Creating a glial scar-free implant-CNS interface by blocking chondroitin sulfate proteoglycan biosynthesis

Ning Zhang, Xuejun Wen.
Clemson-MUSC Bioengineering Program, Department of Bioengineering, Clemson University
173 Ashley Ave., CRI#605B, Charleston, SC, 29425

Statement of Purpose: Following nervous system injury, peripheral nervous system (PNS) and central nervous system (CNS) recover differently. While in most cases, the severed axons of PNS are able to re-extend and re-innervate their targets, eventually leading to functional recovery, a rare return of damaged structures and functions is observed following injuries to CNS. Although the precise mechanism is still unclear, chemorepulsive nature of non-permissive glial scar formed following adult CNS injury plays a major role in inhibiting CNS regeneration. The major components of glial scar are believe to be oligodendrocytes and their secreted myelin-associated glycoprotein (MAG) [1], reactive astrocytes and their secreted chrondroitin sulfate proteoglycan (CSPG) [2], and reactive microglial and macrophage [3]. To ultimately overcome this hurdle in CNS regeneration, this study aims at inhibiting glial scar formation by inhibiting CSPG biosynthesis. Semi-permeable hollow fiber membranes (HFMs) were showing very promising results in promoting axonal regeneration in spinal cord injury models [4]. However, glial scar formation at the distal device−host interface deters regenerating axonal crossing from the device into host spinal cord [4]. To this end, semi-permeable HFMs loaded with bioactive agent, 4-nitrophenyl-β-D-xylopyranoside (PNPX) were fabricated for inhibiting glial scar formation and therefore promoting nerve regeneration.

Methods: Polyacrylonitrile/polyvinylchloride copolymer (PAN/PVC) was used as a model non-degradable polymer as a carrier for PNPX delivery, and poly(DL-Lactide-co-glycolide) (PLGA) 50/50 copolymers was employed as a model degradable polymer for PNPX delivery. Polymer-PNPX were dissolved in dimethyl sulfoxide (DMSO). 1% phthalized-chitosan was added into the solution to enhance the PNPX loading efficiency. PNPX loaded HFMs were fabricated using a wet phase-inversion process. Drug loading efficiency and drug release profiles were studied in vitro using HPLC. Scar suppression effect was examined by implanting HFMs stereotactically into adult Fischer 344 male rat brains (+0.2 mm Bregma, +3.0 mm lateral, 10 mm depth from dura, with nose bar at -3.3 mm). Seven animals from each time point were sacrificed for immunohistological analysis at 2, 4, and 8 weeks following implantation. Horizontal sections of the fixed brains were obtained at a thickness of 50 um using a vibratome. Indirect immunohistochemistry were performed on the sections using antibodies against CSPG, GFAP, Neurofilament, OX-42, ED-1, Reca-1, and 4',6'-diamidino-2-phenylindole hydrochloride (DAPI) to study the glial response, neuronal reaction, foreign body reaction, and angiogenesis to the PNPX loaded HFMs.

Results / Discussion: PNPX loaded HFMs showed a steady release from day one up to four weeks. The extent of astrocytic scarring was examined using anti-GFAP and anit-CSPG antibodies, specific markers for astrocytes and their secreted inhibitory matrix. Increase in GFAP levels as a result of astrocytes proliferation and hypertrophy may occur in injury and disease situations. Constant release of PNPX to local brain tissue greatly suppress the glial scar formation. As shown in Fig. 1, the number of astrocytes (red) and the amount of CSPG secretion (green) are decreased significantly ($P<0.05$). No significant difference was found in neuronal reaction, foreign body reaction, and angiogenesis to the PNPX loaded HFMs. Studies in progress are evaluating axonal outgrowth and regeneration using a spinal cord injury model.

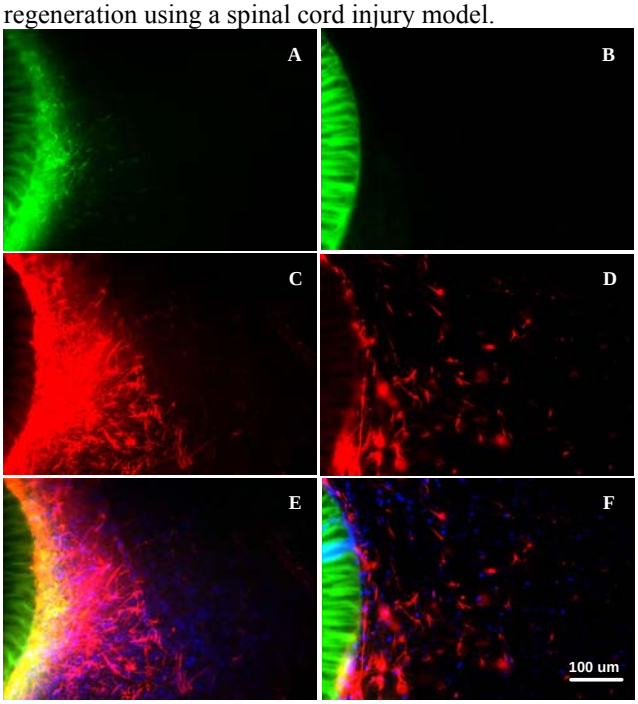

Fig. 1: (A) and (B) show the CSPG pattern surrounding the HFMs. (C) and (D) show the GFAP staining pattern for astrocytes around the implantation zone. The number of astrocytes and the amount of CSPG surrounding the PNPX delivery zone is significantly ($P<0.05$) lower than that for blank control, suggesting that PNPX may suppress glial scar formation after CNS injury.

Acknowledgements: This work was made possible by Wallace H. Coulter Foundation, South Carolina Spinal Cord Injury Research Fund, MUSC URC grant and NIH Grant Number P20 RR-016461 from the National Center for Research Resources (NCRR) and National Science Foundation under Grant No. 0132573.

References:

1. Li S et al. *J Neurosci* **24**, 10511-20 (2004).
2. Morgenstern DA et al. *Prog Brain Res* **137**, 313-32 (2002).
3. Zhang N, Yan H & Wen X. *Brain Res Rev* **49**, 48-64 (2005).
4. Xu XM et al. *Eur J Neurosci* **11**, 1723-40 (1999).