In vitro and *in vivo* characterization of injectable hydrogel scaffolds for spinal cord repair

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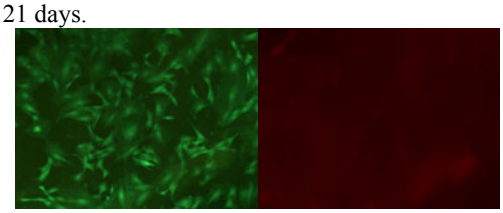
Introduction: Spinal cord injury affects nearly twenty five thousand Americans, with an additional ten thousand new injuries per year[1]. The current treatment options for such injuries are limited due to the body's inability to regenerate neurons and the difficulty in stabilizing the defect created by the injury. Ramon y Cajal showed in the early 1900's that neural cells are capable of regeneration when in the presence of selected neurotrophic factors[2]. Current research has focused on delivering these neurotrophic factors directly or with neural precursor cells injected into the injury site. The disadvantage to this technique is that neither the cells nor the trophic factors are maintained in the local tissue however, entrapping cells within a scaffold would help to prevent their migration.

This project proposes the use of a minimally invasive non-biodegradable polymeric hydrogel that is seeded with bone marrow stromal cells as well as neurotrophin loaded biodegradable microparticles. The hydrogel is made from a thermally responsive, injectable polymer, PNIPAAm-PEG branched copolymer. Below its LCST, typically around 29-32^{0}C, the polymer forms a miscible solution with water, but above its LCST, it becomes hydrophobic, separating from water and forming a semi porous gel. The aqueous polymer solution can be seeded with cells so that the cells are entrapped in the scaffold when it is injected into the defect. Bone marrow stromal cells (MSC) are progenitor cells that produce nuerotrophic factors and serve as a good model for cell transplantation studies[3].

Methods: *Polymer synthesis*- Poly(ethylene glycol Mn-8000) di methacrylate was synthesized prior to reacting it with poly(N-isopropylacrylimide). The ratio of monomers was set to 700:1 NIPAAm:PEG because of its mechanical stiffness and swelling properties. NMR was used to verify the reaction. Solutions of the copolymer at 10 wt% were made using sterile D-MEM/F12 Advanced cell culture media (Invitrogen) containing 16.6% fetal bovine serum (Hyclone). The solution was then sterilized with steam.

Cell studies- Rat marrow stromal cells were incorporated into the aqueous copolymer solution by removing the cells with trypsin/EDTA. The cells were then re-suspended using the 10 wt% copolymer solution and aliqouted into sterile 12-well plates. The samples were incubated at 37^{0}C and 5% CO_2 for 2 hours, then were placed into culture dishes with 5mL of fresh media with 5% penicillin - streptomycin. Samples were removed at desired time points, washed with PBS and incubated for 30 minutes with a dual fluorescent stain (Live/Dead from Invitrogen) and then imaged using a fluorescent microscope. Protein release from the scaffold was evaluated by the addition of nuerotrophin-3 (NT-3) to the copolymer solution prior to re-suspending the cells. Samples were removed from the media at desired times, centrifuged and analyzed using ELISA.

Results/Discussion: The viability of the copolymer-cell system is a critical element to the design and was evaluated using the common dual fluorescent stain for live-dead cells. The assay shows live cells under green fluorescence (Fig 1A) and dead cells under red fluorescence (Fig 1B). Figure 1 shows that after four days of incubation at 5%CO_2 and 37^{0}C the cells are still alive and attaching to the copolymer scaffold with minimal cell death. Similar results were seen up to 21 days.



A B

Figure 1

Release of the protein therapeutic neurotrophin-3 (NT-3) was evaluated for up to 7 days from the copolymer scaffold. Release was evaluated by calculating the total cumulative release and converting it the fraction released at each desired time point (figure 2, n=3). The results indicate that the hydrogel itself acts as a barrier to diffusion of the NT-3 releasing only 30% in the first 24 hrs and 70% after one week.

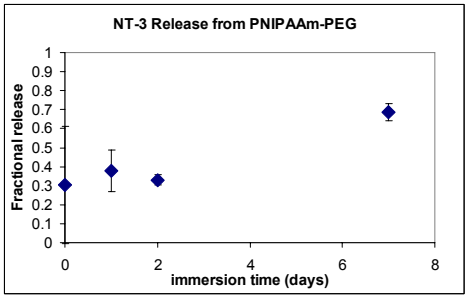


Figure 2

Conclusions/Further studies: It has been shown thus far that the copolymer matrix can form solid hydrogels that physically entrap the marrow stromal cells and allow for their attachment and survival for up to 21 days at physiological conditions. The scaffold slows the release of NT-3 from the system for up to one week but excludes about 30% of the drug almost immediately. Further studies will be done to evaluate the scaffold's ability to promote axon in growth *in vivo*. Female rats will receive a surgical hemi section injury prior to injection of the scaffold. The rats will be kept for 30 days before collecting the spine for histology.

References:

1.Latham, R., *Spinal Cord Injury: Hope Through Research*, 2003, National Institute of Neurological Disorders and Stroke: Bethesda, MD

2.Ramón y Cajal, S., 1959, New York: Hafner Pub. Co. 2 v. (xx, 769 p.).

3.Neuhuber, B., et al.,. Brain Research, 2005. 1035(1): p. 73-85.