address: drwjg@hotmail.com (J. Wang).
[1]These authors contributed equally to this work.

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1. Introduction

Spinal cord injury (SCI) is a global medical concern that is complicated by the spinal cord's inability to regenerate. The lack of regenerative ability is thought to result from several factors, including the immune response and an increase in molecules that inhibit axon regeneration after SCI (Fleming et al., 2006; Kopp et al., 2012). Several experiments have shown that chondroitin sulfate proteoglycans (CSPGs) are secreted in increasing amounts by activated astrocytes and oligodendrocytes and that these CSPGs subsequently inhibit axon regeneration after SCI (Bradbury et al., 2002; Properzi et al., 2003; Matsui and Oohira, 2004).

CSPGs contain two common structures; one is the core protein-containing NG2, and the other is a glycosaminoglycan (GAG) that contains a glycosylation side chain. Chondroitinase ABC (ChABC) can decompose the GAG structure in CSPGs, abate GAG-induced axon inhibition, and indirectly promote axonal regeneration (Ikegami et al., 2005). However, ChABC use in animal experiments has been limited by several problems. One major issue is that traditional administration of ChABC cannot guarantee effective drug concentrations at the injury site. Additionally, as a protein, ChABC remains active for only 3–5 days at 37 °C (Tester et al., 2007). It has been shown that sustained delivery of agents from implanted biodegradable materials can maintain drug concentrations at effective levels and attenuate the side effects experienced with systemic administration. Therefore, we tested the effectiveness of such a drug delivery system for the local release of ChABC.

Poly(propylene carbonate) (PPC), which is biodegradable, is synthesized from carbon dioxide (CO_2) and propylene oxide and is degraded into CO_2 and water (Du et al., 2004). Our previous studies have shown that polymer materials made of PPC *via* electrospun technology have optimal biocompatibility and are biodegradable (Fan et al., 2010; Xia et al., 2013).

In the present study, we used electrospun technology to develop a sustained delivery system for ChABC. Because ChABC is inactivated in the organic solvent that is used to dissolve PPC, we designed a method in which ChABC could be encapsulated by chitosan (CS). CS is obtained by chitin deacetylation and is also biocompatible (Feng et al., 2014). Most SCIs occur because of contusions and, therefore, we designed the delivery system as a membrane instead of a scaffold. This was to enable the attachment of the membrane to the injury site to ensure that it would not cause a new second-injury, especially a crush injury. Here, we developed a PPC micron fiber membrane and combined CS spheres that were interfused with ChABC using electrospun technology. Thus, we established a novel system for delivering ChABC in an experimentally injured spinal cord *via* a PPC-CS sphere membrane. Using this delivery system, we assessed the effects of ChABC in SCI, and the possibility of using electrospun fibers to administer protein agents.

2. Results

Fibers were readily generated from PPC and CS microsphere emulsion using the electrospinning technique. The microspheres and fibers had a smooth surface, relatively uniform morphology, and average diameters of 300 nm (Fig. 1A) and 3 μm (Fig. 1B), respectively.

2.1. *ChABC release from infused micron fibers*

The weight ratio of ChABC to micron fibers was 1:99. We found that micron fibers were effective in supporting the stable release of ChABC over 10 days (Fig. 1B) in PBS at 37 °C and 5% CO_2. The quantity of ChABC released accounted for 89.10%±1.41% of the total amount, whereas active ChABC accounted for 26.29%±0.46% of the total amount (Fig. 1C).

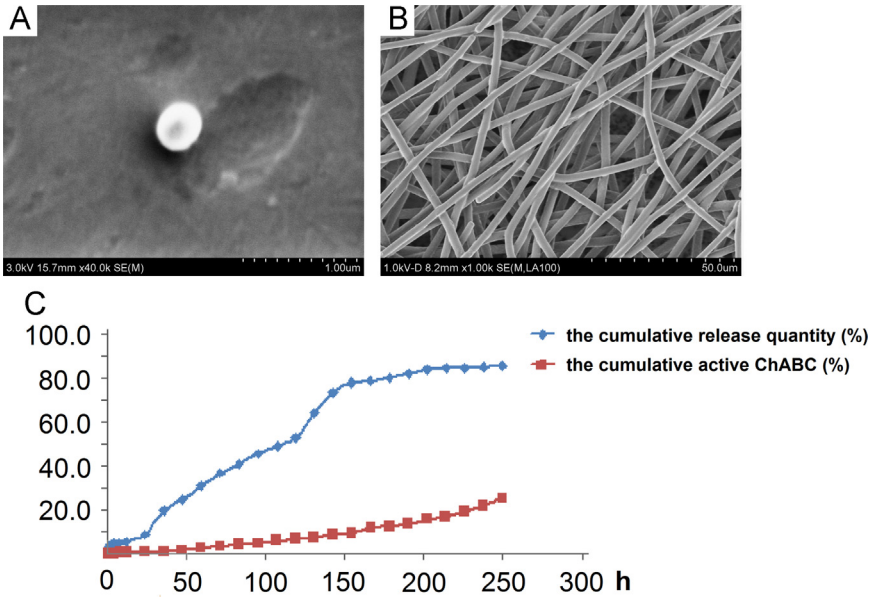

Fig. 1 – (A) Surface and morphology of the chitosan (CS) spheres. (B) Surface and morphology of the micron fibers. (C) Release curve of chondroitinase ABC (ChABC).