Protein Stability upon Encapsulation and Release From Polyanhydride Nanoparticles

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Statement of Purpose: Many current efforts in biotechnology are focused on the creation of vaccines based on peptide or protein subunits; this is why the design of new and effective delivery vehicles is a key objective in this area. One of the most challenging tasks in the development of protein pharmaceuticals is to deal with the physical and the chemical instabilities of the protein, which invariably lead to loss of biological activity. In order to avoid these problems, it is necessary to design vehicles that will minimize the degradation, maximize the *in vivo* activity, and provide controlled release, of the encapsulated protein. There is growing interest in the development of vaccine delivery systems based on biodegradable polyanhydrides as they have been shown to stabilize and provide sustained release of proteins and modulate the immune response[1-2]. However, the mechanisms of protein alteration are protein-specific, so it is necessary to select polymer formulations that can stabilize the specific protein of interest. The purpose of this research is to test the stability of tetanus toxoid (TT), an immunizing antigen for tetanus which caused around 130,000 neonatal deaths in 2004[3], and immunoglobulin G (i.e, anti-TT), which has been studied as a means to provide passive immune protection[4], upon encapsulation and release from polyanhydride nanoparticles.

Methods: *Nanoparticle fabrication.* Polymers based on sebacic acid (SA), 1,6-bis(*p*-carboxyphenoxy)hexane (CPH), and 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) anhydrides were synthesized. An anti-solvent nanoencapsulation method was used to fabricate TT and anti-TT loaded-20:80 CPH:SA and 50:50 CPTEG:CPH microparticles. *Protein release*. Protein release was studied using a conventional *in vitro* extraction method[2] and compared to a dialysis release method. *Protein structure and antigenicity after release.* To study the antigenicity of TT and functionality of anti-TT, an antigen-specific ELISA was used. Additionally, the primary, secondary, and tertiary structures of TT and IgG were evaluated using SDS-PAGE, circular dichroism, and fluorescence spectroscopy, respectively.

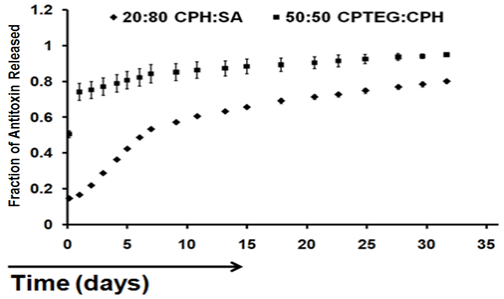

Figure 1. Cumulative release of anti-TT from polyanhydride nanoparticles. Protein release was evaluated using a dialysis release method

Results: Chemistry-dependent release profiles were observed for both proteins using both release methods (**Figure 1**). The results indicated that the proteins were released faster from the less hydrophobic 50:50 CPTEG:CPH nanoparticles. TT was detected outside the dialysis cassette, which may indicate that the protein is breaking down; however, antigenic TT was released from both polyanhydride chemistries as determined by studying the full length anti-toxin with an ELISA. Functional anti-TT was released from 50:50 CPTEG:CPH nanoparticles, however, functionality as well as primary, secondary and tertiary structures of anti-TT were affected by the 20:80 CPH:SA nanoparticles (**Figure 2**).

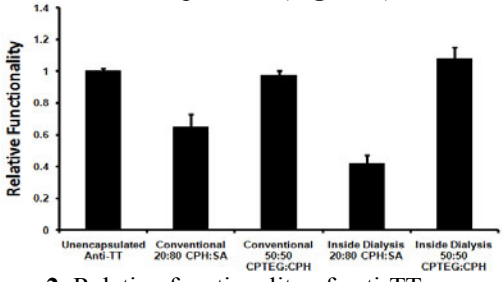

Figure 2. Relative functionality of anti-TT (determined with a TT-specific ELISA) after release from polyanhydride nanoparticles.

Conclusions: While TT was stable upon release from 20:80 CPH:SA and 50:50 CPTEG:CPH nanoparticles, the delivery of IgG as functional protein required the amphiphilic environment provided by the 50:50 CPTEG:CPH nanoparticles. These studies indicated that 50:50 CPTEG:CPH nanoparticles are promising antigen carriers for use in tetanus vaccines.

References: [1]Kipper MJ, et al. J Biomed Mater Res. 2006; 76; 798-810; [2]Carrillo-Conde B, et al. Acta Biomaterialia. 2010;6;3110-3119; WHO. Global Burden of Disease. 2004; Haigwood NL, et al. Immunology. 1996; 23;107-114