Spray-Coating for Biopharmaceutical Powder Formulations: Beyond the Conventional Scale and Its Application

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Purpose: Fluid-bed spray-coating process is widely used to prepare non-protein pharmaceutical solid dosage forms using macro-size seed particles (200–1000 μm) at kilogram batch sizes. In this study we developed a small-scale fluid-bed spray-coating process (20 g) to produce micro-sized vaccine powder formulations (40–60 μm) for epidermal powder immunization (EPI)

Methods: A bench-top spray coater was used to spray two vaccines, diphtheria toxoid (dT) and alum-adsorbed hepatitis-B surface antigen (Alum-HBsAg), onto crystalline lactose particles of 40–60 μm in diameter. Particle properties such as particle size, surface morphology, and degree of particle agglomeration were determined. Protein stability was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The immunogenicity of the vaccine was evaluated *in vivo* by needle injection and epidermal powder immunization (EPI) of mice or guinea pigs.

Results: Coating feasibility was demonstrated for both vaccine formulations containing different excipients. However, the nature of the vaccine antigen appeared to affect coating feasibility in terms of particle agglomeration considerably. Delivery of spray-coated dT and alum-HBsAg through EPI to mice and guinea pigs, respectively, generated significant antibody responses, at a level comparable to liquid formulation delivered subcutaneously through needle/syringe injection.

Conclusions: The new spray-coating process represents an important technical advance and may provide a useful tool for developing high-valued biopharmaceutical powder formulations for novel applications. The strong *in vivo* performance of the coated dT and alum-HBsAg powders by EPI further demonstrated that spray-coating is a viable dry powder formulation process and the skin's epidermal layer presents an efficient vaccine delivery route.

KEY WORDS: spray-coating, powder formulation, epidermal powder immunization, diphtheria toxoid.

INTRODUCTION

As the application of biopharmaceutical dry powder formulations grows, the needs for versatile and efficient particle formation processes increase. Some emerging novel powder formation processes were recently reviewed (1), including spray-freeze-drying (2–4), supercritical fluid methods (5–7), spray-drying (8–14), and fluidized-bed spray-coating. All these methods except spray-coating involve droplet or particle formation, where chemical components in the formulation play a critical role in determining powder characteristics. For applications requiring specific particle properties such as high density and narrow particle size distribution, coating a biopharmaceutical formulation onto a (seed) powder of well-defined particle characteristics represents a rational approach.

Fluid-bed spray coating has long been used in the pharmaceutical industry for oral solid dosage preparations of non-protein-based drugs (15,16) and is a mature technology for coating macro-particles (200-1000 μm) at large-scale production (>> 1 kg). Spray coating using 100-μm seed particles became feasible only after Würster spray coaters were developed (17–19). The only biopharmaceutical formulation investigated for spray coating was recombinant human deoxyribonuclease (rhDNase), which was coated onto lactose seed particles as small as 50–125 μm using laboratory-scale equipment (100–500 g batch size; Refs. 20, 21). For developing high-valued proteins/peptides/vaccine antigens formulations, there is a need to further reduce the batch size, in turn, to reduce development costs. In addition, some applications may require smaller particles (<100 μm) with a narrow particle size distribution. Thus, successful small-scale spray coating using micro-seed particles may extend the application of spray-coating process to pharmaceutical development.

One of the applications of the spray-coated vaccine powder is for epidermal powder immunization (EPI), a novel immunization technology that delivers powder vaccines to the epidermal layer of skin and holds promises for improving the safety and effectiveness of vaccination (22–28). Effective EPI requires powder formulations with unique particle characteristics, including a particle size of 20–70 μm and a high particle density. In this study, two vaccine antigens, diphtheria toxoid and alum-adjuvanted hepatitis-B vaccine (Alum-HBsAg), were used to coat crystalline seed particles of 40-60 μm at a batch size of 20 g using a new laboratory-scale spray coater (Fluid Air, Inc., Aurora, IL, USA).

MATERIALS AND METHODS

Materials

Chemicals and excipients used in this study are summarized in Table I. All except alum-adjuvanted HBsAg (Alum-HBsAg) were used as supplied. Alum-HBsAg was concentrated to 400 μg/mL HBsAg (10 mg/mL alum) by centrifugation (Allergra 6R centrifuge, Beckman Instrument, Palo Alto, CA, USA) before use.

Lactose monohydrate of national formulary (NF) grade was obtained from Amressco (Solon, OH, USA). This crystalline material was separated into two particle size fractions, 40–60 and 80–100 μm, using a particle classifier (Model C-1, Vortec Product Company, Long Beach, CA, USA). This classifier allows accurate separation of spherical or cubical particles of uniform density. Crystalline mannitol of USP-grade was also obtained from Amressco and classified by the same method above and evaluated as a seed powder.

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Table I. Chemicals/Excipients Used in the Study

Chemical	Lot no.	Source	Comment
Diphtheria toxoid (MW 58 kd)	G9334	Accurate Chemical and Scientific (Westbury, NJ, USA)	Manufactured by Statens Serum Institute, Denmark, and provided at 5 mg/mL (1 Lf = 3 μg), used as supplied.
Alum-adjuvanted hepatitis-B surface antigen (HBsAg)		Evans Vaccines (Speke, UK)	20 μg HBsAg adsorbed to 0.44 mg of aluminum/1.5 mg alum hydroxide.
Salmon calcitonin (MW 3,432 da)	T-24103	BACHEM (Torrance, CA)	HPLC purity >99%, used as supplied
Sodium fluorescein	08604MQ	Aldrich (Milwaukee, WI, USA)	Used as yellow dye, used as supplied
Lysozyme	57H7045	Sigma (St. Louis, MO, USA)	Used as supplied
Dextran (MW 1000 da)	E-167	Pharmacocosmos A/S (Viby Sj., Denmark)	Clinical grade, used as supplied
Mannitol	127H0960	Sigma (St. Louis, MO, USA)	Reagent grade, used as supplied
Trehalose dihydrate	28H3797	Sigma (St. Louis, MO, USA)	Reagent grade, used as supplied

Methods

Spray Coating

Coating experiments were performed using a commercially available lab-scale fluid bed coater (Model 0002, Fluid Air, Inc.). The fluid bed was set up for bottom spray coating in a 0.5-L bowl. A peristaltic pump was operated to deliver the liquid formulation at a flow rate ranging from 0.5 to 1.5 g/min through a two-fluid nozzle operated at an air pressure of 20–25 psi to produce droplets of < 15 μm. The drying/fluidization air-flow rate could be controlled up to 12 SCFM. The perforated air-distribution plate produced a spouting action of the fine powder bed in the bowl, where the agitation action was vigorous that particle agglomeration could be minimized. Inlet temperature was set at or below 50°C and the product temperature was kept below 35°C. Filter socks retained the powder with a blow-back system.

Spray Drying

A custom-made spray-dryer was used to produce large particles (>30 μm in median size). It consists of a glass chamber (6 in. in diameter and 5 ft in height) and an ultrasonic atomizer (120 kHz, Sono-Tek Corp., Milton, NY, USA). Directly under the glass chamber was a vacuum paper filter onto which the dry powder was collected. Spray-drying conditions included drying air inlet temperature of 130–140°C, liquid feed of 3.5 mL/min, and drying air outlet temperature of 80–85°C.

Powder Sieving and Agglomeration

The coated powder was weighed and then sieved through a mechanical sieve shaker (Model A200, Retsch Corporation, Haan, Germany) with 8-inch diameter sieves (Fisher Scientific, Hamilton, NH, USA). The coated powder of 40-60 μm was sieved between 53 and 75 μm, and that of 80–100 μm between 75 and 106 μm. The shaker was set at an amplitude of 2.5 mm, an interval of 5 s, and sieving time of 15 min. The powder unable to go through the sieve was weighed, and agglomeration was determined by comparing it with the initial sample weight. The powder-coated with the yellow-dyed formulation was fractionated using a laboratory sonic sifter (Model L3P, ATM Corp., Milwaukee, WI, USA). Powders collected on sieves of 38, 75, and 90 μm were weighed.

Optical Microscopy

Visual analysis of the particles was performed using an optical microscope (Model DMR, Leica, Germany) with 10× eyepiece lens and 10× objective lens. The system was equipped with a Polaroid camera system for image output.

Scanning Electron Microscopy

The external morphology of coated particles was examined using an Amray 1810T scanning electron microscope (Amray, Bedford, MA, USA). The powder sample was first sputtered coated with Au using a Hummer JR Technics unit (Pergamon Corporation, King of Prussia, PA, USA).

Particle Size Analysis

The mean geometric/aerodynamic diameter of the particles in the volume distribution was determined using a dry powder dispersion-based particle size analyzer (Aerosizer, API, Hadley, MA, USA). The mean volumetric size was calculated by the software using the density of 1.3 g/mL. The size of the particle population (volume-distribution) between 10% (D_{10}) and 90% (D_{90}) was reported for particle size distribution. Triplicate measurements were made for each sample. The standard deviation is consistently within 10% from the mean value.

Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

The dT-coated powder was reconstituted with 10 mM potassium phosphate buffer (pH 7.4) and tested for the presence of covalent aggregates via gel analysis (NuPage, San Diego, CA, USA). Bis-Tris gels (4–12%) were used with a MES running buffer. Samples for gel analysis (reduced and non-reduced conditions) were prepared as outlined in the NuPage Manual. Approximately 0.66 μg dT was loaded per lane, with a 30 μL load volume, under reduced conditions. Approximately 0.78 μg dT was loaded per lane under non-reduced conditions, also with a 30 μL load volume.

Animals, Immunization, and Serum Collection

Female 5- to 7-week-old BALB/c mice (Harlen–Sprague–Dawley, Indianapolis, IN, USA) were used to assess the immunogenicity of spray-coated dT formulation. Spray-coated formulation was reconstituted with distilled water and administered by subcutaneous injection into the scruff of the neck using a 26-1/5 needle. Each injection administered 200 μl of solution containing 1 μg of dT protein. Control mice were immunized with the same dose of untreated liquid dT vaccine. A boost immunization was administered on day 28. Prior to each vaccination and 2 weeks after boost, blood was collected via retro-orbital bleeding.

Hairless guinea pigs (Charles River, Wilmington, MA, USA) were used to assess the immunogenicity of spray-coated dT formulation when administered by EPI. The method of immunization has been previously described (22). Briefly, 0.5 mg of powder was dispensed into a trilaminate cassette. The cassette was inserted into a PowderJect® device at the time of immunization. The device was placed against the left inguinal skin of the animals and actuated by releasing the compressed helium at 40-bar pressure from the gas cylinder. Control animals were immunized with 0.20 ml of dT vaccine in saline by intramuscular injection using a 26½-gauge needle. Blood was collected via the kerotid blood vessel before each vaccination and two weeks post boost. The immunogenicity of spray-coated alum-adsorbed HBsAg was determined in mice by EPI as previously described (22).

Enzyme-Linked Immunosorbent Assay (ELISA)

The antibody response to dT or HBsAg in the mouse sera was determined using a modified ELISA method (22). A 96-well plate (Costar, Fisher Scientific Products, Pittsburgh, PA, USA) was coated with 0.1 μg of antigen in 30 mM phosphate-buffered saline (PBS), pH 7.4, per well overnight at 4°C. Plates were washed 3 times with tris-buffered saline, pH 7.4, containing 0.1% Brij-35, and incubated with test sera diluted in PBS containing 5% dry milk for 1.5 h The plates were then washed and incubated with biotin-labeled goat antibodies specific for mouse immunoglobulin IgG (1:8,000 in PBS, Southern Biotechnology Associate, Birmingham, AL, USA) for 1 h at room temperature. After three additional washes, plates were incubated with streptavidin-horseradish peroxidase conjugates (Southern Biotechnology) for 1 h at room temperature. Finally, plates were washed and developed with TMB substrate (Bio-Rad Laboratories, Melville, NY, USA). The endpoint titers of the sera were determined by 4-parameter analysis using the Softmax Pro 4.1 program (Molecular Devices, Sunnyvale, CA, USA) and defined as the reciprocal of the highest serum dilution with an OD reading above the background by 0.1. A reference serum with a pre-determined titer to dT was used on every plate to calibrate the titers and adjust assay-to-assay and plate-to-plate variation.

Antibody titers in the guinea pig sera were determined using a similar ELISA procedure with the exception that biotin-labeled goat anti-guinea pig IgG immunoglobulin (Accurate Chemical and Scientific Corp., Westbury, NJ) was used as the secondary antibody.

RESULTS

Selection of Seed Particles

As an inert carrier, the seed powder needs to satisfy some criteria such as good particle strength/integrity to sustain attrition during fluidization, appropriate surface properties/morphology to encourage fluidization, affordability, and commercial availability. Among the commonly used seed powders, such as lactose crystals (19–21), calcium carbonate (17), phenacetin (18), nonpareil (17,18), and microcrystalline cellulose (Avicel, FMC Corp., Chicago, IL, USA), crystalline lactose appears to be most appropriate for spray coating of vaccines for EPI. Crystalline lactose particles were air classified into 2 size fractions, 40–60 and 80–100 μm, and used for preparation of spray-coating formulations in Table II.

The sieved seed particles were analyzed using the time-of-flight method (Aerosizer assuming particle density of 1.3 g/mL). The measured size was consistently smaller than the sieved size for both the 40- to 60- and 80- to 100-μm fractions A difference between the measured size and the sieved size were also seen with the 80-100 μm fraction. This difference might be attributed to the non-sphericity of the crystalline lactose.

Fluorescein (Yellow-Dyed) Formulations

To aid visualization, three fluorescein-containing liquid formulations (A–C in Table II) were coated onto 40- to 60-

Table II. Excipient Composition for Spray-Coated Powder Formulations

	Lactose		Liquid formulation		
Formulation	Size (μm)	Batch size (g)	Volume (mL)	Solid content (w/v%)	Composition
A	40–60	50	10	10	Mannitol (5%) + trehalose (5%) + fluorescein (0.03%)
B	40–60	50	100	11	Lysozyme (1%) + mannitol (5%) + trehalose (5%) + fluorescein (0.03%)
C	40–60	20	25	10	Mannitol (3%) + trehalose (3%) + dextran (4%) + fluorescein (0.03%)
D	40–60	20	20	10.2	dT (0.2%) + trehalose (3%) + mannitol (3%) + dextran (4%) + Tween 80 (0.02%)
E	80–100	20	20	10.2	Same as D
F	40–60	20	23	10.3	Alum-HBsAg (0.3%) + trehalose (3%) + mannitol (3%) + dextran (4%) + Tween 80 (0.02%)
G	80–100	20	23	10.3	Same as F

μm lactose particles at the batch size of 50 g for formulations A (F–A) and B (F–B) and 20 g for formulation C (F–C). Two base excipient compositions, mannitol:trehalose (1:1, w/w) or mannitol:trehalose:dextran (3:3:4), were used throughout the study. Lysozyme was added to F–B as a model protein. The powder turned yellow and the color became more intense during coating. All three powders after coating were free flowing. Under optical microscopy, there were no obvious large agglomerates for all formulations (Fig. 1B for F–A and Fig. 1C for F–B). Particle size analysis for powders before and after coating confirms this observation although the coated particles are slightly larger (Table III). However, small particles were visible, particularly in F–A (Fig. 1B). After sonic sieving, approximately 13% of F–A passed the 38 μm sieve while only 3% for Formulation B, further demonstrating the presence of small particles. The feasibility of 20-g batch size was first tested using F–C as the coating solution consisting of trehalose, mannitol, and dextran (MW 1000 Da). Few small spray-dried particles were observed. However, the coated particles were slightly agglomerated (Fig. 1D).

Scanning electron microscopy was performed to verify the presence of the coating. The un-coated crystalline lactose shows a rough surface morphology (Fig. 2A). Based on simple calculation, as 50 g of the seed powder were coated with 1 g of F–A (Table II), the thickness of coating is < 1 μm. In F-B, the seed powder receiving 10 g of the coating material might have a coating thickness of approximately 2 μm. Although the theoretical coating thickness is quite thin, the coating is somewhat discernible because the surface of the coated particles appears to be smooth after coating (Fig. 2B for F–A and Fig. 2C for F–B).

Table III. Particle Size Analysis (Aerosizer Based on Time-of-Flight) for Powder Formulations Listed in Table II Before and After Coating

Formulation	Sieved size range (μm)	D_{10} (μm)	D_{50} (μm)	D_{90} (μm)
Seed particles	40–60	23.3	33.5	45.5
Seed particles	80–100	42.5	57.8	72.5
A	40–60	25.5	38.7	53.1
B	40–60	23.8	38.5	52.0
C	40–60	26.0	39.5	53.5
D	40–60	30.0	42.1	56.1
E	80–100	46.8	61.4	76.5
F	40–60	32.6	48.6	62.4
G	80–100	58.2	75.6	94.9

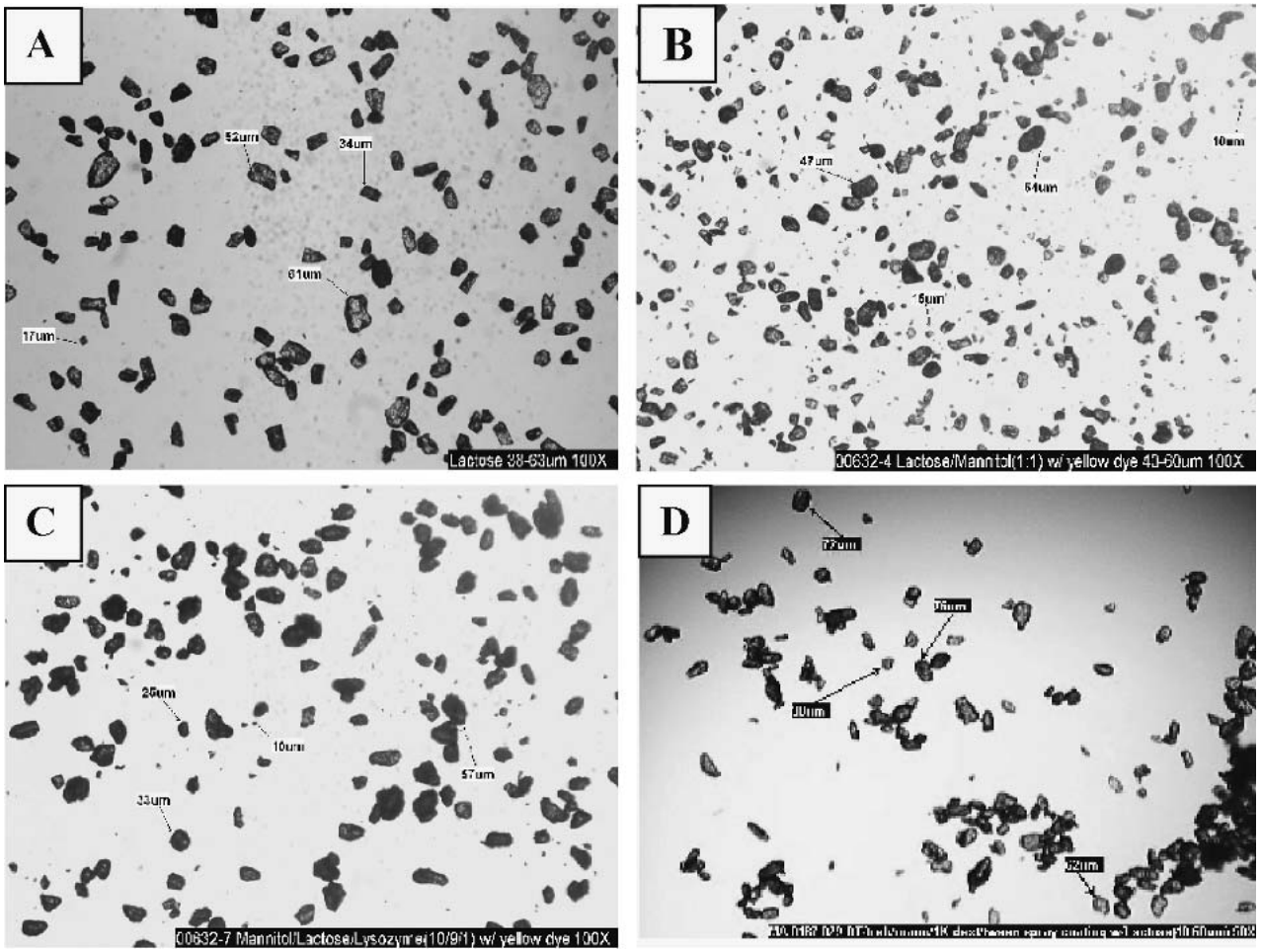

Fig. 1. Optical micrograph for the lactose powder classified into the 40- to 60-μm particle size range (A), coated with Formulation A (Table II; B), coated with Formulation B (Table II; C), and coated with Formulation C (Table II; D).

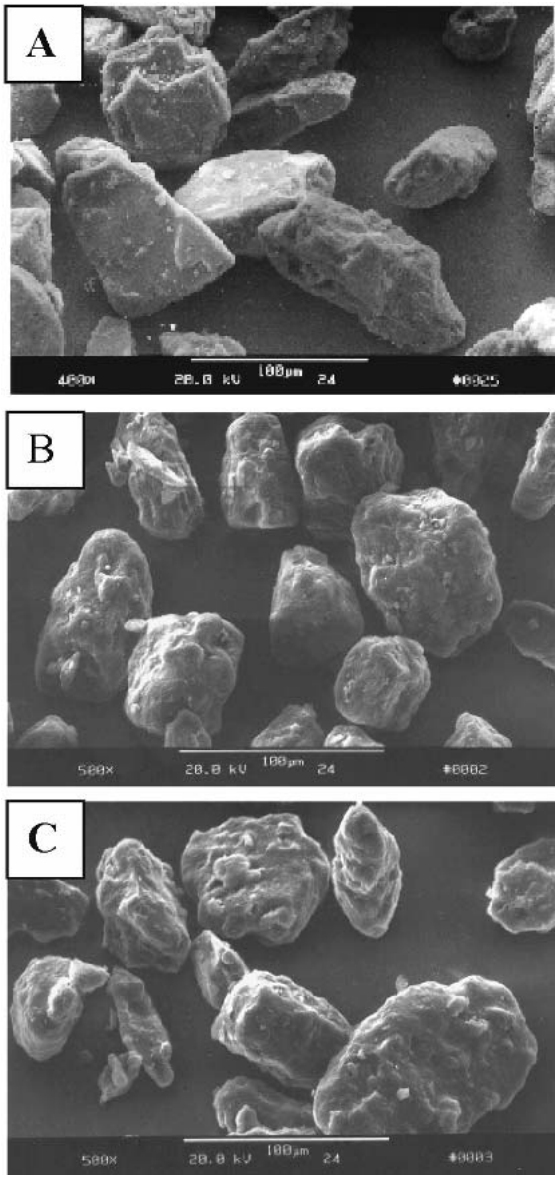

Fig. 2. Scanning electron micrographs for the lactose powder classified into the 40- to 60-μm particle size range (A), coated with Formulation A (Table II; B), coated with Formulation B (Table II; C).

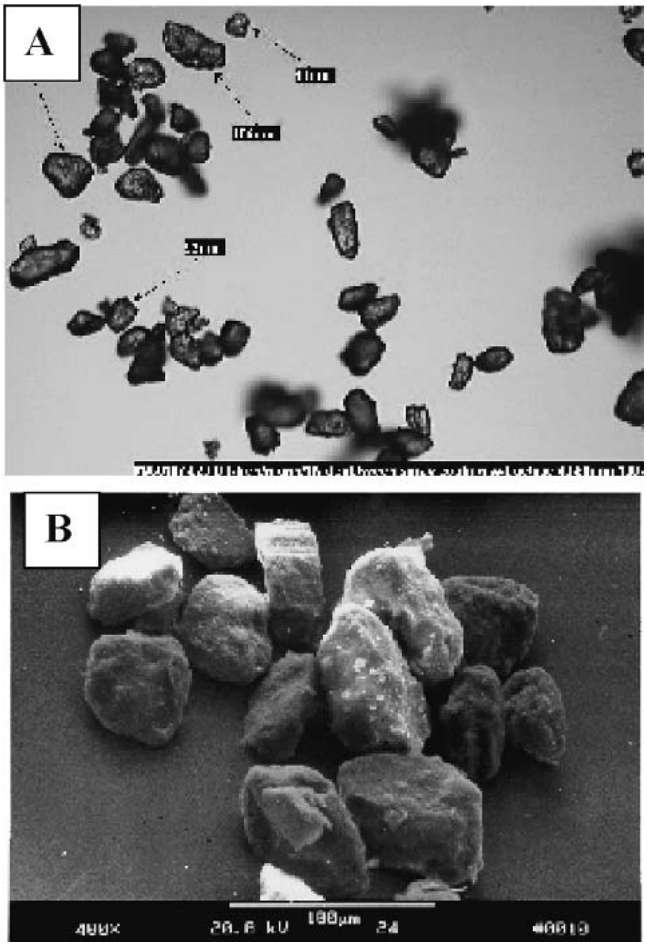

Fig. 3. Optical micrograph for the lactose powder coated with Formulation D (Table II; A) and for the same powder in (A) Scanning electron micrographs for the lactose powder coated with Formulation D (Table II; B).

Diphtheria Toxoid (dT) Formulations

As a model protein vaccine, dT formulation (trehalose:mannitol:dextran, 3:3:4) was spray coated onto lactose particles of 40–60 (F–D in Table II) and 80–100 μm (F–E in Table II), respectively. Interestingly, a small quantity of dT (0.2 w/v%) appeared to reduce the powder flow. Frequent mechanical vibration to the fluid-bed chamber was introduced to maintain fluidization during the experiment. The coated powder was slightly agglomerated. However, there was no evidence of spray-dried particulates (Fig. 3A for F–D). Sieving the coated powder resulted in < 10% (w/w) of the particles larger than 75 μm (F–D) and ≈20% of particle larger than 125 μm (F-E). These agglomerated particles were readily desegregated upon aerosolization during time-of-flight analysis where the coated particles and the particle sizes were only slightly larger than the respective starting seed powder (Table III). Coating morphology of the dT-containing formulation was smooth (SEM in Fig. 3B). According to chemical analysis by SDS-PAGE, no additional bands other than the monomeric dT (MW 58 kDa) were observed, suggesting that the coating process did not denature dT. The amount of dT recovered from the coating was consistent with the theoretical content, 2 μg per 1 mg of powder, indicating coating uniformity.

Alum-Adsorbed HBsAg Formulations

HBsAg adsorbed onto aluminum hydroxide forms a gel suspension in the liquid formulation. It presents a completely different formulation compared to the water-soluble dT formulation. Before the coating experiment, spray-drying was performed to evaluate the drying property of Formulation F (F–F in Table II). The spray-dried alum gel-containing powder was free flowing with a spherical morphology with $D_{50} = 31$ μm (Fig. 4A), which is comparable with the same formulation without alum, suggesting that aluminum hydroxide had little effect on spray-drying. However, the introduction of aluminum hydroxide-adsorbed HBsAg formulation appeared to decrease the fluidizing ability of the seed powder during spray coating (40–60 μm for F–F and 80–100 μm for

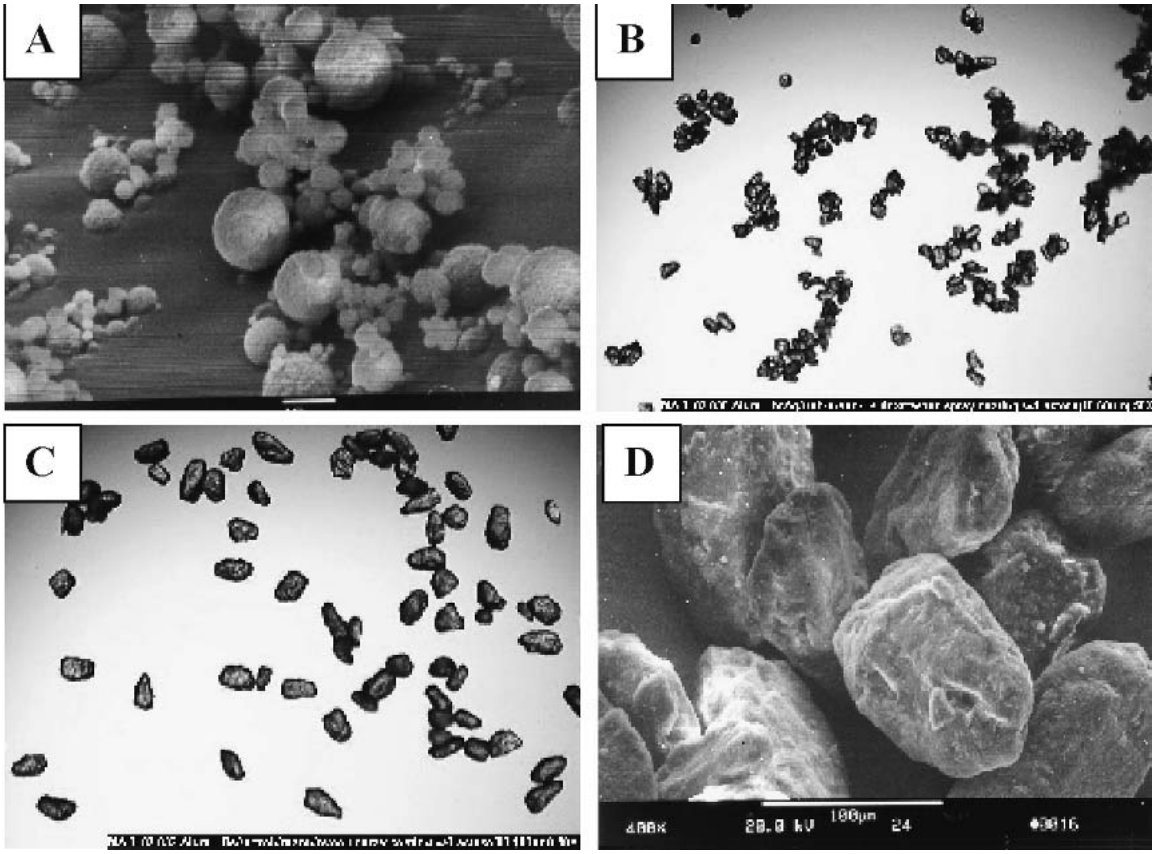

Fig. 4. Scanning electron micrographs for a spray-dried powder prepared from aluminum hydroxide (0.5%)/trehalose (7.0%)/mannitol (12.0%)/dextran 10 kDa (10.5%)/Tween 80 (0.02%; A). Optical micrograph for the lactose powder coated with formulation F (Table II; B), and with formulation G (Table II; C). Scanning electron micrographs for the lactose powder coated with Formulation F (Table II; D).

F–G in Table II), indicating the change of surface properties of the seed particles during coating. This difficulty could be partially corrected by reducing the liquid feed rate from 1.2 g/mL to 0.4 g/mL and diluting the alum concentration by 50%, from 0.3 to 0.15 w/v%. The resultant coated particles (40–60 μm for F–F) remained agglomerated (Fig. 4B). Using larger seed particles (80–100 μm, F–G) for spray coating of the same vaccine formulation improved particle flowability and reduced particle agglomeration (Fig. 4C). Particle size analysis by the time-of-flight method shows particle size increase after coating, 75.6 vs. 57.8 μm (D_{50}) for 80- to 100-μm particles and 48.6 vs. 33.5 μm for 40- to 60-μm particles, suggesting some level of aggregation. The coating appears to be uniform based on scanning electron microscopic examination (Fig. 4D). Chemical stability of HBsAg after coating was analyzed using SDS-PAGE. All bands appeared on the nonreducing and reducing gels matched those of the starting material (gel data not shown). Quantitatively, the amount of HBsAg approached the theoretical value.

Immunogenicity Study

The antibody titers against dT measured by ELISA are shown in Fig. 5A. Low but consistent antibody responses were seen in all animals following prime immunization with the reconstituted spray-coating formulation; and these titers in the post-boost sera were significantly higher. The spray-coated formulation and the untreated liquid vaccine elicited comparable dT-specific antibody titers following prime (day 28) or boost (day 42) immunization.

EPI with the spray-coated dT formulation effectively induced dT-specific antibody responses (Fig. 5B). The antibody titers increased following each immunization. Comparable antibody titers were elicited by the spray-coated formulation and the spray-dried formulations at each time point, suggesting that the spray-coated powders are suitable for EPI.

The immunogenicity of spray-coated Alum-HBsAg formulation was demonstrated in a mouse study. Mice ($n = 8$) received 0.05 μg of antigen on days 0 and 28 by EPI with spray-coated formulation (Formulation G in Table 2) or subcutaneous injection with untreated liquid vaccine. Analysis of the sera collected on day 42 by ELISA revealed that spray-coated formulation elicited a mean antibody titer to HBsAg (3000 ELISA units) that was not significantly different from the titer elicited by the untreated liquid vaccine (5000 ELISA units; $p > 0.05$, t test). This data suggest that spray-coated Alum-HBsAg formulation is stable and suitable for EPI.

DISCUSSION

The first reason for us to develop a small-scale spray-coating process using micro-seed particles is to develop powder formulations for EPI. EPI delivers vaccine powders to the skin epidermis via pressurized helium gas released from a powder delivery device. By targeting vaccines to the immune cells in the skin, EPI may offer an important efficacy advan-

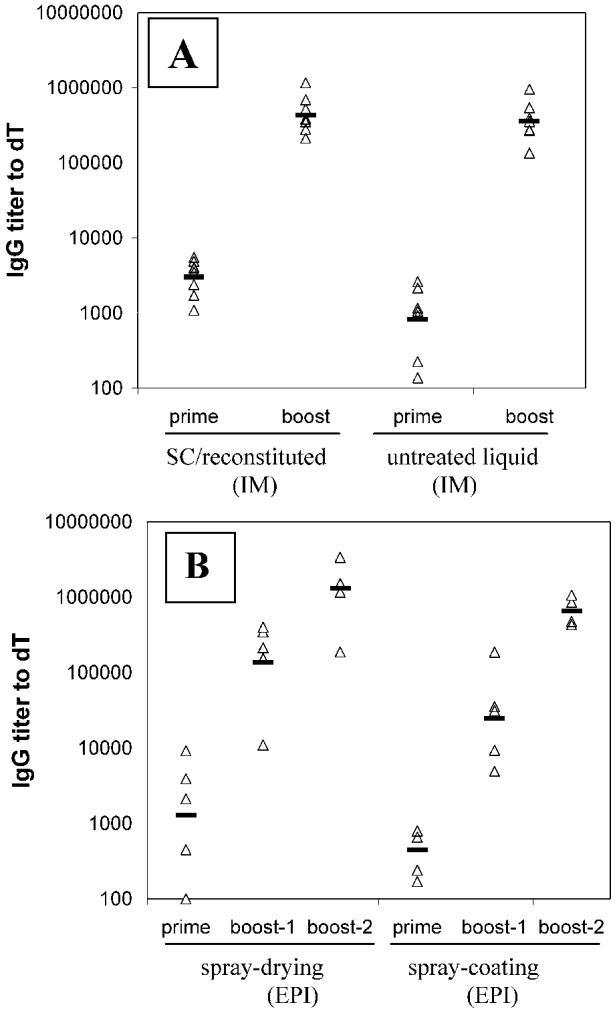

Fig. 5. Antibody responses to diphtheria toxoid following subcutaneous injection of the untreated dT liquid and the reconstituted powder spray-coated with dT (formulation D in Table II) to mice (A) or after EPI of the same spray-coated powder formulation and a spray-dried formulation, (0.1%)/trehalose (7.0%)/mannitol (12.0%)/dextran 10 kDa (10.5%)/Tween 80 (0.02%), to hairless guinea pigs (B).

tage over traditional needle injection. Another advantage of EPI, as a needle-free immunization method, is to reduce and even eliminate the discomfort to the patients (26) and the risk of transmitting blood-borne pathogens (27,28) that are associated with needle injection.

For tolerability and efficacy reasons, powder for EPI should possess some unique physical characteristics, including small sizes (<70 μm) to avoid tissue injury, narrow particle size distribution for uniform acceleration and penetration to the target tissue, and a high density (>1 g/mL) for efficient acceleration by helium gas. To satisfy these criteria, spray coating using appropriate seed particles was considered a suitable process for developing powder formulations for EPI. Despite the numerous types of seed powders available for spray coating, only a few are acceptable for parenteral use, and our search suggested that crystalline lactose might meet the above criteria and was selected as the seed powder for coating in this study.

Secondly, spray coating might offer the advantage of preserving the physical stability of alum-adsorbed vaccines. It is well documented that alum-adsorbed vaccine products usually lose potency when subjected to traditional drying or freezing process (29,30). We believe that during drying/freezing, alum particles brought to close proximity produces strong inter-particle attraction and results in alum particle coagulation (30). Once coagulated, the original gel could not be reproduced. It was found that the coagulated alum adjuvant suffers significant loss of the adjuvant activity (30–32). To avoid/minimize such coagulation, methods that can prevent alum particles from forming a large-scale coagulated matrix should be pursued (33). Spray-coating would theoretically satisfy this need because the atomized droplets containing alum particles would spread over the surface of the seed particle in a form of thin layer before drying. Such a thin layer could prevent the alum adjuvant from coagulating to the same extent as that in the spray-dried particle. We have shown that spray-coated alum-containing vaccines could still return to its original gel form upon reconstitution and were immunogenic when administered to animals. Thus, spray-coating might present an effective method in preparing stable alum-vaccine powder formulations.

Like many other protein or peptide-based pharmaceuticals, vaccines are very expensive, which might constitute a significant portion of the product development costs. A small-scale process would be cost-effective. To our knowledge, there is no mature small-scale spray-coating process and spray coating of seed particles of <100 μm may have not been attempted probably because of the lack of appropriate instruments and technical difficulties involved. Maintaining good seed-particle fluidization during spray coating is critically important for achieving coating uniformity and reducing aggregation of coated particles. Particle fluidization, which is the result of particle inertia arising from fluidizing air overcoming inter-particle cohesion and adhesion between particles and the contacting surfaces, is dependent on the humidity of the coating chamber and the surface properties of the particles and coating surface. In the small-scale process, chamber humidity tends to rise quickly, which may change particle surface properties, resulting in the loss of particle fluidization and disruption of the coating process. From the perspective of spray-coating mechanism, the atomized droplets have to contact the seed particle, spread over it to form a thin film, and dry instantaneously due to the large surface area. The inherent problem with coating on small particles is the relative sizes of the atomized droplets and the seed particles that encourage particle agglomeration. The droplet size, determined by atomization conditions, is usually 10 μm or greater. Smaller droplets are technically difficult to produce. As the size of the droplet approaches that of the seed particle, film-forming may result in a thicker film, which requires longer drying time. When the film is wet and viscous, coated particles in contact tend to form agglomerates and result in powder formulations of poor properties. Therefore, controlling the level of particle agglomeration is a significant challenge for a small-scale spray coating of micro-seed particles. We realized that a spray coater design providing optimum air flow patterns and dynamics is the key to effective fluidization and minimization of particle agglomeration. As the first small-scale commercial product, the spray coater used in the study appears to satisfy our general needs. However, formulation components and compositions may also play a vital role.

Another challenge for coating micro-seed particles is to

minimize spray-drying of atomized droplets. Effective spray-coating requires the droplets to contact and spread over the lactose particles before major water removal by the drying air. Two liquid properties affecting spread-ability are wettability between the droplet and the seed particle and droplet viscosity. Wettability is often determined by measuring the contact angle between the droplets on the substrate. Good wettability, that is, small contact angles, would promote effective spreading of the droplet on the seed particle effectively. However, at a high viscosity, the spread-ability of a droplet on the seed particle might be deterred because the drying rate may outpace the spreading rate, thereby resulting in poor coating uniformity. At the molecular level, these two characteristics are determined by the collective properties of the individual components in the formulation and are often difficult to predict. However, we made an attempt to evaluate several excipients and vaccine antigens in this feasibility study.

Only parenterally approved pharmaceutical excipients such as trehalose, mannitol, dextran, etc. were tested in this study because epidermal powder immunization is considered a parenteral application. These excipients are commonly used as protein stabilizers or bulking agents in the spray-dried and freeze-dried formulations. Because F–A (the combination of mannitol and trehalose) might not possess appropriate wetting properties, an amphiphilic model protein, lysozyme, was added in F–B. The addition of lysozyme appears to improve the coat-ability of the mannitol/trehalose formulation as demonstrated by minimum formation of small particles.

Another excipient, dextran (MW 1000 daltons), was added to F–A to form the coating solution denoted as F–C. Dextran is capable of enhancing the glass transition temperature of the dry protein formulation, thereby improving the protein's biochemical stability (34). F–C also resulted in successful coating morphology although dextran is not known as a good wetting agent. This formulation seems, however, to generate agglomerated particles although these particles were only weakly agglomerated and breakable upon sieving or aerosolization by the time-of-flight particle size analysis (Table III). As discussed earlier, particle agglomeration during coating is a result of the thin-film on the surface of the seed particle remains wet and viscous before coming in contact with other particles. Particle agglomeration is intended in a fluid-bed pharmaceutical process called "granulation", commonly used in the oral solid-dosage form. Granulation often needs a binding agent such as polyvinylpyrrolidone to promote particle agglomeration. Dextran might act like a binding agent in this process, and its binding ability might increase with increasing molecular weight, which justified the selection of the 1-kd molecular weight. Sticky films during coating not only promote particle agglomeration but also increase the chance of coated particles adhering to the encountered coater surfaces. The loss of fluidized particles to coater surfaces would make maintaining stable fluidization difficult. Therefore, periodic vibration was introduced to the coating chamber to offset electrostatic forces and potential surface adhesion. Overall, F–C represents the first successful formulation to be spray coated on 40- to 60-μm lactose particles at a batch size as small as 20 g. However, it's not within the scope of this feasibility study to optimize any of these coating formulations. In a final note, we found that spray drying and spray coating were poorly correlated, suggesting that there might be other factors governing drying characteristics with these two drying technologies.

CONCLUSIONS

The ability of a fluid-bed spray-coating process to coat seed particles of 40–60 μm at the batch size of 20 g has been successfully demonstrated. Although formulation composition affects coating performance, the low drying temperatures associated with spray-coating are well tolerated by the antigens tested in this study. Both dT and alum-HBsAg are potent and immunogenic after coating, which provides an effective drying alternative to alum-adjuvanted vaccines. This novel coating process would serve as a valuable research tool to prepare dry powder biopharmaceuticals.

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