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Mixing is required for uniform reconstitution of filter-dried protein antigens in a single-injection vaccine formulation

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ABSTRACT

Ambient temperature filter dried vaccine formulations have been proposed to simultaneously achieve thermostability and offer a ready-to-use immunisation device that combines reconstitution and injection. Vaccine concentration should be uniform at the point of injection, but the uniformity following direct reconstitution of filter-dried vaccines has not been reported. We present here a study of vaccine mixing and release following dissolution of filter-dried model protein and toxoid antigens within a single syringe, filter and needle unit. Release was better for filters made from glass than cellulose. Without additional mixing, uniformity was poor and only 41% of input protein was released from protein filter-dried onto glass fiber. In contrast, adding a simple glass bead and mixing by inversion, 100% release antigen solution was achieved, with uniform concentration at exit from the needle throughout a simulated injection. Adsorption onto alum adjuvant had no detectable effect on vaccine dissolution and mixing. The uniformity and yield of low doses of diphtheria and tetanus toxoid was also improved by mixing, albeit with a lower yield of 60–68%. We conclude that uniformity and mixing should be studied to ensure safety and efficacy of directly reconstituted filter-dried vaccine formulations.

1. Introduction

A significant proportion of the cost of vaccination programs lies in safe refrigerated storage and distribution, and costs of trained healthcare professionals for administration. This has driven extensive research into drying and thermostabilisation technology for vaccines [1]. Traditionally freeze-dried vaccines still require refrigerated storage, although excipient optimization can achieve thermostable lyophilized vaccines [2]. Freeze-dried vaccines still require careful reconstitution and preparation prior to administration, typically reconstitution in a freeze-drying vial, followed by transfer to a syringe and needle for injection. Whilst the widespread use of liquid formulations distributed in pre-filled syringes for many common vaccines reduces staff time and skill level, liquid vaccine preparations are inevitably less stable than freeze-dried formulations, with solid formulations more stable and easier to handle [3,4] because chemical (e.g. hydrolysis) and physical (e.g. foaming or aggregation) instability have far faster kinetics in aqueous form. Pre-filled syringes could be argued to be more expensive formulations than traditional lyophilized vials, but in reality freeze-drying is already a labour intensive and slow process constrained by capacity of industrial freeze-driers [3], and the processes used for manufacture of vaccines in pre-filled syringes are now well developed. Furthermore, lyophilized vaccine vials still require water for injection plus disposable syringe and needle, which whilst low-cost components, increase the complexity of the supply chain.

The ideal vaccine formulation must therefore not only be produced from low cost components, but must also have cost-effective scalable manufacturing process, and the simplest possible format for the end-user administrating the vaccine dose. To give the product stability and avoid cold-chain storage, this device would contain a dry physical form produced using a scalable drying method which maximises thermostability. Whilst alternatives to injection such as dried oral solid dosage forms [5] offer benefits, the overwhelming majority of current vaccines require injection. Reconstitution of dried vaccine to a liquid for injection therefore becomes an important engineering consideration.

The cost of freeze-drying, and burden of cold-chain storage and complex preparation for administration has driven research into a range of novel formulations, and modelling has shown that thermostable vaccines would deliver significant improvements to the vaccine supply chain[6]. Multiple alternatives to freeze-drying have been explored, such as spray- or foam- drying [7].

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A long-established and intensively studied example is the use of ambient temperature drying to form an amorphous sugar glass from high glass transition temperature carbohydrates such as trehalose [8–10]. This method has been combined with simplified formulation to deliver a thermostable, single-component vaccine formulation and injection format that shows great promise [11,12]. The vaccine is dried to form a sugar glass on a non-woven fibre filter substrate, which is then encased in a filter housing which is connected between a syringe filled with water for injection, and the needle [13,14]. The sugar glass vaccine dissolves in flow as the syringe is depressed, and the dissolved vaccine injected directly into the patient. This approach is suitable to fragile live attenuated recombinant viral vaccines including modified vaccinia and adenovirus, which were preserved on the membranes for at least 6 months storage at high temperature (45 °C) [13]. Similarly, hepatitis B vaccine dried onto filters are stable and immunogenic at 55 °C for 7 weeks [9].

As this highly promising "all-in-one" formulation is now being developed for use in new human vaccine products, we questioned if the dissolution characteristics of reconstitution from the filter has been studied in enough detail. The uniformity of any drug prior to injection into patients is critical to safe and controlled administration and function, and we wished to understand if this novel formulation can deliver a uniform concentration of reconstituted vaccine. Whilst fluid flow through the filter matrix will generate a degree of turbidity, and some mixing will occur between reconstitution from the filter and the end of the needle, there is no mechanism driving axial redistribution if flow is unidirectional from syringe, through filter, into needle.

The filter units reported for thermostable filter-dried viral vaccines [13] are not freely available, and no design or specification has been published. Therefore, to study the efficiency of reconstitution from ambient temperature filter-dried vaccine formulations, we developed a simple model formulation using an unmodified plastic syringe, which made mixing and reconstitution easy to monitor and quantify. Likewise, complex and fragile live attenuated vaccines are expensive and challenging to visualize, making study of mixing and reconstitution unnecessarily complex. Tetanus and diphtheria toxins derived from *Clostridium tetani* and *Corynebacterium diphtheriae*, respectively are chemically inactivated to form toxoids that retain antigenic structure and potently induce a protective toxin-neutralizing immunity. Whilst very stable when compared to live attenuated whole viral vaccines, structure can still be lost on storage or during processing or formulation [15,16]. Toxoids such as tetanus toxoid are among the most heat-stable biological vaccine components. However, World Health Organisation (WHO) recommend that tetanus and diphtheria toxoids still require storage at 2–8 °C for years, with reduced stability of months at 25 °C and only weeks at 37 °C [15]. In this initial proof-of-concept study, we opted to explore recovery and mixing using the simplest single-protein vaccine to permit simple visualisation, and therefore selected these two toxoids as a model to study reconstitution from filter-dried vaccines.

We present here a pilot study of mixing following reconstitution of filter-dried protein antigens. We used an extremely simple and low-cost injection rig to explore the feasibility and effectiveness of active mixing within a syringe prior to injection. To permit not only precise quantitation of vaccine dissolution but also direct imaging of uniformity of vaccine solution, we studied fluorescently labelled protein and alum-adsorbed protein plus diptheria and tetanus toxoid as model vaccine antigens. We found that the filter dried vaccine is unlikely to be mixed effectively following reconstitution by addition of water alone, and that additional mixing by addition of a glass bead combined with agitation not only increased uniformity of vaccine delivered, but increased the yield.

2. Materials and methods

2.1. Materials

To make fluorescent model protein antigen, Bovine Serum Albumin (BSA) at ≥2 mg/ml in 0.5 M of carbonate buffer (pH 9.5) and 10 mg/ml FITC in anhydrous dimethyl sulfoxide (DMSO) were combined a ratio of 80–160 μg FITC per mg of BSA. After incubation at room temperature for 1 h, conjugates were purified by size-exclusion chromatography. BSA, FITC and Sephadex G-50 superfine were from Sigma-Aldrich (Dorset UK). Phosphate buffer saline tablets (Sigma, Dorset UK) were dissolved in ultrapure water and ProClin 300 (Sigma, Dorset UK) was added as a preservative. Adjuvant adsorbed antigen was prepared following manufacturers direction, briefly Imject Alum (Thermo Fisher UK, Paisley UK) was added to FITC-BSA at a final volume ratio of alum to immunogen of 1:3, then mixed together for 30 min for efficient adsorption. Non-adsorbed diphtheria and non-adsorbed tetanus toxoid were used as model antigens in a simulated combined vaccine, and were obtained from the National Institute for Biological Standards and Control (NIBSC, Potters Bar, UK) as well as monoclonal and polyclonal antibodies for sandwich ELISA antigen quantitation; all were reconstituted following the supplier's recommended method. Two types of filter were compared for filter drying of model vaccine antigens: cellulose filters were Whatman™ qualitative filter papers grade 1 (Cat No 1001-055) with 180 μm thickness; glassfibre filters were grade 8964 with 429.3 μm thickness (Ahlstrom, Lyon, France). Glass beads for mixing were taken from 1.2 mL Sodium Heparin S-Monovette® blood collection tubes (Sarsedt, Leicester UK).

2.2. Preparation of ambient temperature dried model antigen filters and combined injection device

A simple prototype combined injection device was made using two filter-dried vaccine antigen squares loaded into the barrel of a 2 mL plastic disposable syringe (BD, Berkshire UK). To promote mixing, a 3 mm diameter glass mixing bead taken from a Monovette® 1.2 mL blood collection tube (Sarstedt; Leicester UK) was added with the filters (Fig. 1A). To make filter-dried antigen squares, 10 μL of fluorescein, fluorescent protein or antigen solution was added per 5 mm square filter, and dried at ambient temperature (typically 20 °C) in a petri dish until completely dry, which took a minimum of 1 h (drying time was based on previous thermogravimetric studies of vaccine drying onto polymer films [17], and confirmed by kinetic analysis of mass loss). To establish methods and directly image reconstitution, 1 mM sodium fluorescein solution was dried onto filters. This was followed by study of filters loaded with fluorescently labelled BSA, used as a model protein to more accurately reflect vaccine antigen dissolution. A 3.2 mg/mL solution of BSA-FITC was found to be the lowest concentration suitable for quantitative imaging in the chamber. To determine if a real toxoid vaccine antigen behaved similarly to the high concentration of model protein used, both diphtheria and tetanus toxoids were diluted in ultrapure water to the lowest concentration that permitted quantitation by ELISA. A concentration of 4 lf/ml was selected for both toxoids mixed in a ratio of 1:1, and 10 μL was pipetted on a 5 × 5 mm square of filter to yield 0.02 lf of diphtheria and tetanus toxoid per filter. Toxoid concentrations are presented in flocculation value per ml (lf/mL). When two filters in a syringe were reconstituted with 500 μL ultrapure water, the maximum concentration of reconstituted vaccine expected was 0.08 lf/mL.

2.3. Measurement of mixing and dissolution of filter dried model vaccine antigens

To observe fluorescent protein mixing directly after reconstitution, filters with dried 1 mM sodium fluorescein were imaged in a

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