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Immunogenicity of pulsatile-release PLGA microspheres for single-injection vaccination

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ABSTRACT

The World Health Organization's Expanded Programme on Immunization has led to a dramatic rise in worldwide vaccination rates over the past 40 years, yet 19.4 million infants remain underimmunized each year. Many of these infants have received at least one vaccine dose but may remain unprotected because they did not receive subsequent booster doses due to logistical challenges. This study aimed to develop injectable controlled release microparticles with kinetics that mimic common vaccine dosing regimens consisting of large antigen doses administered periodically over the course of months in order to eliminate the need for boosters. Sixteen poly(lactic-co-glycolic acid) (PLGA) microsphere formulations containing bovine serum albumin (BSA) as a model vaccine antigen were screened in vitro to determine their respective release kinetics. Three formulations that exhibited desirable pulsatile release profiles were then selected for studying immunogenicity in mice. Two low-dose microsphere formulations induced peak anti-BSA IgG antibody titers of 13.9 ± 1.3 and 13.7 ± 2.2 $\log_2$ compared to 15.5 ± 1.5 $\log_2$ for a series of three bolus injections delivered at 0, 4, and 8 weeks with an equivalent cumulative dose. Similarly, high-dose formulations induced peak antibody titers that were 16.1 ± 2.1 $\log_2$ compared to 17.7 ± 2.2 $\log_2$ for controls. All three microparticle formulations studied in vivo induced peak antibody titers that were statistically similar to bolus controls. These results suggest that pulsatile antigen release from polymeric microparticles is a promising approach for single-injection vaccination, which could potentially reduce the logistical burden associated with immunization in the developing world.

1. Introduction

Despite the immense increase in vaccine coverage worldwide over the past four decades, vaccine-preventable infectious diseases still claim the lives of approximately 1.5 million children each year [1]. However, these deaths are not due to inadequate vaccine function, but rather inadequate distribution and administration of vaccines – especially in some areas of the developing world. Although nearly 86% of infants are fully immunized against diphtheria, tetanus, and pertussis, 19.4 million infants remain underimmunized against these pathogens [2]. Of these infants, 6.6 million have received at least one dose of the vaccine, but remain at-risk because they did not receive a full series of doses (DTaP3) due to limited healthcare access or other socioeconomic factors [3–5]. Unfortunately, a single bolus administration is not typically adequate to ensure robust and durable immunity [6].

Microparticle-based controlled release of vaccines may present an option for achieving immunity after only one administration [7]. These devices, which release antigen over time, could eliminate need for booster injections, thereby reducing the logistical barrier by two-thirds and completely eliminating dropout for many vaccines [8]. Over the past 35 years, researchers have attempted to create polymeric systems capable of extended antigen release to provide immunity after only one injection [9]. Poly (lactic-co-glycolic acid) (PLGA) microparticles have been widely used in these systems owing to their precedence in existing biomedical products and tunable release kinetics [10,11]. These microspheres can be delivered in a single injection and release their contents over days, weeks, or months depending on their properties. Further, depending on their composition and

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fabrication parameters, PLGA microspheres can be designed to obtain near zero-order, first-order, or pulsatile release kinetics [12–18]. Although there is some evidence that alternative antigen presentation kinetics result in strong immune responses [19,20], pulsatile antigen release that best mimics bolus dosing regimens known to be safe and effective may be desirable [21].

Several groups have reported on pulsatile release from PLGA microspheres in vitro [16–18], but equivalent in vivo studies have only begun recently [22]. Herein, we describe the development, in vitro release kinetics, and in vivo immunogenicity of PLGA microsphere formulations that release bovine serum albumin (BSA) in a series of pulses after administration. Pulsatile microsphere development focused on utilizing the inherent bulk eroding properties of PLGA, which yield tri-phasic release kinetics [21,23]. The initial burst can be attributed to the release of antigen from the microparticle surface, the second to antigen diffusion through porous microparticles, and the third to antigen release during structural degradation of microparticles. We hypothesized that by changing polymer composition (e.g. lactic-to-glycolic acid ratio, end group), polymer molecular weight, and antigen loading, we could adjust these bursts to occur at desired intervals. Serum antibody titers from animals treated with BSA-loaded microspheres were compared to those from animals treated with a series of bolus BSA injections representative of a common immunization schedule.

2. Materials and methods

2.1. Materials

Poly(D,L-lactic-co-glycolic acid) (PLGA Resomer® RG 502 H, RG 503 H, RG 504 H, and RG 752 H) and BSA were purchased from Sigma-Aldrich (St. Louis, MO). Poly(vinyl alcohol) (PVA, Mw = 25,000) was purchased from Polysciences, Inc. (Warrington, PA). Dichloromethane (DCM) and 2,2,2-trifluoroethanol (TFE) used in this study were reagent grade.

2.2. Microsphere fabrication

Sixteen formulations of PLGA microspheres containing BSA (Table 1) were fabricated using a spontaneous single-emulsion/solvent evaporation method previously reported [24,25]. Briefly, 200 mg of PLGA were dissolved in 10 mL of 4:1 DCM:TFE and mixed with 300 μL of BSA in water. Mixing formed a clear, single-phase solution that was subsequently added to 200 mL of 5% (w/v) PVA in water. The emulsion formed spontaneously and was stirred for 3 h at room temperature. Particles were then centrifuged, washed five times with water, and lyophilized. When prepared for in vivo use, PLGA and BSA solutions were filtered through 0.2 μm polytetrafluoroethylene filters (Whatman, Little Chalfont, England) prior to forming the emulsion and mixed in a sterile laminar flow hood.

2.3. Microsphere characterization

Microsphere size distribution was determined using a Multisizer 3 Coulter Counter (Beckman Coulter, Brea, CA). Histograms were created using a bin size of 0.39 μm and smoothed using central moving average with a window size of ±5 bins. Scanning electron microscope (SEM) images were collected using a JSM-5600LV SEM (JEOL, Tokyo, Japan) at an acceleration voltage of 5 kV. Prior to imaging, samples were coated with Au/Pd using a Hummer 6.2 Sputtering System (Anatech, Battle Creek, MI) to prevent surface charging.

2.4. In vitro BSA release

Ten milligrams of microspheres were dispersed into 1 mL phosphate-buffered saline (PBS) in capped tubes and incubated on a rotating platform at 8 RPM and 37 °C. At each time point (day one, then weekly for 1–13 weeks), samples were centrifuged at 1500 RCF for 5 min, after which the supernatant was collected. Samples were then resuspended in fresh PBS and returned to the incubator for sampling at subsequent time points. BSA release from microspheres was quantified using a BCA assay kit (Thermo Scientific Pierce, Rockford, IL) and normalized to the total amount released by the end of the study. Samples were run in triplicate and data reported as mean ± standard deviation.

2.5. In vivo administration of BSA microspheres

All animal work was approved by MIT's Committee on Animal Care. Briefly, female BALB/c mice 6–8 weeks of age received injections of (1) BSA-loaded microspheres, (2) empty microspheres, (3) bolus BSA, or (4) saline. While mice in the first two groups received only one injection, those receiving a bolus BSA or saline only were injected again at 4 and 8 weeks to match the amount and timing of BSA release from PLGA microspheres in vitro. Twenty mg of BSA-containing PLGA microparticles were suspended in 400 μL of saline and half of the solution was injected subcutaneously into each hind limb. At this particle mass, mice in the low dose (0.5%) groups received 64 μg (Formulation C) or 71 μg (Formulation G) of total

Table 1
Microsphere formulations and size characterization.

Formulation	BSA (% w/w)	PLGA M_w (kDa)	PLGA ratio	Particle size (μm)	90% Threshold diameter[a] (μm)
A	5	7–17	50:50	10.5 ± 6.8	18.5
B	3	7–17	50:50	10.6 ± 6.4	18.1
C	0.5	7–17	50:50	10.3 ± 6.2	22.1
D	0	7–17	50:50	10.5 ± 5.9	17.4
E	5	24–38	50:50	8.6 ± 6.7	21.4
F	3	24–38	50:50	11.4 ± 8.3	21.4
G	0.5	24–38	50:50	12.1 ± 8.2	23.1
H	0	24–38	50:50	11.4 ± 7.2	20.2
I	5	38–54	50:50	14.1 ± 9.4	25.4
J	3	38–54	50:50	12.0 ± 7.1	20.3
K	0.5	38–54	50:50	11.9 ± 6.8	20.6
L	0	38–54	50:50	11.9 ± 6.4	19.7
M	5	4–15	75:25	11.3 ± 7.0	19.7
N	3	4–15	75:25	12.2 ± 7.4	21.2
O	0.5	4–15	75:25	12.4 ± 7.5	21.1
P	0	4–15	75:25	11.7 ± 6.5	19.9

[a] 90% of particles in the formulation are smaller than this threshold.

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