Elastin-like Polypeptide Microspheres: A Stimuli-Responsive Vehicle for Controlled Drug Delivery

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Statement of Purpose: Current treatment choices for liver cancer such as surgical resection or systemic chemotherapy have limited effect, in part, due to rapid tumor recurrence and undesired side effects associated with high-dose chemotherapy. Because of liver's atypical blood circulation, wherein the hepatic artery feeds cancer cells, delivering large amounts of drug directly to the tumor is possible by chemoembolization, by using large, drug-laden microspheres placed directly into the blood vessel feeding the tumor. The objective of this study is to synthesize microspheres of tunable size and release kinetics that can be used to deliver drugs and biologics in a loco-regional fashion. A novel protein dehydration methodology of Microglassification™ is used to fabricate microspheres[1] composed of a thermally responsive recombinant biopolymer – elastin-like polypeptide (ELP). ELPs have an intrinsically controllable insoluble-soluble switch that can be exploited to stabilize microspheres and control drug release by coacervation at body temperature.[2] The effects of fabrication parameters on particle size, thermal transitions, and protein secondary structure have been analyzed. Moreover, the tunability of microsphere dissolution has been realized using kosmotropic salts.

Methods: To evaluate the feasibility of microglassifying ELP, a single ~100 µm droplet of ELP dissolved in DI water (2.0 mM) was expelled in the dehydrating medium (n-octanol) using a micropipette, and the time-dependent dissolution of the droplet was recorded. A microfluidics platform was employed to bulk-produce microspheres, and the effect of ELP concentration (0.5-2.0 mM) on microsphere size and was analyzed using ImageJ. The flow rates of both phases were independently varied to obtain emulsion droplets, which underwent Microglassification™ to form dehydrated particles. The microspheres were recovered by centrifugation, washed in n-hexane, and stored under vacuum. The secondary structure of ELP before and after Microglassification™ was determined by FTIR. To control microsphere dissolution, we incorporated $(NH_4)_2SO_4$, a kosmotropic salt (5-50 mM), during microsphere synthesis. ELP transition temperature (T_t) was determined by heating 0.5 mM of ELP microspheres in PBS in the range of 10-50 °C at 2 °C/min using DSC (n=5). Dissolution profile was evaluated by immersing 12.5 mg (0.5 mM) of dried ELP microspheres in 1 mL of pre-warmed PBS at 37 °C for 15 days (n=5). 20 µL of the immersing solution was replaced with PBS at regular time intervals, and ELP concentration was determined using UV absorbance spectroscopy.

Results: Micropipette analysis established that ELP microdroplets were successfully Microglassified™ in the form of a solid microsphere having a concentration of ~1100 mg/mL within 49s. Image analyses of microfluidics-based microsphere formation (Fig. 1a) showed that the variation in particle size was concentration dependent; the sizes of ELP microspheres obtained using 0.5, 1.0, and 2.0 mM ELP solution were 15 ± 4, 19 ± 4, and 21 ± 5 µm, respectively. FTIR analyses of ELP before and after Microglassification™ showed an expected shift in Amide I position towards lower wavenumber for the latter sample; however, the secondary structure was restored upon rehydration. The average T_t of ELP microspheres decreased with increasing salt concentration; from 31.9 °C in the absence of salt, to 31.1, 29.2, and 27.8 °C in presence of 5, 25 and 50 mM salt, respectively. Dissolution studies showed that the presence of salt in ELP microspheres suppressed burst dissolution (<20%) in a concentration-dependent manner (Fig. 1b). Moreover, all samples continued to exhibit controlled material dissolution throughout the incubation period. After 15 days of incubation, samples prepared using 5, 25, and 50 mM $(NH_4)_2SO_4$ showed cumulative dissolution of 82, 69, and 50%, respectively.

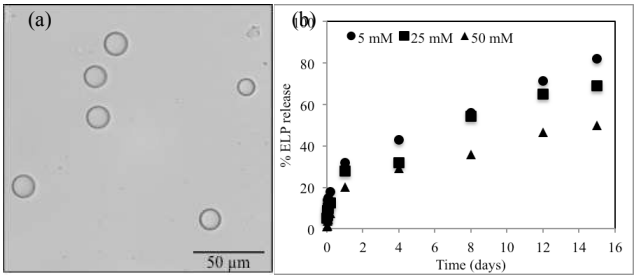

Fig. 1: (a) Optical images of Microglassified™ ELP microspheres produced on a microfluidics platform; (b) Dissolution profile of ELP microspheres in PBS.

Conclusions: Results of the study establish the feasibility of using Microglassification™ to fabricate stimuli-sensitive ELP microspheres with precise control over particle size and morphology. Single-particle analysis showed that each water-in-oil type ELP emulsion microdroplet formed in n-decanol readily lost bulk water until the water activities of both phases were in equilibrium. The T_t of ELP microspheres decreased with increasing kosmotropic salt concentration, which may have been due to the polarization of interfacial water molecules involved in hydrating amide groups on ELP.[3] Finally, ELP microspheres demonstrated low initial burst, and remarkably controllable dissolution profile upon thermal trigger in physiological media over 15 days. It is expected that ELP microspheres, upon conjugation with chemotherapeutics, can serve as a programmable drug delivery platform for local delivery to treat liver cancer.

References:

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[2] A Chilkoti. Adv Drug Deliv Rev. 2002; 54(5):613-630
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