PLGA Nanospheres for Delivery of Chondroitinase ABC to the Glial Scar

Au AY[1], Garma PD[1], Osterhout DJ[2], Stelzner DJ[2], Hasenwinkel JM[1]
[1]Syracuse University Department of Biomedical and Chemical Engineering, 121 Link Hall, Syracuse, NY 13244
[2]SUNY Upstate Medical University, Syracuse, NY 13210

Introduction: There is an upregulation of inhibitory molecules, including chondroitin sulfate proteoglycans (CSPGs), in the glial scar that forms following a spinal cord injury. CSPGs are highly negatively charged molecules composed of glycosaminoglycans (GAGs) covalently attached to a protein core. The GAGs are hypothesized to be the inhibitory component of CSPGs as they prevent axonal regrowth and the formation of functional synaptic connections between neurons.[1] The enzyme chondroitinase ABC (cABC) cleaves the GAGs from the CSPG protein core and renders the molecule more permissive to axonal regrowth.[2] Thus, the goal of the current project was to successfully fabricate and characterize nanospheres composed of the biodegradable polymer poly(D,L lactide-co-glycolide) (PLGA; 85:15 copolymer ratio) that release biologically active cABC when injected into the glial scar. To better target the nanospheres to the negatively charged CSPGs, surface modification was performed with didodecyldimethyl-ammonium bromide (DMAB).[3] Nanospheres were studied using *in vitro* assays for size, affinity to CSPG-rich substrates, release of bovine serum albumin (BSA), and the potential to promote neurite outgrowth. Nanospheres were also evaluated for their *in vivo* efficacy in a rat spinal cord contusion model. We hypothesize that biodegradable nanospheres are an effective and targeted delivery system for cABC in the glial scar.

Methods: Nanospheres were prepared using 85:15 PLGA (94.5 kDa) by employing a modified emulsification-diffusion method with either polyvinyl alcohol (PVA, 5% w/v, Sigma) or DMAB (4% w/v, Sigma) as the stabilizer to create uncharged or cationic nanospheres, respectively.[4] The stabilizers, BSA (Sigma), cABC (0.2 Units/ml, Sigma), and coumarin-6 fluorescent dye (Polysciences Inc.) were added to the internal water phase under magnetic stirring prior to emulsion with the external oil phase composed of PLGA (2% w/v) in ethyl acetate. The emulsion was homogenized for 10 minutes at 25,000 rpm and added to a secondary water phase to allow the organic solvent to diffuse out of the droplets. Nanospheres were collected via centrifugation and stored in a dessicator overnight to remove residual solvent. Nanospheres were then stored at -20°C until use.

An atomic force microscope (Multi-Mode AFM-2 with Nanoscope III controller, Digital Instruments) was used to determine mean particle diameter of nanospheres on mica substrates. Surface adhesion of nanospheres was determined by coating 24-well plates with poly-lysine and either laminin alone, laminin + CSPG, or CSPG alone. Nanospheres (10 mg/ml) were plated for 30 min at 22°C and washed with phosphate buffered saline (PBS). Remaining nanospheres were imaged with Image-Pro® 3D Suite (Media Cybernetics, Inc.) to determine fraction area coverage of adherent nanospheres. A BCA Reagent Kit (Pierce Biotechnology) was used to determine BSA release over time from nanospheres suspended in PBS at 37°C. Western immunoblotting was used to determine cABC digestion of CSPGs following release from nanospheres. Nanospheres were evaluated in an *in vitro* neurite outgrowth assay using rat embryonic cortical neurons plated on CSPG-rich substrates. CSPG digestion was measured *in vivo* by staining with the CS-56 (intact CSPGs) and 2B6 (core protein) antibodies. ANOVA and Student-Neuman-Keuls post-hoc tests were performed using SigmaStat 3.1. Differences were considered significant when $p<0.05$.

Results/Discussion: Nanosphere compositions had average diameters from 111 to 253 nm, significantly decreasing in size as cABC concentration increased ($p<0.001$). DMAB stabilized nanospheres showed significantly more adhesion to the negatively charged CSPG substrates versus PVA stabilized nanospheres (Fig 1). BSA was released from the nanospheres for more than 45 days and bioactive cABC was released for at least two weeks, as measured by Western immunoblotting. Treatment with cABC nanospheres significantly increased process outgrowth and axon elongation for cells grown on CSPG substrates. Antibody labeling of the core protein significantly increased from 5 to 14 d *in vivo*, while CS-56 labeling significantly decreased over the same time.

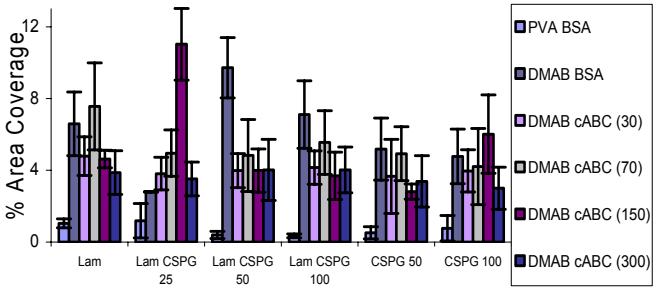

Figure 1. Effect of stabilizer and cABC concentration on nanosphere adhesion to CSPG-rich substrates

Conclusions: cABC nanospheres composed of 85:15 PLGA were successfully fabricated with two different stabilizers. *In vitro* assays indicated nanospheres released BSA and bioactive cABC for weeks. *In vivo* studies have shown digestion of CSPGs in the glial scar for over two weeks. DMAB stabilized nanospheres also have a greater potential for targeting to the CSPG-substrates. Thus, these nanospheres have the potential to serve as a delivery vehicle for cABC to promote axonal regeneration.

References:

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