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

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Introduction:

Administration of brain-derived neurotrophic factor (BDNF) has been shown to promote neural stem/progenitor cell survival, growth and differentiation, and encourage synaptic plasticity in animal stroke models. These combine to enhance learning, memory and sensorimotor recovery, making BDNF a potential treatment for stroke in humans [1]. However, current BDNF delivery techniques are inefficient, invasive, or both [2]. A minimally invasive strategy is required that will deliver BDNF 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 physically blended hyaluronan (HA) and methyl cellulose (MC). PLGA nanoparticles are easily made, biodegradable, and slow the release of drug. HAMC is shear thinning and inversely thermal gelling, which allows it to be injected and gel upon injection into physiological environments. Surrounding the drug-loaded nanoparticles with HAMC reduces the initial burst release that is characteristic of nanoparticle drug delivery [3], and also allows precise placement of the composite on the surface of the cortex. We hypothesize that BDNF will diffuse from the composite, through the brain tissue and promote neurogenesis by stimulating endogenous neural stem/progenitor cells.

Materials and Methods:

BDNF Nanoparticle Encapsulation

A double emulsion water/oil/water technique was used to encapsulate BDNF in PLGA nanoparticles. An aqueous phase of aCSF containing BDNF and bovine serum albumin (BSA) was added to an organic phase consisting of dichloromethane, PLGA and Pluronic F-127 to create the first emulsion. This mixture was then vortexed and sonicated. Polyvinyl alcohol (PVA) (2.5 wt%) was added to create the second emulsion, which was vortexed and sonicated a second time. The final mixture was added to additional 2.5 wt% PVA and left to stir overnight to allow solvent evaporation. The particles were collected and washed via centrifugation, and they 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 immunosorbant assay (ELISA) and comparing the achieved loading to the theoretical loading. Total protein encapsulation efficiency was determined in a similar manner using a micro bicinchoninic acid (μBCA) assay.

In Vitro Release Characterization

HAMC hydrogels were prepared by blending hyaluronan and methyl cellulose in artificial cerebrospinal fluid (aCSF). The nanoparticles were loaded into HAMC by physical mixing and the composite was injected into the bottom of an eppendorf tube. For the release media, aCSF was added on top of the composite and the tube was placed in a 37^{o}C incubator. BDNF release into aCSF was measured by ELISA over a period of 16 days. The cumulative quantity of BDNF was determined at

each sample point and plotted against time to characterize the release profile of the drug from this specific complex *in vitro*.

Results:

Nanoparticles were created with a BDNF encapsulation efficiency of 20.3%, and a total protein encapsulation efficiency of 73.7%. Particle size was determined to be 158.6 nm with a PDI of 0.049. The *in vitro* release study of BDNF from the HAMC-PLGA composite showed a slight burst release, followed by a fairly linear trend (Figure 1).

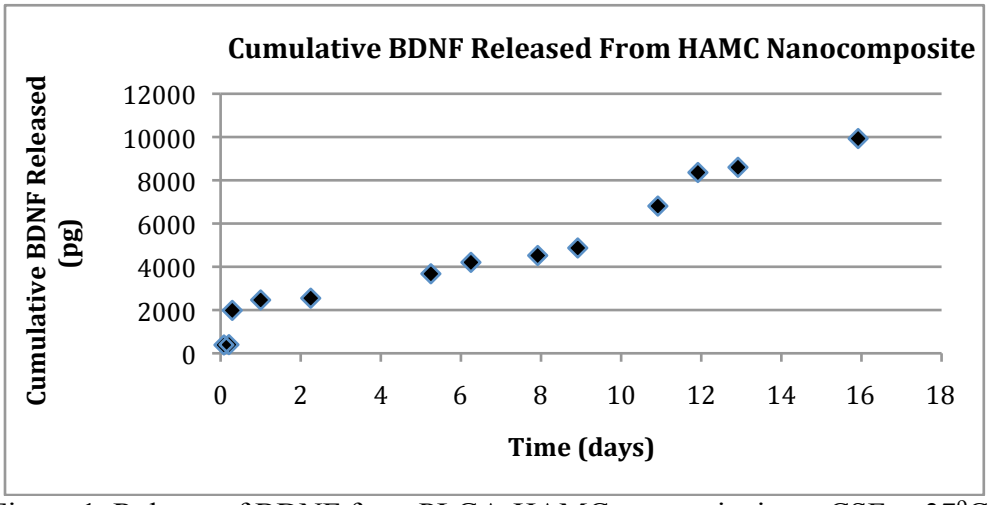

Figure 1. Release of BDNF from PLGA-HAMC composite into aCSF at 37°C.

Discussion:

The preliminary studies are promising and will be repeated in order to perform the appropriate statistics for the data. There are many variables that affect the encapsulation efficiency of BDNF, such as sonication time, and additives like magnesium carbonate for buffering of PLGA degradation. There is also the possibility that the extraction procedure is detrimental to the protein. Future studies will investigate these factors with goal of achieving increased drug loading and release of bioactive protein. The release profile is consistent with what was expected, based on previous studies; a slight burst release followed by sustained, linear release that implies first order release kinetics. In ongoing studies, the release of bioactive BDNF will be studied both in vitro and in vivo.

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