Controlled Delivery of Epidermal Growth Factor for Neural Stem Cell Stimulation after Stroke

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Statement of Purpose: Stroke is a neurodegenerative disorder resulting from lack of blood supply and oxygen in the brain. It is the 3rd leading cause of death worldwide[1]. There is currently no cure for stroke, and the only approved treatment, tissue plasminogen activator (tPA), has limited therapeutic benefit. Regeneration of lost brain tissue is a treatment approach that may facilitate functional recovery. This may be achieved *via* the delivery of growth factors to stimulate endogenous brain neural stem cells in the subventricular zone (SVZ) lining the lateral ventricles. Epidermal growth factor (EGF) is a potent mitogen that has shown therapeutic benefits towards this end[2]. The clinical relevance of this treatment, however, is hampered by the inability of current drug delivery systems (DDS) to administer protein drugs to a target site in the brain in a minimally invasive manner, and their failure to control delivery to ensure maximum efficacy. Polymeric scaffolds, often used in drug delivery for their biocompatibility and tunable drug release and mechanical properties, may be used to overcome these difficulties. Our *goal* is to develop a minimally invasive biodegradable polymeric DDS to localize and sustain the delivery of EGF in the brain to stimulate tissue regeneration after stroke.

Methods: A composite polymeric DDS was developed where EGF is encapsulated in poly(lactide-co-glycolide) (PLGA) nanoparticles through double-emulsion, solvent evaporation to temporally sustain delivery. An aqueous solution of EGF was emulsified into an organic phase of PLGA in dichloromethane, and this primary emulsion was again emulsified into an outer aqueous phase. The protein-containing nanoparticles were incorporated into a biodegradable injectable hydrogel blend of hyaluronan (HA) and methylcellulose (MC) (HAMC) to localize EGF delivery. The *in vitro* delivery of EGF was monitored over time using the microBCA colorimetric protein assay. The stability of EGF during the encapsulation process was monitored over time as the quantity of soluble protein remaining. To improve the stability of EGF during the encapsulation process, poly(ethylene glycol) (PEG) was conjugated to the protein in a N-terminus site-specific manner using aldehyde-amine chemistry. The bioactivity of EGF released from the system over time was monitored *in vitro* using a neurosphere assay and an MTT metabolic assay with mouse neural stem cell cultures.

Results: The effects of various parameters on the microencapsulation process were examined. The volume of the inner aqueous phase, the initial loading of EGF, the concentration of PLGA in the organic phase, and the composition of the outer aqueous phase were controlled to yield the optimum EGF encapsulation efficiency and release profile. EGF may be encapsulated in our PLGA nanoparticles with a loading of 1.4% protein/polymer ratio and an encapsulation efficiency exceeding 50%, both exceeding previously reported values[3]. A therapeutically relevant 7-day linear release may also be obtained from the composite DDS *in vitro* (Fig. 1), which does not exceed the *in vitro* degradation time of HAMC.

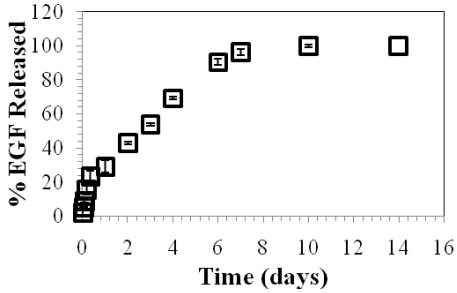

Figure 1. EGF release profile *in vitro*.

Additionally, the conjugation of PEG to EGF was found to improve the protein stability during the encapsulation process, as shown by the amount of soluble protein remaining over time (Fig. 2). Furthermore the EGF released remained bioactive over the 7 days (Fig. 3).

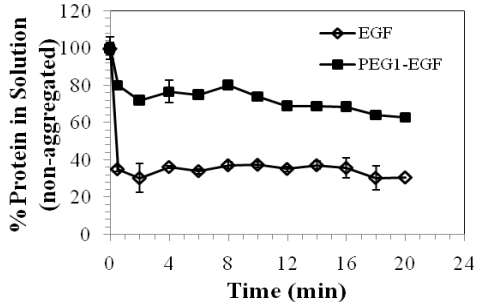

Figure 2. Stability of EGF during microencapsulation

	# of Neurospheres / Well (24 well plate)	
	Day 1	Day 7
EGF incubated at 37°C	90	79
EGF released from PLGA particles	52	15
EGF solution prepared fresh	86	75

Figure 3. Bioactivity of EGF released from DDS

Conclusions: We found that EGF may be encapsulated in our composite DDS with high encapsulation and loading, and a desired release profile may be obtained. Site-specific conjugation of PEG improved the stability of EGF, and the bioactivity of the protein is preserved over the 7-day release period.

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References: 1. Wiltrout C. Neurochem Int 50 (2007) 1028 – 1041. 2. Kolb B. Jour Cereb Blood Flow and Metabolism (2007) 27, 983 – 997. 3. Kim T. H. Biomaterials 23 (2002) 2311–2317.