Characterization of Polyelectrolyte-Coated Liposomes for Delivery of Estradiol

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Statement of Purpose: Encapsulation of drugs into nanoparticles is often used in pharmaceutical formulations to control release rates, affect pharmacokinetics, provide stability and decrease toxicity *in vivo*. Lipids, natural and synthetic polymers have been used to encapsulate a variety of therapeutics, including antibiotics and cardiovascular drugs (Westedt U. J Control Release. 2007; 119. 41-51). Recently, a delivery vehicle was proposed, which utilizes both lipid and polymer materials to deliver proteins (Haidar ZS. Biomaterials. 2008; 29. 1207-1215). It is comprised of a liposomal-core and polyelectrolyte (PE) multilayer shell. PE multilayers are fabricated by 'layer-by-layer', which is the stepwise adsorption of oppositely charged polymers, such as alginate and chitosan, on a charged colloidal suspension. 17β-estradiol (E2) has been shown to affect cellular apoptosis, proliferation and chemotaxis in the bone marrow stem cell niche and dose-dependently mobilize endothelial progenitor cells, which mediate cardiovascular repair (Chandrasekar B. Tromb Haemost. 2005; 94, 1042-1047). We hypothesize that a controlled delivery system of 17β-estradiol would promote progenitor cell recruitment and improve cardiovascular healing after myocardial infarction. The research in this abstract details the early design and characterization of the liposomal-core and biopolymer-shell based system for E2 delivery.

Methods: Liposomes were prepared by thin film hydration. Briefly, 1, 2-dialmitoyl-sn-glycero-3-phosphocholine (DPPC), cholesterol, didecyldimethylammonium bromide and E2 were dissolved in a solvent mixture of chloroform:methanol (4:1). Solvent was removed by rotary evaporation to achieve a thin film on a round bottom flask. The flask was then placed under a stream of nitrogen to ensure complete drying and solvent removal.

Liposomes were rehydrated with ultrapure water (UPW) for 30 min under constant rotation (60 rpm). Next, liposomes were sonicated in an ultrasonic water bath (Bransonic 2510, Branson Ultrasonic, USA) for 1 hr at 60°C and extruded through polycarbonate membrane filters (0.2 μm) with an Avanti® Mini-Extruder (Avanti Polar Lipids, USA). Blank particles without estradiol were prepared as controls. To coat the liposomes, sodium alginate was dissolved in UPW to obtain the desired concentration (1 – 2.5 mg/mL) and filtered through 0.22 μm syringe filters to remove debris and/or agglomerates. Then, liposomes were incubated with alginate (1 h). The suspension was centrifuged (15 min, 15 000g) to remove unadsorbed polymer and washed three times with UPW. Size and zeta (ζ) potential were determined using a ZetaPALS analyzer (Brookhaven Instruments, USA).

Results: Mean particle size and ζ-potential were first determined at each step in the liposome formulation protocol (Table 1). Results show that DPPC liposomes can be successfully formulated around 200 nm in diameter with a cationic charge, as determined by the ζ-potential. Particles loaded with E2 did not show any differences in ζ-potential or size compared to blank particles (data not shown).

Table 1. Particle size and ζ-potential of liposomes

DPPC Liposomes	Zeta Potential (mV)	Particle Size (nm)
Rehydrated	48.27 ± 2.46	507	0.3 ± 464.5
Sonicated	51.76 ± 1.84	769	.3 ± 194.1
Extruded	43.93 ± 1.36	218	.6 ± 13.1

Next, several alginate concentrations were investigated to determine effects on particle size and ζ-potential. After incubation with alginate and washing, liposome sizes ranged from 800 to 1200 nm (Figure 1), which is likely due to the sponginess of alginate. In addition, ζ-potential was reversed, indicating that alginate was effectively adsorbed onto the liposome surface (Figure 1). Based on these results, it was determined that an alginate concentration of 2 mg/mL produced particles which show both charge reversal and minimize particle size.

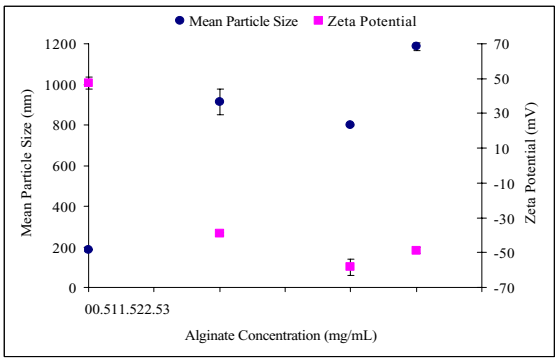

Figure 1 – Particle size and ζ-potential of alginate coated liposomes as a function of alginate concentration

Conclusions: Results from this study show that cationic liposomes can be produced with a mean size of 218.6 ± 13.6 nm after processing. Liposomes were successfully coated with alginate, as demonstrated by a reversal of ζ-potential and particle size increased significantly. Future studies will further characterize the adsorption of alginate and introduce chitosan to build PE multilayers. In addition, E2 loading and release kinetics will be investigated with the final aim of producing a controlled delivery system to promote cardiovascular healing.

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