Polymersomes: Towards Treatment of Neurodegenerative Disorders through Enzyme Replacement Therapy

Jessica M. Larsen[1, 2, 3], Elizabeth E. Pearce[2], Douglas R. Martin[1, 3], Mark E. Byrne[2, 3, 4]

[1] Scott-Ritchey Research Center and Department of Anatomy, Physiology, and Pharmacology, College of Veterinary Medicine

[2] Biomimetic & Biohybrid Materials, Biomedical Devices, and Drug Delivery Laboratories, Department of Chemical Engineering

[3] US Dept of Education GAANN Graduate Fellowship Program in Biological & Pharmaceutical Engineering. Auburn University

[4] Biomimetic & Biohybrid Materials, Biomedical Devices, and Drug Delivery Laboratories, Department of Biomedical Engineering, Rowan University

Statement of Purpose: Delivery of therapeutics to the brain through noninvasive administration is a difficult task due to the presence of the blood-brain barrier (BBB) [1]. Currently explored treatment methods for GM1 gangliosidosis involve invasive cranial injections of adeno-associated viral vectors in various neural locations in a feline model. Although success has been demonstrated, intravenous delivery of the missing enzyme, β-galatosidase, would be ideal treatment for the juvenile patients. We are designing and characterizing polymersomes, due to their high physiological stability and tunable release mechanisms, for enzyme delivery via intravenous methods. When coupled with either mannitol or a low density lipoprotein receptor, delivery through the BBB and into the lysosome of neural cells is expected. The ability to encapsulate and maintain stability is an important consideration with bio-therapeutic delivery. Mannitol and inulin encapsulation was explored as potential lyoprotectants for polymersome and enzyme.

Methods: With DI water as a solvent, polyethylene glycol-b-poly(lactic acid) (PEG-b-PLA) block copolymer has been proven to self-assemble, forming vesicle structures. Particle size distributions (PSD) were determined using dynamic light scattering (DLS) techniques using a Malvern Zetasizer Nano. The use of 0.45 and 0.80 µm microporous membranes was employed for polydisperse sample separations. After formation, assembly was stopped by liquid nitrogen. Structural confirmation was obtained using transmission electron microscopy. After freeze-drying, PSDs were repeated. Analysis of encapsulant loading at 2, 5, and 8 wt%/v was done using Fourier transform-infrared (FT-IR) spectroscopy, DLS, and Karl Fischer titration. Stability studies were completed in a physiologic buffer of Dulbecco's Phosphate-Buffered Saline with 10% serum, tris buffer with a pH of 7.4 to mirror physiologic conditions, and an acetate buffer with a pH of 4 to mirror lysosomal conditions. Measurements were taken every five minutes for a total period of one hour.

Results: PEG-b-PLA has been proven to self-assemble, forming vesicle structures at an average minimum diameter of 216.2 nm ± 12.9 nm. Empty polymersomes lost their size properties after lyophilization. Measuring the PSD of empty polymersomes before and after lyophilization showed a normalization value of 4.63 ± 0.01. This measurement allows for the maintenance of properties during necessary processing to be determined[2]. This discrepancy led to the introduction of both mannitol and inulin at 2, 5, and 8 wt%/b to aid in the control of diameters of polymersomes. FT-IR spectroscopy was performed, showing broad peaks in the OH bond region of 3800 to 3000 cm^{-1} with inulin and mannitol introduction, but no peak in this thumbprint region with empty polymersomes. Figure 1 shows that the PSDs of empty polymersomes and polymersomes with mannitol and inulin at all concentrations after the use of membranes. The smallest average diameter found when inulin and mannitol were encapsulated was 188.6 ± 38.6 nm and 148.2 ± 20.9 nm respectively. Karl Fischer titration determined the lowest moisture content after lyophilization, 1828 ± 362 ppm, to occur with 8 wt%/v inulin. The lowest moisture content found with mannitol-filled polymersomes was 5155 ± 1987 ppm at a concentration of 2 wt%/v. Studies in physiologic buffer show that transmittance remains at one at 200 nm with all concentrations of mannitol encapsulation and concentrations of polymersomes in serum at 2, 4, and 8 mg/mL. Preliminary data suggests serum stability of empty and encapsulated polymersomes.

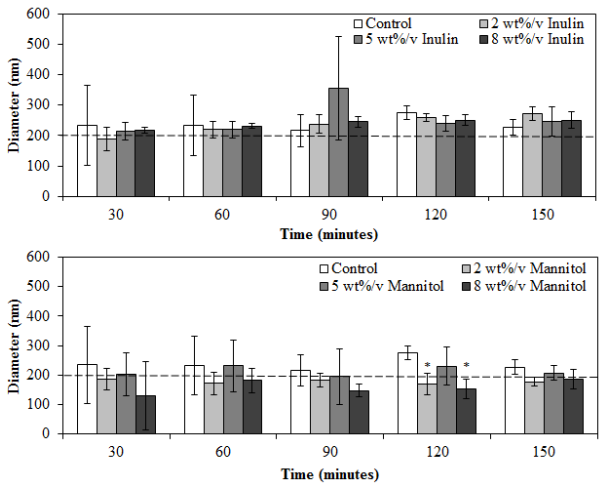

Figure 1. PSD of Polymersomes with Mannitol and Inulin

Conclusions: Incorporation of both mannitol and inulin was confirmed using Fourier transform- infrared spectroscopy, with absorbance increasing with increasing molecule concentration. The PSDs of polymersomes were not negatively affected by the incorporation of molecules, with no statistically larger diameters found over a period of 2.5 hours when compared to empty polymersomes. At 120 minutes, the incorporation of mannitol at both 2 wt%/v and 8 wt%/v lead to polymersome diameters statistically smaller than empty polymersomes at the same time point. Karl Fischer titration showed that increasing inulin concentration decreased the moisture content found after lyophilization, which is beneficial to encapsulation of enzymes, whose activities are vastly affected with crystallization. The introduction of these molecules led to reproducible effects with respect to size control, allowing for the best opportunity to cross the BBB.

References: [1] Khawli L & Prabhu, S. Mol Pharm. 2013. [2] Ayen W. Eur J Pharm Sci. 2012.