

Minimally Invasive Delivery of Brain-Derived Neurotrophic Factor to the Brain

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Statement of Purpose: Over 15 million people suffer a stroke each year, making this disease a leading cause of permanent disability¹. The main cause of stroke is an interruption in blood flow to the brain, which destroys brain tissue and results in death or memory and function deficits. Though there are ways to limit the damage caused by stroke, there are no treatments that promote repair of damaged brain tissue¹. Administration of brain-derived neurotrophic factor (BDNF) has been shown to promote neural stem/progenitor cell survival, growth and differentiation, encourage synaptic plasticity in animal stroke models, as well as promote the survival of mature neurons that surround the stroke site². However, current BDNF delivery techniques are inefficient, invasive, or both³. A minimally invasive strategy is required that will deliver BDNF to the brain in a localized and sustained manner.

The Shoichet lab has developed a novel drug delivery strategy consisting of protein-loaded poly(lactic-co-glycolic acid) (PLGA) nanoparticles dispersed in HAMC, a hydrogel composed of hyaluronan (HA) and methyl cellulose (MC). We hypothesize that placing this BDNF-loaded PLGA-HAMC composite on the surface of the cortex will result in sustained release of bioactive BDNF with minimal trauma to the surrounding tissue. We further hypothesize that this will promote neural tissue repair and improve functional recovery in a rat model of stroke injury.

Methods: A double emulsion technique was used to encapsulate BDNF in PLGA nanoparticles. The particles were collected and washed via centrifugation, and were characterized by dynamic light scattering. Encapsulation efficiency was determined by extracting BDNF from a sample of the particles, determining the quantity of BDNF via an enzyme-linked immunosorbent assay (ELISA) and comparing the achieved loading to the theoretical loading.

HAMC hydrogels were prepared by blending hyaluronan and methyl cellulose in artificial cerebrospinal fluid (aCSF). The nanoparticles were mixed with the HAMC, the composite was injected into the bottom of an eppendorf tube, and aCSF was added on top of the composite. The release was carried out at 37 °C. The released BDNF was measured by ELISA and the cumulative mass of BDNF at each sample point was plotted against time.

The bioactivity of the released BDNF was demonstrated using a dorsal root ganglion (DRG) bioassay. DRG were incubated with samples of released BDNF for 24 hr, then imaged to quantify process outgrowth.

Results and Discussion: Nanoparticles were created with a BDNF encapsulation efficiency of $47\% \pm 7\%$. Particle size was determined to be $158 \text{ nm} \pm 7 \text{ nm}$ with a distribution of 0.049. The *in vitro* release study of BDNF from the PLGA-HAMC composite is shown in Figure 1.

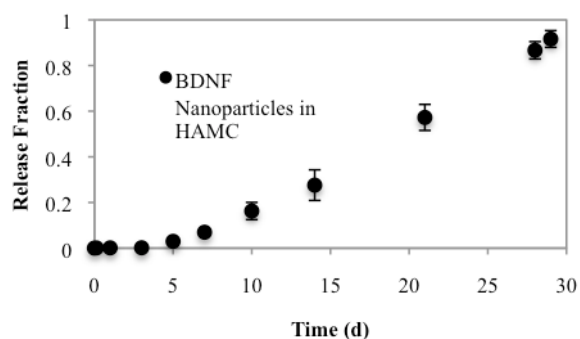


Figure 1. Release of BDNF from the PLGA-HAMC composite into aCSF at 37 °C.

DRG cultured with BDNF release samples showed increased process outgrowth compared to the controls that were not cultured with BDNF, shown in Figure 2.

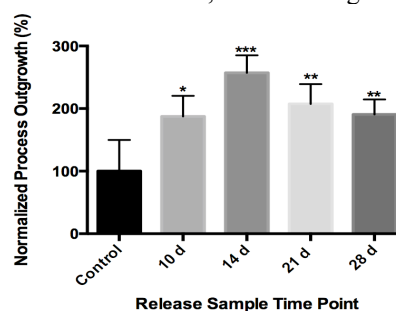


Figure 2. Normalized process outgrowth from DRG cultured with BDNF release samples for 24 hr.

The *in vitro* release of BDNF from the PLGA-HAMC composite is sustained, with no initial burst release. BDNF is steadily released into aCSF over the 28-day time period, with a 4-day delay in the onset of release, potentially due to the interaction of BDNF with the PLGA nanoparticles.

DRG cultured with BDNF release samples were shown to have increased process outgrowth, demonstrating that the released BDNF is bioactive. The processes were confirmed to be neurites through immunocytochemical staining with NF200.

Conclusions: These results validate the potential of this delivery strategy for sustained, local delivery of bioactive BDNF to the brain. Future studies will investigate the diffusion of BDNF from the composite through rat brain tissue and the consequent therapeutic benefit.

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References:

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