Combination Therapy of Stem Cell Derived Neural Progenitors and Drug Delivery of Anti-Inhibitory Molecules for Spinal Cord Injury

Thomas Wilems, Shelly Sakiyama-Elbert
Washington University in St. Louis

Introduction: Spinal cord injury (SCI) is a major medical problem affecting 270,000 Americans, with 12,000 new cases annually. Secondary injury results in the formation of a glial scar surrounding a cystic cavity or cellular void. The goal of this project is to develop a method to limit inhibition from the glial scar and replace cells lost due to primary and secondary injuries. Toward the goal a combination therapy of stem cell-derived neural progenitors and sustained delivery of the NEP1-40 peptide and chondroitinase ABC (ChABC) enzyme from fibrin scaffolds to overcome inhibitory signals was developed and studied.

Methods: PLGA microspheres loaded with NEP1-40, a peptide that blocks myelin-associated inhibition, were formed using a water in oil in water suspension. Highly labile ChABC, an enzyme that removes inhibitory cues from chondroitin sulfate proteoglycans (CSPG), was stabilized in trehalose and loaded into dried DC9,6PC lipid microtubes. The release profiles and bioactivity of released anti-inhibitory molecules were tested in vitro. Mouse embryonic stem cells were modified to express the puromycin resistance gene under the Olig2 transcription factor promoter. The stem cells were allowed to form into embryoid bodies for 2 days and then differentiated into progenitor motor neurons (pMNs) using retinoic acid and purmorphamine. The Olig2 transcription factor is up regulated in pMNs and therefore the cells were treated with puromycin from days 4-6 of induction to kill undifferentiated cells. The NEP1-40 loaded microspheres, ChABC loaded microtubes, and selected pMNs were incorporated into fibrin scaffolds containing a heparin binding delivery system (HBDS) that promotes slow release of the neurotrophic factors NT-3 and PDGF. The fibrin scaffolds were transplanted into adult female Long-Evans rats two weeks after an initial T8 dorsal hemisection. Two weeks post-transplantation, the spinal cords were harvested and immunohistochemistry performed to determine the effect on CSPG deposition, axonal extension/sprouting, and incorporation of the transplanted cells into the host environment.

Results: Release profiles of fluorescently labeled NEP1-40 in PLGA microspheres and fluorescently labeled ChABC in microtubes showed sustained release for over two weeks (Fig. 1). Bioactivity assays indicate that released NEP1-40 or ChABC remain active the ability to limit myelin-associated inhibition and CSPG inhibition, respectively (Fig. 2). Transplantation of fibrin scaffolds with the modified HBDS containing neurotrophic factors, loaded PLGA microspheres, loaded microtubes, and selected pMNs resulted in improved axonal extension and decreased formation of the glial scar.

Conclusions: The loading of PLGA microspheres with NEP1-40 and lipid microtubes with ChABC provide a mechanism for sustained release of bioactive molecules. The microspheres and microtubes can be incorporated into modified fibrin scaffolds capable of cell encapsulation and the slow release of neurotrophic factors. The transplanted fibrin scaffolds allow for a combination therapy that not only limits inhibition around the injury site but also repopulates the cellular void.


Figure 1: Release profile of fluorescently labeled NEP1-40 (A) and release profile of fluorescently labeled ChABC (B) show sustained release when loaded into microspheres or microtubes compared to either molecule directly released from fibrin scaffolds.

Figure 2: A) Enzymatic activity assay shows active ChABC released from microtubes in fibrin scaffolds for over one week compared to ChABC directly released from fibrin. B) The average neurite length of neurons plated on inhibitory myelin spots is significantly increased when media containing released NEP1-40 is used instead of control media containing no released NEP1-40.

Figure 3: Immunohistochemistry of sectioned spinal cords two weeks after transplantation of fibrin scaffolds. β-tubulin staining suggests a significant increase in axonal extension around the injury site when treating with neurotrophic factors, NEP1-40, ChABC, and selected pMNs. No change in non-degraded CSPG deposition (CS56) or GFAP staining was measured but degraded CSPGs (C-S-4) were significantly increased in the group with neurotrophic factors, microtubes and spheres, and selected pMNs compared to neurotrophic factors alone.