Microspheres for sustained delivery of NEP1-40 and chondroitinase ABC for treatment of spinal cord injury

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Introduction: Spinal cord injury (SCI) is a major medical problem affecting 230,000-310,000 Americans, with 12,000 cases occurring annually. SCI typically results in partial or complete loss of function below the initial site of injury, leaving thousands of people without the ability perform basic daily activities[1]. Current research focuses on combinatorial strategies that utilize cell transplantation, delivery of growth factors, and blocking inhibitory signals. The formation of a glial scar, which contains chondroitin sulfate proteoglycans (CSPGs) and residual myelin-associated growth inhibitors, greatly limits axonal regeneration into and across the injury site. Therefore, the ability to provide sustained release of bioactive factors at the site of injury could potentially improve recovery after spinal cord injury. Poly(lactic acid-*co*-glycolic acid) (PLGA) microspheres have previously been used to deliver bioactive molecules over weeks to months. The goal of this project was to deliver NEP 1-40 and chondroitinase ABC from PLGA microspheres to reduce the inhibitory environment after SCI.

Methods: To mimic the release of NEP1-40 peptide from microspheres, 1mg/mL of fluorescent dextran (Mw = 4kDa) in water was loaded into PLGA microspheres formed using a water in oil in water suspension. The microspheres were added to PBS, and the release profile was measured using a fluorimeter over two weeks. After two weeks, the microspheres were degraded using 1M NaOH to determine the amount of fluorescent dextran still loaded in the microspheres. The highly labile protein chondroitinase ABC (chABC) was dissolved in 0.5 M trehalose, 0.1M glutathione, 0.01M Tris-base (pH 8.0), and 0.5% w/v BSA at 50 units/mL and loaded into PLGA microspheres[2,3]. The enzymatic activity after release from the microspheres was determined by the ability of chABC to degrade chondroitin sulfate, and the resulting absorbance of unsaturated disaccharides was measured using a spectrophotometer at 232nm.

Results: The release profile of fluorescent dextran from PLGA microspheres showed a burst release of 30 ± 6% of the loaded dextran after 24 hours in PBS with 68 ± 9% of the loaded dextran being release over a two week period (figure 1). This indicates that peptides, such as NEP1-40 can be released from PLGA microspheres for up to two weeks. The PLGA microspheres were loaded with chABC in a solution that thermostabilizes the enzyme to maintain enzymatic activity. ChABC was loaded into the microspheres using several types of thermostabilizing solutions that were previously reported to enhance activity of various thermolabile enzymes. The enzymatic activity of the released protein was measured after formation of the microspheres, and the result suggests that 90% of the released enzyme remains active (figure 2).

Conclusion: PLGA microspheres loaded with 4 kDa dextran are capable of sustained release up to two weeks, which suggests that NEP1-40 peptide can be released from the material to inhibit myelin associated growth inhibitors. The protein solution used to dissolve the chABC was capable of preventing excessive denaturing to the enzyme resulting in 90% of the released enzyme remaining active. Therefore, suggesting that chABC can be loaded into and released from PLGA microspheres to provide a mechanism that removes the inhibition of CSPGs at the glial scar within spinal cord injuries.

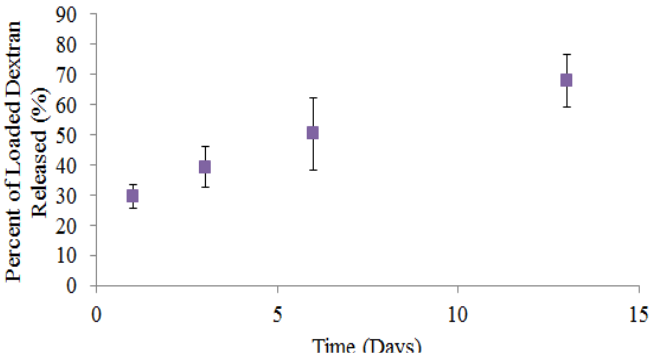

Figure 1: Release profile of fluorescent dextran (Mw = 4kDa) from PLGA microspheres formed using a water in oil in water suspension. n = 5

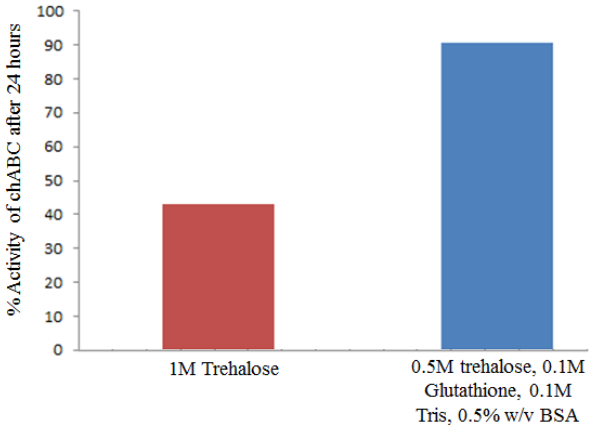

Figure 2: Percent of enzymatic activity after 24 hours of release from PLGA microspheres for chABC.

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References: 1.McDonald et al. 2004. Journal of Neurotrauma. (21):383-393.
2. Lee, McKeon, Bellamkonda. (2010). PNAS 107: 3340-3345.
3. Genta et al. 2001. Journal of Controlled Release. 77: 287-295