Development of Biomaterials for Sustained Delivery of Bioactive Molecules in Spinal Cord Injury

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Introduction: Spinal cord injury (SCI) is a major medical problem affecting 270,000 Americans, with 12,000 new cases occurring annually[1]. 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[2]. The formation of a glial scar, which contains chondroitin sulfate proteoglycans (CSPGs) and residual myelin-associated growth inhibitors, greatly limit axonal regeneration into and around the injury site. A recent publication illustrates the potential of combination therapies using anti-myelin inhibitors and chondroitinase ABC (ChABC) treatment after spinal cord injury using microinjections and intrathecal pumps and cannulas to deliver drugs which requires multiple surgical sites[3]. The goal of this project is to overcome the need for multiple surgical sites by providing sustained delivery of the NEP1-40 peptide using Poly(lactic acid-*co*-glycolic acid) (PLGA) microspheres and sustained delivery of ChABC using hollow lipid microtubes encapsulated in fibrin scaffolds.

Methods: PLGA microspheres loaded with either 1 mg/mL fluorescent dextran (Mw = 4kDa) or NEP1-40 (Mw = 4625Da) were formed using a water in oil in water suspension. The microspheres were encapsulated into 10 mg/mL fibrin scaffolds and the release profile of fluorescent dextran was measured using a fluorimeter for two weeks. After two weeks, the microspheres were degraded using 1M NaOH to determine the amount of fluorescent dextran still in the microspheres. The highly labile protein ChABC was dissolved in 1 M trehalose and loaded into dried $DC_{8,9}PC$ lipid microtubes[4]. The enzymatic activity after release from microtubes encapsulated into fibrin scaffolds was determined by the ability of ChABC to degrade chondroitin sulfate, and the resulting absorbance of unsaturated disaccharides was measured using a spectrophotometer at 232 nm. The bioactivity of fibrin scaffolds containing ChABC, NEP1-40, or both was measured using dissociated embryonic chick dorsal root ganglia cells (DRGs) plated on inhibitory spots containing CSPGs, myelin, or CSPGs and myelin.

Results: The release profile of fluorescent dextran from PLGA microspheres showed a burst release of 13 ± 4% of the loaded dextran after 24 hours with 41 ± 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. ChABC was loaded into lipid microtubes in a thermostabilizing solution. The enzymatic activity of the released protein was measured and the result suggests that the released enzyme remains active for at least one week (figure 2). Inhibitory spot assays with media from fibrin scaffolds containing loaded microtubes and microspheres showed that releasing both ChABC and NEP1-40 significantly increased neurite growth compared to either factor alone (figure 3).

Conclusion: The loading of PLGA microspheres with NEP1-40 and $DC_{8,9}PC$ lipid microtubes with ChABC provides a mechanism for sustained release of bioactive molecules from fibrin scaffolds that may provide improved functional recovery after spinal cord injury by mitigating inhibitory effects caused by CSPGs and myelin associated inhibitors.

References: [1] 2012. *Journal of Spinal Cord Medicine*. 35(6):480-481. [2]McDonald et al. 2004. *Journal of Neurotrauma.* (21):383-393. [3]Zhao, et al. 2013. *Eur J Neurosci.* 38(60):2946-2961. [4]Lee, McKeon, Bellamkonda. (2010). *PNAS* 107: 3340-3345.

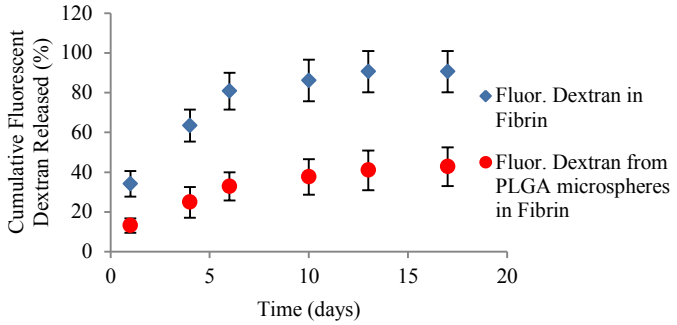

Figure 1: Release profile of fluorescent dextran (Mw = 4kDa) from PLGA microspheres in fibrin scaffolds formed using a water in oil in water suspension.

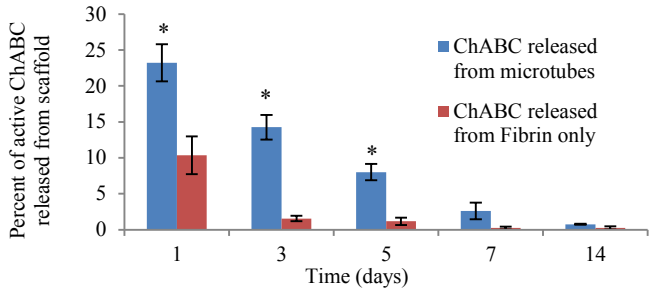

Figure 2: Release profile of active ChABC from $DC_{8,9}PC$ lipid microtubes in fibrin scaffolds shows active ChABC for at least one week. Error bars are standard deviation. * denotes significance from ChABC in fibrin only ($p<0.05$).

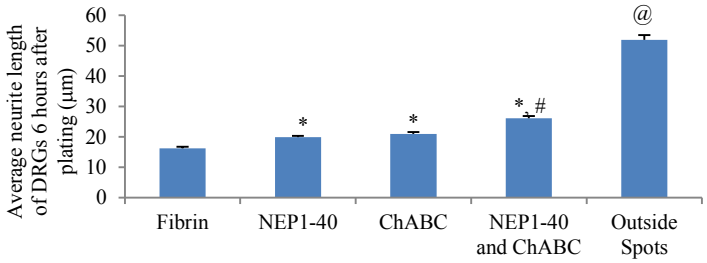

Figure 3: Day 1 results of releasing NEP1-40 from PLGA microspheres and ChABC from lipid microtubes when encapsulated in fibrin scaffolds. Error bars are standard error of the mean. * denotes significance from fibrin ($p<0.05$). # denotes significance from NEP1-40 and ChABC groups ($p<0.05$). @ denotes significance from all other groups ($p<0.05$).