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VACCINE DELIVERY USING THE NEMAURA SOLID DOSE INJECTOR

In this article, Joanne Broadhead, PhD, Product Development, Nemaura Pharma; and Karmen Cheung, MSc, Department of Chemical Engineering, Loughborough University, UK, report an investigation into the delivery of two vaccines using Nemaura's solid-dose injection technology.

Vaccines are widely recognised as one of the greatest achievements in public health, yet incorrect handling and administration can compromise their effectiveness. The process of storage, handling, preparation and

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administration can be complex, particularly where vaccines are being distributed in developing countries in which security of the cold chain can be difficult to assure. In addition, administration procedures can be a source of error.

For conventional vaccines supplied in vials, there can be contamination and stability risks associated with the advance preparation of syringes ("pre-drawing"). This is why the US Centers for Disease Control & Prevention (CDC) encourages the use of unit dose, ready to administer vaccines.¹

It is clear even from the brief discussion above that a vaccine which does not require cold-chain handling and which is single use, unit dose and ready to use, requiring no specialised training to administer, would offer a significant step forwards in maximising the benefits of vaccination.

Microneedle delivery systems have been extensively investigated as a means to address this unmet clinical need. Both removable and biodegradable systems have been evaluated.² The former were associated with safety concerns because of the perceived risk of needles fracturing and remaining in the skin. This led to greater interest in biodegradable systems. However, these present significant challenges, particularly in terms of their fabrication.²

As an alternative to the microneedle concept, Nemaura has developed a novel solid dose injector device which enables the controlled delivery of solid dose vaccine formulations. This offers similar advantages to microneedle systems in terms of avoidance of cold chain and simplicity of use but, due to its larger size, presents fewer fabrication challenges. In the context of vaccine administration, where only one (or a few) doses are required, the larger size is not considered limiting. Here we present the results of a proof of concept study in mice in which

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Figure 1: Prototype Nemaura solid dose delivery device.

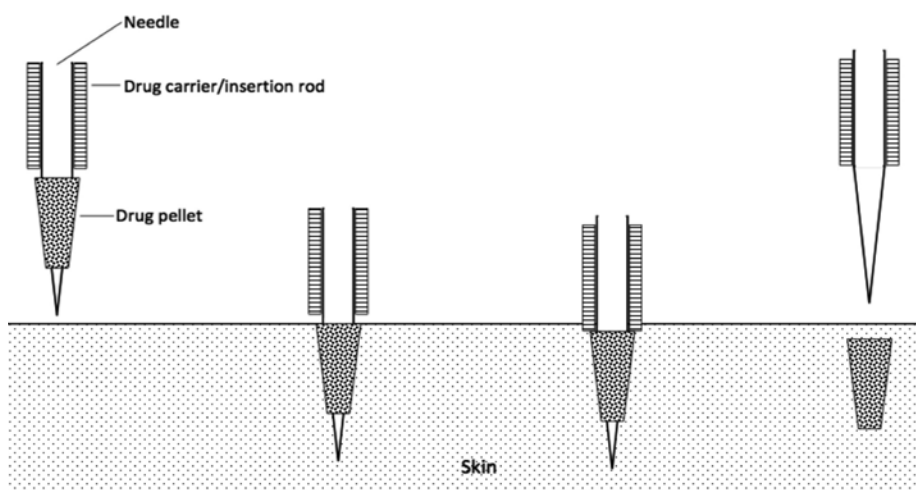


Figure 2: Delivery using the Nemaura solid dose delivery system. Needle is inserted into the skin to the desired depth, pellet accompanies needle into the skin then needle is removed leaving the solid micro-dose formulation inside the skin.

a prototype device was used to deliver a tetanus vaccine and a diphtheria, tetanus and pertussis (DTaP) vaccine.

NEMAURA SOLID DOSE DELIVERY DEVICE

Figure 1 shows a prototype version of the Nemaura solid dose delivery device. The principle is based upon the initial insertion of a super sharp stainless steel needle to breach the tough outer barrier of the skin followed by delivery of the solid dose formulation in pellet form which is inserted alongside the needle. The needle is subsequently retracted leaving the solid dose formulation in the skin (Figure 2). In this particular prototype, a frustoconical-shaped pellet was used but various pellet geometries are feasible.

The device design can be adapted to allow the solid-dose formulation to be inserted efficiently into the skin either intradermally or subcutaneously depending on the dosing requirements. The device is designed to be easy to administer and includes safety features such as the complete retraction of the needle into the device following insertion of the solid-dose pellet, thus reducing the risk of needlestick injuries.

IN VIVO PROOF-OF-CONCEPT STUDY

A proof-of-concept study in mice provided a preliminary evaluation of the immunogenicity of the solid-dose vaccine using a Nemaura prototype solid-dose injector.

“The study reported here is an encouraging step forward in developing a vaccine delivery system which is straightforward to manufacture, a low-cost disposable system, easy to use and avoids the problems associated with cold-chain delivery of vaccines, and potentially other biologics.”

MATERIALS

Tetanus vaccine was selected for one arm of the study. Freeze-dried inactivated tetanus toxoid was purchased from the National Institute for Biological Standards and Control (NIBSC; Potters Bar, UK).

Infanrix® vaccine (sourced from GSK) was used for the DTaP arms of the study. The DTaP vaccine contains tetanus toxoid, pertussis and diphtheria and is a component of the childhood vaccination programme. This vaccine is supplied as a liquid and was converted to a dry state by freeze-drying with suitable excipients. Pharmacopoeia-grade excipients were obtained from reputable excipient suppliers.

METHODS

Manufacture & Characterisation of Pellets

An optimised excipient blend was developed which, when compressed, resulted in pellets with physical properties in the desirable range. Excipients were selected based on their suitability for use in parenteral products.

Freeze-dried vaccine formulations were incorporated into the optimised excipient blend using geometric mixing. The solid-dose formulations were individually compressed into pellets using a bespoke direct compression micro-press. The pellets had a base diameter of 1.6 mm, a tip diameter of 0.8 mm and a height of 2 mm (approximate dimensions). The average mass was approximately 4 mg. Compressed solid-dose pellets were mounted on the prototype device and packaged in a nitrogen environment using a moisture-impermeable barrier material.

Solid Dose Pellet Formulation	Test Conducted	
	Hardness (N) (SD)	Disintegration (seconds) (SD)
High Dose Tetanus Pellet	6.44 (0.68)	27.5 (5.24)
Low Dose Tetanus Pellet	No data ¹	No data ¹
Placebo Pellet	No data ¹	53.0 (7.86)
DTaP Formulation One	7.88 (1.82)	49.0 (19.70)
DTaP Formulation Two	6.48 (1.78)	96.0 (21.63)

Table 1: Hardness and disintegration data.

Test Conducted	Time point (weeks) at temperatures				
	25°C			40°C	
	0	2	4	2	4
Hardness (N)(SD)	6.21 (1.25)	4.59 (1.06)	5.32 (1.06)	3.13 (0.61)	5.04 (1.53)
Disintegration (seconds) (SD)	75.67 (15.16)	Not tested	54.67 (2.66)	Not tested	55.4 (2.41)

Table 2: Hardness and disintegration stability data.

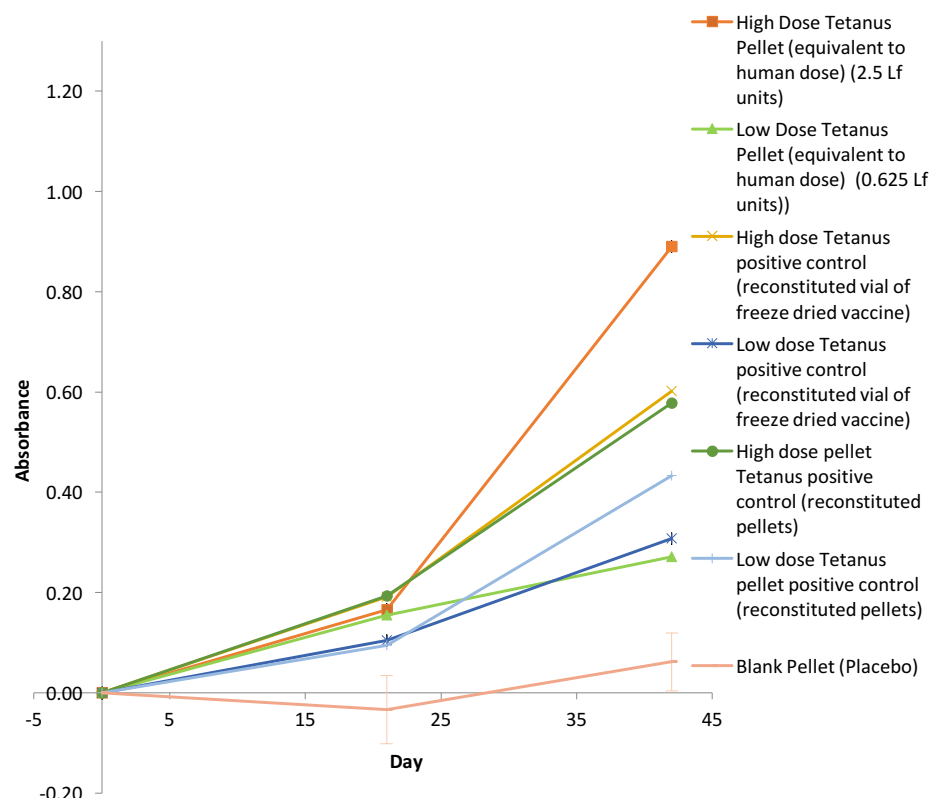


Figure 3: Tetanus immune response (Tetanus Study).

Tetanus pellets were formulated at a “high” dose (2.5 Lf units, equivalent to ¼ of a human dose) and a “low” dose (0.625 Lf units). Relatively high doses were selected because of the lack of adjuvant in the tetanus formulations.

DTaP pellets contained all the components of the original Infanrix® vaccine including the adjuvant. Two different freeze-dried formulations, containing different stabilisers, were used in the manufacture of the pellets. Pellets contained approximately 2% of the normal human dose of all components.

Pellet hardness was evaluated using a MultiTest 2.5-i compression instrument (Mecmesin, Sleaford, UK). Friability was evaluated using an in-house friability tester and disintegration time was determined by measuring the time taken for an individual pellet to disintegrate in 1 ml of water.

Immunogenicity Study

The immunogenicity study was conducted at a third-party CRO site. For tetanus arms, pellets were administered to ten mice in each dose group. For DTaP arms, 20 mice per dose group were used as each mouse could only provide sufficient serum for two ELISA assays.

Control groups for the tetanus study were administered reconstituted pellets or an equivalent dose of reconstituted, freeze-dried tetanus toxoid as supplied from NIBSC. Control groups for the DTaP study were administered an equivalent dose of reconstituted freeze-dried vaccine or an equivalent dose of Infanrix®. A constant dosing volume of 500 µL was used for all control groups. Animals were dosed on day 0 (prime) and day 28 (boost). Blood samples were taken on days 21 and 42.

The pellets were inserted into the skin of the lower back of the mouse in a lateral direction to ensure the pellet was administered subcutaneously. Daily physical and general behaviour of the mice throughout the study was monitored including weight, faecal matter and general movement. Body weight and feed intake of all the animals was evaluated weekly. No mortalities occurred during the study.

ELISA kits (GenAsia Biotech, Shanghai, China) were used to determine the antibody response to the administered vaccine solid dose pellets. Samples from all test animals except the naïve (untreated) control group were diluted five-fold prior to assay. The ELISA assays were performed according to kit instructions.

RESULTS

Physical Properties of Pellets

Hardness and disintegration data for sample pellets are shown in Table 1. Previous Nemaura data indicated that a hardness of 3N was sufficient for skin penetration. All tested pellets were sufficiently hard and disintegrated rapidly. Only one pellet was noted to break during the course of the immunogenicity study.

Table 2 provides physical stability data for pellets stored at 25°C and 40°C. Friability testing was also performed and less than 3% weight loss was observed for all tested pellets. This study was conducted using the DTaP Formulation Two pellets and provided assurance that the pellets were sufficiently robust to withstand storage and shipping conditions.

Animal Observations

Basic observations of the animals were determined to be normal. No mortality occurred and the weight gain of the mice was normal. Minor observations of erythema and oedema were noted at the injection site.

a) Immunogenicity: Tetanus Study

Figure 3 shows the anti-tetanus immune response. The results are expressed as an optical density value and are adjusted for the negative control (naïve mouse). Values for blanks, positive and negative assay controls were very similar at both 21 and 42 days enabling the 21- and 42-day data to be directly compared.

The results clearly show that there is an increased effect at day 42 compared with day 21 with all the high-dose groups showing a stronger response than the low-dose groups. The high-dose tetanus pellet gave a stronger response than both positive controls (reconstituted pellets and reconstituted freeze-dried vaccine) which could suggest a dose-sparing effect associated with the solid formulation. However, further studies are required to evaluate this further. All dose groups show a clear response compared with the placebo group.

b) Immunogenicity: DTaP Study

Figures 4 to 7 show the immune responses to the DTaP pellets. As for the tetanus study, the results are expressed as an optical density value and are adjusted for the negative control.

The anti-tetanus signal was weaker for the triple vaccine compared with the tetanus

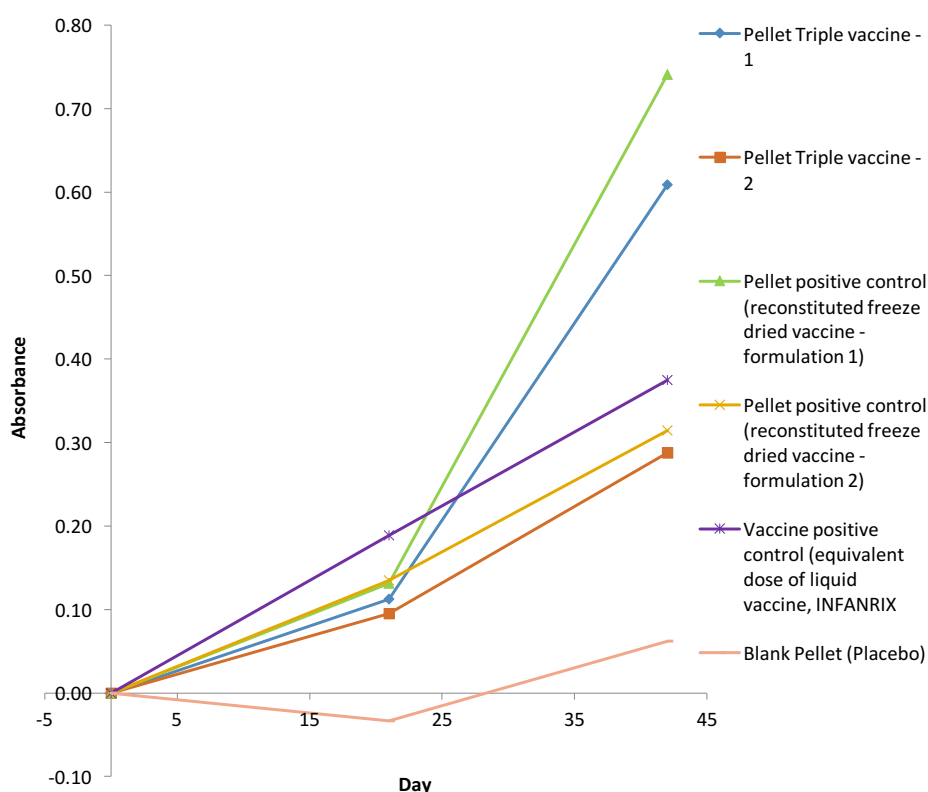


Figure 4: Tetanus immune response (DTaP Study).

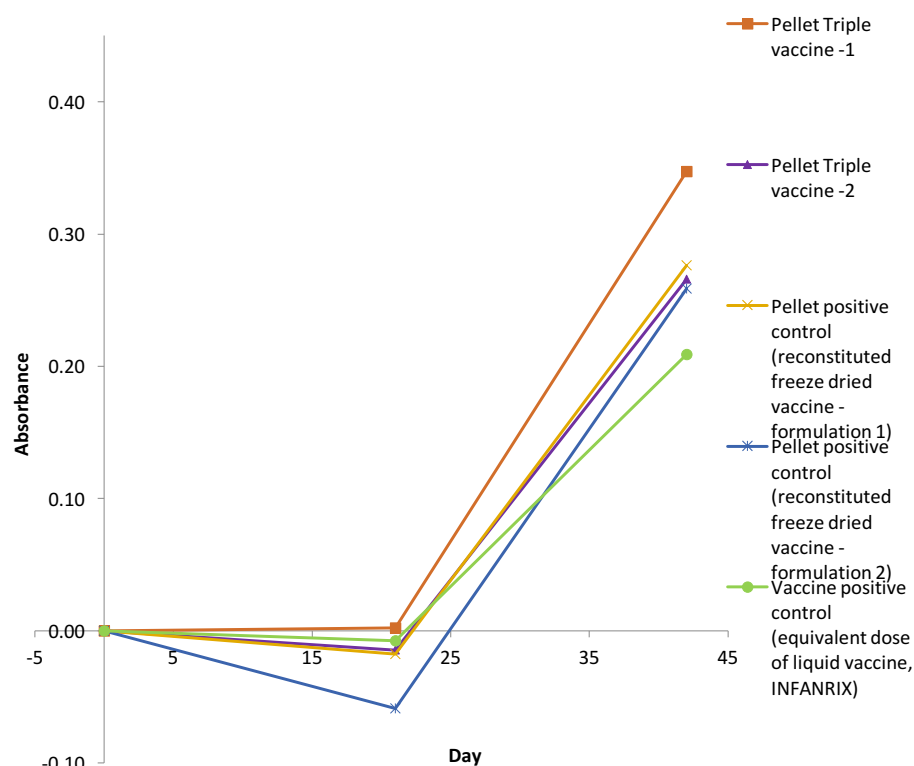


Figure 5: Diphtheria immune response (DTaP Study).

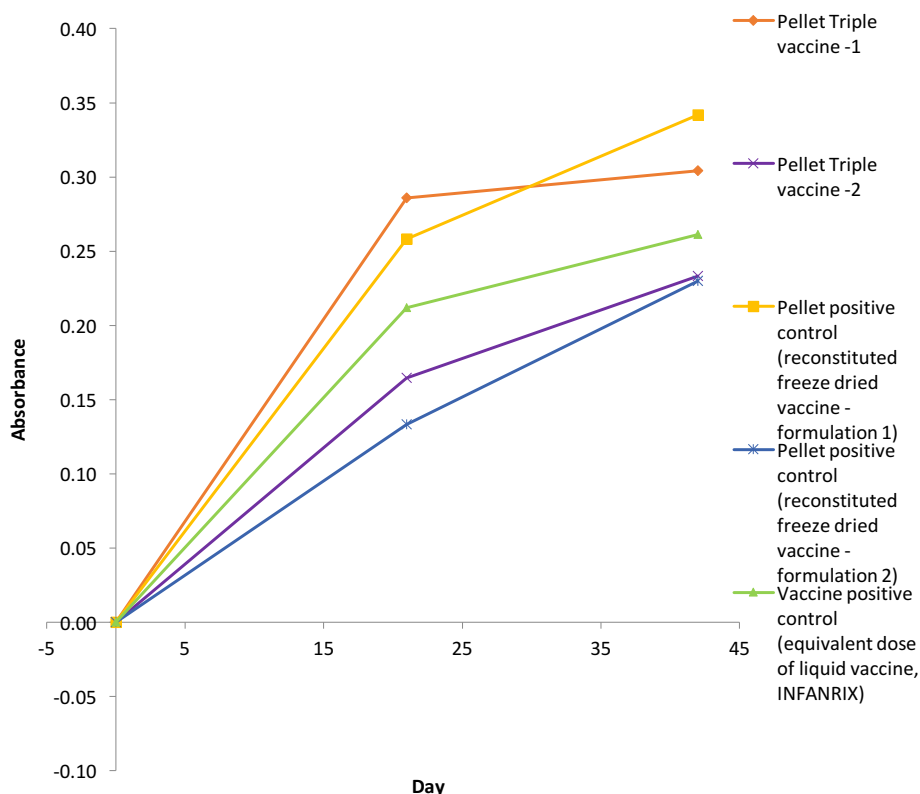


Figure 6: Pertussis toxoid immune response (DTaP Study).

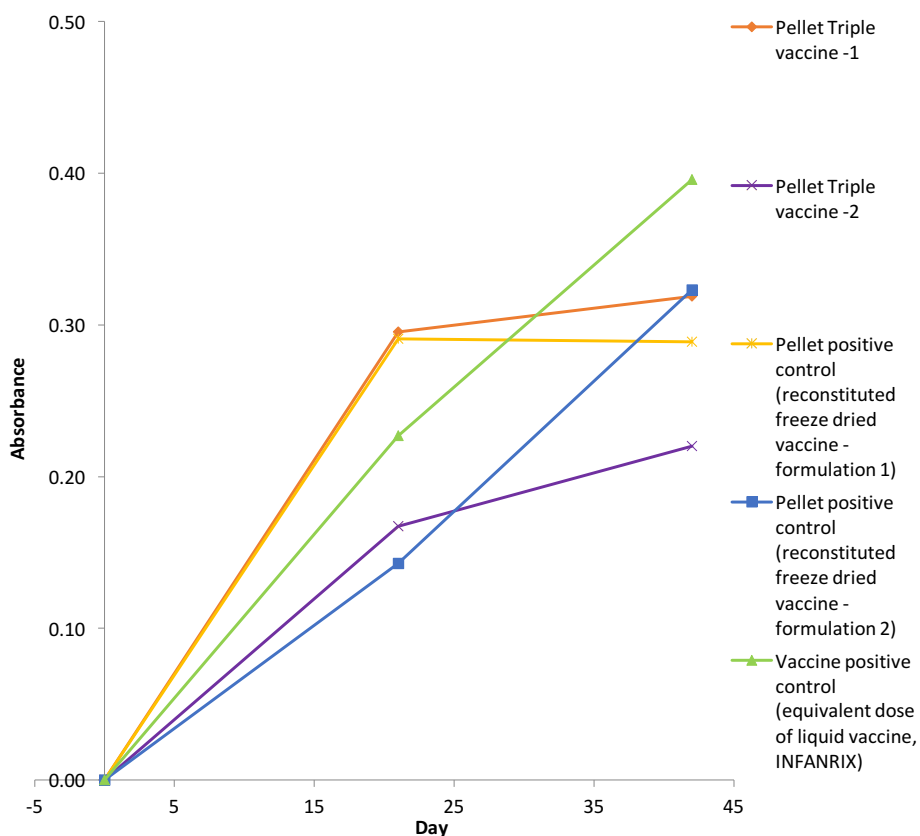


Figure 7: Pertussis immune response (filamentous haemagglutinin (FHA) vaccine arm) (DTaP Study).

pellets. However, the dose of triple vaccine was significantly lower (approximately 2% of a human dose compared with 25% and 6% for the high- and low-dose tetanus-only groups). The anti-tetanus response was very low at 21 days and no response was seen to diphtheria at this time point. However, a response to both was seen at 42 days after the boost dose. In contrast, the pertussis response was evident at 21 days with no significant increase at 42 days.

DISCUSSION

The purpose of this study was to demonstrate the potential for solid-dose vaccine delivery using the Nemaura proprietary solid-dose injector technology. The data clearly show that solid-dose delivery of tetanus and DTaP vaccines resulted in immune responses. The tetanus study showed a dose-response effect with some indication of a possible dose-sparing effect when delivery was in the solid form. In addition, the DTaP study demonstrated that the freeze-drying process which was used to convert the vaccine to a solid form did not result in any observable loss in potency.

The study reported here is an encouraging step forwards in developing a vaccine delivery system which is straightforward to manufacture, a low-cost, disposable system, easy to use and avoids the problems associated with cold-chain delivery of vaccines and potentially other biologics.

Industrial processes for pellet manufacture are currently in progress and pellets of a significantly smaller size than those used for this preliminary study have also been developed by Nemaura. The technology is currently being evaluated for a number of molecules, and collaboration and business development enquiries should be sent to David Scott: bd@nemaura.co.uk.

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