In vivo analysis of poly(N-isopropylacrylamide)-poly(ethylene glycol) Branched Copolymer as an Injectable Scaffold for Local Delivery of Neurotrophins and Cellular Transplants

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Statement of Purpose: Most of the functional deficits after spinal cord injury (SCI) result from the inability of the central nervous system to regenerate neurons and due to a lack of trophic support (Tetzlaff W. Prog Brain Res. 1994;103:271-286). In order to successfully repair the damaged tissue following SCI and to promote functional recovery, the non-permissive injury environment needs to be adjusted by modulating the immune reaction that follows the initial injury and leads to inflammation and further expansion of the initial injury and also by replacing lost or damaged cells. This work investigates a non-degradable branched copolymer based on poly(N-isopropylacryalmide) (PNIPAAm) and poly(ethylene glycol) (PEG), to fill the injury gap and to serve as a delivery vehicle for cellular transplants and neurotrophins. Aqueous solutions of this copolymer undergo a phase transition around 32^{o}C, allowing for easy implantation, as it can be injected as a viscous liquid and solidifies *in situ* (Vernengo J. J Biomed Mat Res. 2008;84b:64-69). The PNIPAAm-PEG branched copolymer, loaded with rat fibroblasts expressing brain-derived neurotrophic factor (BDNF), a neural precursor cell (NPC) suspension, or BDNF to promote axonal regeneration, is evaluated in a rodent model of SCI, to determine if it gels *in situ* to fill the defect site without contributing to an injury-related inflammatory response, for its ability to allow for survival and differentiation of the Alkaline Phosphatase (AP) NPCs, as well as rat fibroblasts, and its permissivity to axonal ingrowth.

Methods: N-isopropylacrylamide (NIPAAm) monomer was polymerized in the presence of PEGDM (MW=8000) to create PNIPAAm-PEG branched copolymers. The polymer was dissolved at 10wt% in tissue culture medium to create a hydrogel. To investigate the *in vivo* performance of PNIPAAm-PEG, a lumbar enlargement was performed at the L4/L5 dorsal root in adult female Sprague-Dawley rats and was compared to a commercially available matrix, Vitrogen PureCol™ (Inamed Biomaterials, Fremont, CA). After sacrifice and perfusion, AP histochemical staining was performed to assess the presence and location of graft-derived cells in the cavity. Fluorescence immunochemistry was also performed by incubating tissue sections in their primary antibody, including rabbit anti-GFAP for reactive astrocytes and glial scar formation; mouse anti-RT-97 for neurofilament staining of host and graft axons; mouse anti-CSPG for glial scar formation; and rabbit anti-IBA1 for macrophages. Tissue sections were incubated in their corresponding secondary fluorescent antibody conjugated to rhodamine or FITC and imaged using a Leica DM 5500B Microscope (Leica Microsystems, Bannockburn, IL) with Slidebook software (Intelligent Imaging Innovations, Denver, CO).

Results: Images from AP histochemical staining show cell survival of grafted NPCs in both the PNIPAAm-PEG and Vitrogen control, suggesting that the hydrogel supports cell survival and successfully delivers neural cells *in vivo*, without further damaging the injury site. The *in vivo* inflammatory response (IBA1) suggests that the hydrogel does not elicit a greater inflammatory response than the Vitrogen control. GFAP staining (Figure 1, green) shows that the hydrogel has a comparable presence of astrocytes in the normal tissue and around the injury site, indicating that there is comparable glial scar formation to the Vitrogen control. Glial scar formation was also verified using CSPG staining, and images indicate that scar formation is only present around the cavity, not within, in both the hydrogel and the control. RT-97 staining for neurofilaments suggests that PNIPAAm-PEG loaded with BDNF is permissive to host tissue ingrowth, indicative in Figure 1 by the red neurofilament staining within the cavity (yellow dotted line). In comparison to Vitrogen, results suggest that there is a greater presence of neurofilaments in the cavity of animals with PNIPAAm-PEG.

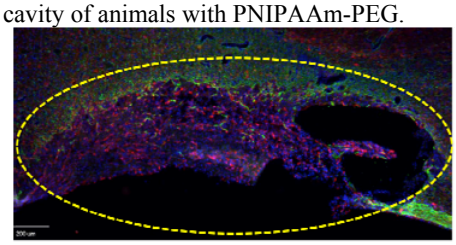

Figure 1: PNIPAAm-PEG allows for axonal ingrowth (red) in the cavity. Glial scar formation (green) is seen around the cavity.

Conclusion: Because of the injectable nature of PNIPAAm-PEG, neural precursor cells, rat fibroblasts, and BDNF were separately and successfully mixed with the hydrogel and delivered locally at the spinal cord injury site. In addition, PNIPAAm-PEG does not elicit a greater host response than the commercially available matrix and is a feasible local delivery vehicle for BDNF as well as cellular grafts *in vivo*. The hydrogel is also permissive to host tissue ingrowth, shown by neurofilament staining within the cavity. Future work includes encapsulating BDNF in micron-sized degradable particles, designed for controlled release, which in turn allows for cell support within the matrix. Future work will also evaluate PNIPAAm-PEG for axon permissivity and cell survival, by combining a NPC cell suspension and BDNF into one multifunctional platform for neural regeneration. By applying multiple therapeutic strategies, this platform for neural regeneration is thought to eventually provide for substantial improvement in the quality of life for SCI patients.