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High-throughput analysis of protein stability in polyanhydride nanoparticles

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ABSTRACT

Polyanhydrides are a class of surface eroding biomaterials with applications in vaccine and drug delivery. With the complexity and fragile nature of many protein molecules used in therapeutic treatments and vaccines, devices capable of protecting and preserving the functionality of these proteins are essential. In addition, the half-lives of many vaccine antigens and therapeutic proteins are often short, especially at elevated temperatures. In this work a high-throughput methodology has been developed to rapidly assess the effects of polymer chemistry and the various steps during protein delivery (i.e. encapsulation, storage and release) from polyanhydride nanoparticles on the stability of a model protein, bovine serum albumin. Additional factors including microenvironment pH were also investigated in this multi-parametric approach to evaluate protein stabilization. The findings indicate that the microenvironment pH caused by the acidic polymer degradation products was the most detrimental factor affecting protein stability. Nanoparticles based on 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane and 1,6-bis(*p*-carboxyphenoxy)hexane maintained protein antigenicity over a range of temperatures for 1 month. These nanoparticles were also successful in preserving protein structure and emerged as viable candidates for use in future drug/protein delivery applications. The combinatorial approach developed in this work allowed for a 25-fold decrease in time and a 10-fold decrease in the amount of materials needed for the investigation of protein stability when compared to conventional methods.

1. Introduction

The delivery of expensive and fragile protein-based drugs is challenging and poses many hurdles, including limited availability and drug stabilization upon storage and administration. Short shelf lives expedited by elevated storage temperatures limit protein functionality and use; this is especially pronounced in developing countries where resources are limited. The administration of proteins for vaccination, disease treatment and anti-cancer therapy all require protein stabilization. It is known that small structural changes can be detrimental to protein function, leading to a decrease in the efficacy of the intended treatment [1,2]. In addition, repeated drug administration or surgical implantation might pose problems with patient compliance. Thus, there is a need for delivery devices that can stabilize fragile protein molecules as well as provide a sustained release to eliminate the need for repeated administration. With the large database of readily available biodegradable polymers intended for protein stabilization and delivery, new high-throughput methodologies are needed for the discovery, testing and design of these biomaterials [3]. These approaches are emerging in the field of biomaterials [3–12] to study large numbers of biomaterials in parallel for use as drug and vaccine delivery vehicles.

Polyanhydrides are a versatile class of biodegradable materials with applications in drug and vaccine delivery [10,13–16]. The polyanhydrides of interest in this work are based on 1,8-bis(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG), 1,6-bis(*p*-carboxyphenoxy)hexane (CPH) and sebacic acid (SA); the chemical structures of the monomers are shown in Fig. 1. These polymers are composed of anhydride bonds, which undergo hydrolysis in the presence of water, as shown in Fig. 1. They erode primarily by surface erosion, which is demonstrated by the near-linear release profiles of proteins from these polymers [9,16–18,20,23]. Moreover, the protein release profiles are consistent with the erosion profiles exhibited by the polymer carriers [16,33]. Materials based on these monomers have shown promise for applications in drug delivery with their ability to stabilize and release proteins in a controlled manner ranging from days (CPTEG-rich) to weeks (SA-rich) to months (CPH-rich) [9,16–20]. Additionally, the polyanhydrides of interest can be rapidly fabricated into micro- or nanoparticles allowing for non-invasive administration via inhalation or injection. These micro- and nanoparticles are promising for applications in vaccine administration, as they possess adjuvant characteristics that may be necessary for efficacious vaccination with poorly immunogenic protein antigens. However, stabilization of the antigen is a primary concern because, without the ability to deliver a

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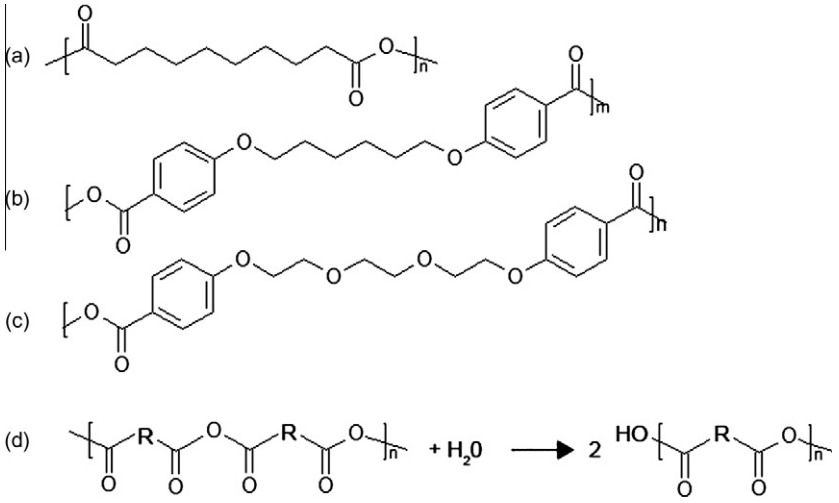

Fig. 1. Chemical structures of (a) SA, (b) CPH and (c) CPTEG; (d) polyanhydride hydrolysis mechanism.

fully functional antigen, protection may not be viable. Polyanhydride microparticles have been previously shown to stabilize protein antigens, specifically tetanus toxoid (TT), which confirmed the ability of TT-loaded CPH:SA microparticles to deliver functional antigen and modulate the immune response [13]. Given that polyanhydrides are versatile and can be tailored for specific properties (e.g. protein stability, protein release, immune activation, adjuvant capabilities) [9,10,13,16,18,19,21,22], the use of traditional, "one-sample-at-a-time" approaches has and will continue to expend large amounts of time and resources for the necessary optimization of these materials for their intended applications.

There is an urgent need to develop high-throughput approaches to screen and design delivery systems to keep pace with the rapid increase in the number of expensive, fragile, protein drugs that are in development for disease therapy and treatment [16,23]. The combinatorial approach for biomaterials design has emerged in the past decade as a viable method which allows for the use of reduced amounts of expensive proteins and accelerates the throughput and development of materials for numerous applications, including but not limited to drug delivery, vaccine design, tissue engineering and gene therapy [4–11,24–32]. More recently, combinatorial studies have been carried out for the investigation of polyanhydrides in the areas of phase behavior, drug/protein release kinetics, cytotoxicity and immune activation [10–12,32]. However, to the best of our knowledge, this approach has not been applied to study protein stability in the polyanhydride (or any other biodegradable polymer) system.

In this work, the stability of a model protein (bovine serum albumin, BSA) upon release from polyanhydride nanoparticles of various chemistries was investigated at high-throughput with an antigenicity assay. Since it is well known that protein stability could be affected by the fabrication, storage and release conditions, high-throughput methods were developed to assess BSA stability upon nanoparticle fabrication/protein encapsulation (solvent exposure, sonication and vacuum), storage (shelf life at different temperatures) and release (polymer chemistry and pH). The multi-parametric nature of this problem and the proposed high-throughput methodology is illustrated in Fig. 2. This combinatorial method allowed for a 25-fold decrease in time and a 10-fold decrease in the materials used to fabricate libraries of polyanhydride nanoparticles and enabled the simultaneous investigation of the effect of polymer chemistry, shelf life, storage temperature and microenvironment pH on protein stability. To better understand the mechanisms of instability caused by the chemistries that were detrimental to protein antigenicity, a high-throughput multi-level structural analysis was carried out, utilizing previously described automated synthesis and fabrication techniques to determine the source of protein degradation and improve the throughput of the experiments. The use of the combinatorial approach will lead to a more in-depth understanding of the relationship between protein stability and the multiple steps in the delivery process, ranging from fabrication to administration. This will therefore help expedite the rational design and development of biomaterial carriers for protein stabilization and delivery.

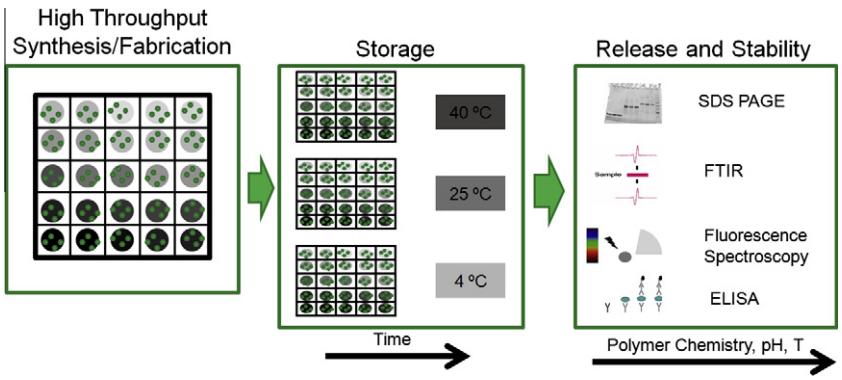

Fig. 2. Schematic describing the high-throughput methodology for studying protein stability with polymer nanoparticle libraries from the initial synthesis/fabrication to storage over time at variable temperatures to the release and subsequent level of stability as affected by pH, temperature and polymer chemistry. This process allows for a 25-fold savings in time and 10-fold savings in materials compared with conventional techniques.