High Throughput Evaluation of Protein Stabilization Upon Encapsulation in Polyanhydride Nanoparticles

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Statement of Purpose: Polyanhydrides are surface eroding polymers which have been studied extensively as vehicles for drug, protein, and vaccine delivery [1-3]. The polyanhydrides of interest are based on sebacic acid (SA) and 1,6-bis(*p*-carboxyphenoxy)hexane (CPH). Many drugs and vaccines are composed of fragile macromolecules which can be better stabilized by polymer carriers. However, it is imperative that the protein maintain its functionality upon encapsulation into the delivery vehicle, during storage, and upon release. Taken together with the diversity of polymer chemistries that can be used as delivery vehicles, a large parameter space must be investigated to design the delivery devices. To screen this large parameter space, the use of combinatorial libraries and high throughput techniques are invaluable resources. We have developed a high throughput method for investigating the stability of a protein upon encapsulation, storage, and release from CPH:SA nanoparticles. Using bovine serum albumin (BSA) as a model protein, we rapidly assessed structural and antigenic alterations.

Materials/Methods: Discrete polyanhydride libraries of various CPH:SA compositions were deposited at high throughput into multi-well substrates by utilizing programmable pumps in conjunction with linear actuators as described previously [5]. All libraries were characterized by high throughput FTIR with an automated mapping program. BSA was encapsulated into the polyanhydride nanoparticles via a high throughput nanoprecipitation method followed by solvent removal [5]. The dried protein-loaded particles were stored at 3 different temperatures (4, 25, and 40°C) for either 0, 1, 2, 3, or 4 weeks. After incubation for the desired amount of time, the nanoparticles were dispersed into PBS buffer (pH 7.4, 0.1 M) to allow protein release for 2 days. To investigate the effect of the acidic polymer degradation products on the stability of BSA, the protein was incubated in saturated monomer libraries for 7 days. Structural alterations of the released protein and the protein incubated with the monomer were assessed by SDS PAGE (primary structure), FTIR (secondary structure) and fluorescence spectroscopy (tertiary structure). A BSA-specific ELISA was employed to study protein antigenicity and all data was normalized by the actual protein concentration as determined by the micro-BCA assay.

Results: In this work, the effect of polyanhydride chemistry, storage conditions, and nanoparticle fabrication conditions on the stability of proteins encapsulated into and released from the nanoparticles was investigated at high throughput. Initial studies examined the stability of BSA after exposure to the nanoparticle fabrication conditions (solvent exposure, sonication, and vacuum) in which slight antigenic alterations were observed. Further studies investigated the effect of storage conditions (temperature and time) on the stability of BSA in the CPH:SA system. The CPH:SA nanoparticles proved to be a suitable protein carrier, as negligible protein alterations were observed from week 1 to week 3. However, as shown in Figure 1, after a 4 week incubation, the CPH- rich nanoparticle

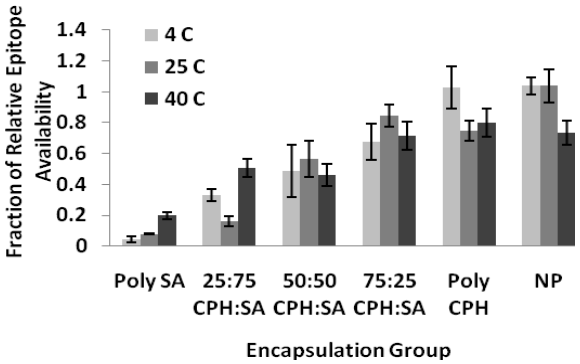

Figure 1: Antigenicity of released BSA from a library of CPH:SA nanoparticles after 4 weeks of incubation at three different temperatures.

chemistries demonstrated the ability to preserve the protein structure (compared with the native protein), while the SA-rich chemistries reduced the antigenicity of BSA. This is most likely caused by the acidic micro-environment produced by the sebacic acid.

To assess the mechanisms altering protein antigenicity during release, the pH effect was investigated by incubating the protein with saturated solutions of SA and CPH monomers for 7 days. Once again, decreasing trends in protein antigenicity were observed with high SA monomer content. Further structural analysis was carried out which indicated that significant changes were taking place in the secondary and tertiary structure of the protein. These results correlate with previous studies on PLGA and structural alterations of BSA [4].

Conclusions: The stability of encapsulated proteins is essential for the viability of a drug/vaccine delivery system. Here we have outlined a multiplexed approach for the rapid screening of the multiple parameters affecting protein stability upon encapsulation, storage and release from polyanhydride nanoparticles. This high throughput approach allowed for a detailed investigation into protein stability in which the conditions of nanoparticle fabrication and the acidic micro-environment proved to be important factors affecting protein stability. The studies identified CPH-rich chemistries as robust protein carriers capable of preserving BSA antigenicity at various temperatures, storage durations, and upon release. These high throughput methods can be invaluable in the rapid discovery and rational design of biomaterial carriers for drug, protein, and vaccine delivery.

References: [1] (Shen, EE. Biomat. 2001;22:202-210.) [2] (Determan AS. JCR. 2004;100:97-109.) [3](Kipper, M. JBMR. 2006;76A:798-810 [4] (Fu, K. Biomat. 1999;58:357-66.) [5] (Petersen, LK. JCC. 2009; accepted)