In vitro Loading and Release Study of Liposome-based Nanoparticles for their potential use in Protein Delivery

Ziyad Haidar[1, 2], Fereshteh Azari[2], Reggie Hamdy[3], Maryam Tabrizian[1, 2].
[1] Faculty of Dentistry [2] Faculty of Medicine [3] Shriner's Hospital for Children; McGill University, Montréal (Québec) Canada

Statement of Purpose: Polymeric drug carriers for the controlled release of different drugs have attracted increasing attention in recent years. Entrapment within natural polymers remains among the most popular due to their biocompatibility, biodegradability, gentle formulation conditions and cost-effectiveness, ensuring high retention of protein viability (Mooney DJ. *Nat Biotechnol*. 2001;19**:**1029-1034). For this study, core-shell nanoparticles based on natural polymers were formulated via the layer-by-layer self-assembly technique on nanoscaled liposomes. The system was lyophilized and then loaded with a model protein. The aim of this study is to evaluate the loading capacity, encapsulation efficiency and release profile of the formulated nanoparticles.

Methods: Liposomes were prepared by the thin-film hydration technique, using an extrusion method that quickly and effectively produces reproducible unilamellar vesicles. The positively charged liposomes (core) were coated with alternating layers of negatively charged alginate and positively charged chitosan, up to a total of six layers (shell). Size and zeta potential were measured and reported elsewhere (Haidar ZS *et al. CBS*. 2006). Atomic Force Microscopy (AFM) confirmed the size and morphology of these spherical nanoparticles. Bovine serum albumin (BSA), a commonly used model protein, was incorporated upon rehydration of the lyophilized particles. For quantitative evaluation of BSA loading and encapsulation, the BSA-loaded particles were separated from the un-adsorbed protein by ultracentrifugation. Un-adsorbed BSA remaining in the supernatant was quantified using a colorimetric method (BCA protein assay – Pierce Biotechnology, Inc. USA) by reading the absorbance at 562 nm (µQuant, Bio-Tek Instruments, Inc. USA).

Results/Discussion: Liposomes with a mean diameter of 194±2.9 nm were formulated. The buildup of the hybrid coating was, evidently, accompanied by an increase in particle size, up to 341±3.6 nm after 6 polymeric layers. (Haidar ZS *et al. CBS*. 2006). AFM confirmed the size and morphology of the coated nanoparticles (Figure 1). The BSA encapsulation efficiency (EE) was affected by the initial BSA concentration, reaching its optimum with 0.5 mg/ml BSA (37.4%). However, the protein loading (LC) was enhanced by increasing the initial BSA concentration, reaching 45% for 2 mg/ml BSA (Figure 2). The BSA *in vitro* results (Figure 3) indicated that the core-shell nanoparticulate system provides a continuous release of the entrapped protein for up to 8 days.

Conclusions: Liposome-based polymeric nanoparticles demonstrated good capacity for the entrapment of proteins and provided continuous release for extended periods of time. Further work aims at optimizing the loading capacity, encapsulation efficiency and release kinetics of our system, both, *in vitro* and *in vivo*.

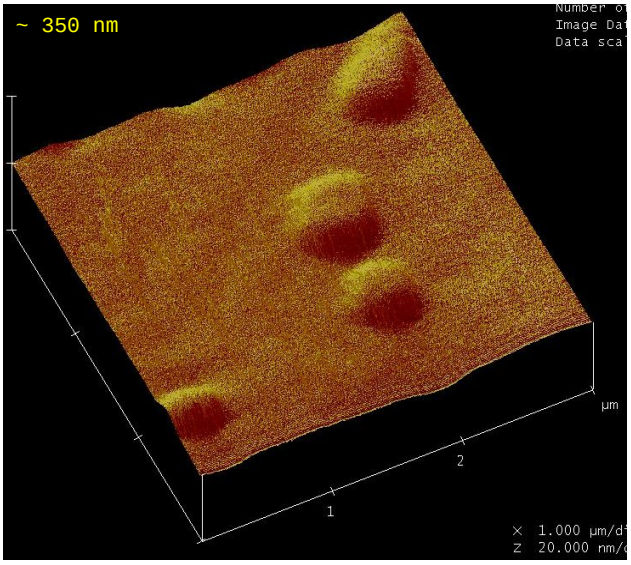

Figure 1. AFM (contact mode in liquid medium) of the liposomes coated with 6 alternating layers of alginate and chitosan

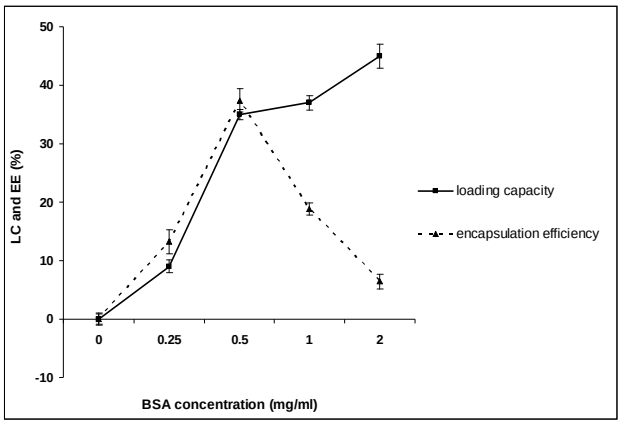

Figure 2. BSA loading capacity and encapsulation efficiency with various BSA concentrations

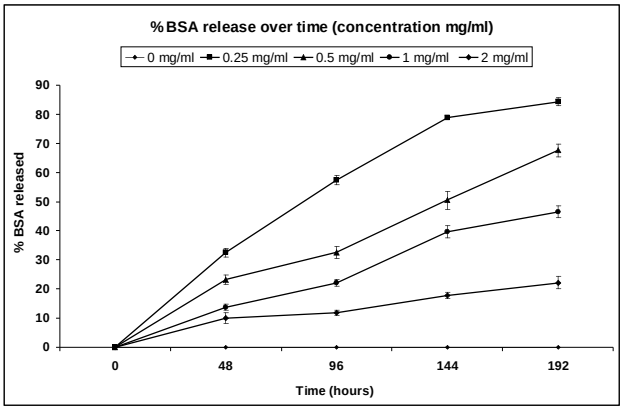

Figure 3. BSA release over a period of 8 days for different concentrations of BSA