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Safety, tolerability, acceptability and immunogenicity of an influenza vaccine delivered to human skin by a novel high-density microprojection array patch (Nanopatch™)

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ABSTRACT

Background: Injection using needle and syringe (N&S) is the most widely used method for vaccination, but requires trained healthcare workers. Fear of needles, risk of needle-stick injury, and the need to reconstitute lyophilised vaccines, are also drawbacks. The Nanopatch (NP) is a microarray skin patch comprised of a high-density array of microprojections dry-coated with vaccine that is being developed to address these shortcomings. Here we report a randomised, partly-blinded, placebo-controlled trial that represents the first use in humans of the NP to deliver a vaccine.

Methods: Healthy volunteers were vaccinated once with one of the following: (1) NPs coated with split inactivated influenza virus (A/California/07/2009 [H1N1], 15 μg haemagglutinin (HA) per dose), applied to the volar forearm (NP-HA/FA), n = 15; (2) NPs coated with split inactivated influenza virus (A/California/07/2009 [H1N1], 15 μg HA per dose), applied to the upper arm (NP-HA/UA), n = 15; (3) Fluvax® 2016 containing 15 μg of the same H1N1 HA antigen injected intramuscularly (IM) into the deltoid (IM-HA/D), n = 15; (4) NPs coated with excipients only, applied to the volar forearm (NP-placebo/FA), n = 5; (5) NPs coated with excipients only applied to the upper arm (NP-placebo/UA), n = 5; or (6) Saline injected IM into the deltoid (IM-placebo/D), n = 5. Antibody responses at days 0, 7, and 21 were measured by haemagglutination inhibition (HAI) and microneutralisation (MN) assays.

Findings: NP vaccination was safe and acceptable; all adverse events were mild or moderate. Most subjects (55%) receiving patch vaccinations (HA or placebo) preferred the NP compared with their past experience of IM injection with N&S (preferred by 24%). The antigen-vaccinated groups had statistically higher HAI titres at day 7 and 21 compared with baseline ($p < 0.0001$), with no statistical differences between the treatment groups ($p > 0.05$), although the group sizes were small. The geometric mean HAI titres at day 21 for the NP-HA/FA, NP-HA/UA and IM-HA/D groups were: 335 (189–593 95% CI), 160 (74–345 95% CI), and 221 (129–380 95% CI) respectively. A similar pattern of responses was seen with the MN

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assays. Application site reactions were mild or moderate, and more marked with the influenza vaccine NPs than with the placebo or IM injection.
Interpretation: Influenza vaccination using the NP appeared to be safe, and acceptable in this first time in humans study, and induced similar immune responses to vaccination by IM injection.

1. Introduction

Most vaccinations are performed by injection using the needle and syringe (N&S). However, this method has drawbacks, including fear of needles, risk of needle-stick injury, the need for safe disposal of sharps waste, the need to reconstitute lyophilised vaccines, and the requirement for healthcare professionals to administer injections. Several groups are developing microarray patches (MAPs) for vaccine delivery to overcome these limitations [1]. Various MAP formats have been tested extensively for vaccine delivery in preclinical models [2]. MAPs have also been evaluated in clinical trials for delivery of non-vaccine drugs [3], and in a limited number of clinical studies evaluating the tolerability and acceptability of MAPs, but without vaccine antigen [4–7]. However to date, only two clinical studies using MAPs to deliver a vaccine to human skin have been published, both of which used dissolving microneedles (DMNs) and trivalent split influenza virus vaccines [8,9].

Vaxxas Pty Ltd is developing the Nanopatch™ (NP), a MAP with a high density of solid microprojections onto which the vaccine formulation is dispensed and dried. The current configuration of the NP used and its applicator were identical to those studied previously [7]. In preclinical studies, the NP has been shown to deliver vaccine to the viable epidermis and dermis, and to be dose-sparing [10–14] and adjuvant sparing [15] compared with N&S IM delivery. The high density of microprojections might have a physical immune-enhancing effect, resulting in dose-sparing [11] but this feature means that high velocity application with an applicator is required to achieve skin penetration. The safety and tolerability of the NP (without vaccine) was recently evaluated in humans. NPs were found to be safe and well-tolerated, and preferred to N&S by the majority of subjects [7]. Here we report the first clinical study evaluating vaccine delivery with the NP.

2. Methods

2.1. Nanopatch manufacture

The NP and applicator were identical to those used in a previous study [7]. Briefly, NPs were manufactured from silicon wafers, dry-etched, and diced to give individual patches of 10 × 10 mm with microprojection arrays (10,000/cm^2) of 250 μm length (Fig. 1A and B). The separate applicator was a hand-held spring-powered, auto-disabling device (Fig. 1C and D). The applicator delivered NPs onto the skin at a velocity of 20 m/s.

2.2. Vaccine

Vaccine-coated NPs for the NP-HA/FA and NP-HA/UA groups were produced in an aseptic process. cGMP-grade, monovalent, inactivated, split influenza A/California/07/2009 (H1N1)-like virus antigen (Seqirus Pty Ltd, Australia. MPH Lot # M09061498100V) was combined with excipients: 1% w/v hypromellose (Shin-Etsu Chemical Company Ltd, Japan); 0.72% w/v trehalose (as dihydrate) (Pfanstiehl, Germany); and Dulbecco's phosphate-buffered saline (DPBS) (Sigma Aldrich, USA) to give a final formulation of 0.976 μg/μL haemagglutinin (HA). The resulting solution (41 μL, containing 40 μg HA) was applied onto each NP and dried using sterile filtered nitrogen [16]. Preclinical and *in vitro* studies demonstrated that approximately 20% (i.e. approximately 8 μg) of the vaccine was delivered into the skin, with the rest of the material remaining on the base of the NP [17]. Placebo NPs (for the NP-placebo/FA and NP-placebo/UA groups) were coated with formulation excipients without antigen and gamma-sterilised (≥25 kGy, Steritech, Australia). Following coating, NPs were placed into aluminium MediCan containers (Amcor, UK), foil-sealed, and stored at 2–8 °C before use (Fig. 1C). The A/California/07/2009 HA antigen coated onto NPs was shown to be stable (the average HA content of coated NPs was ≥70 and ≤130% of the potency of the original coating solution) for ≥3 months at 2–8 °C following coating onto NPs by enzyme-linked immunoassay [18]. The investigational A/California/07/2009 (H1N1) vaccine on the NP met its target specifications and was administered to patients within three months of production.

The vaccine for the IM-HA/D group was the commercially-available trivalent Fluvax® 2016 Southern hemisphere formulation (Seqirus Pty Ltd, Australia batch number 0906-46202), containing A/California/07/2009 (H1N1-like), A/New Caledonia/71/2014 (NYMC X-257A) (H3N2-like) and B/Brisbane/60/2008, all at 15 μg HA per dose. Pre-filled syringes containing sterile saline solution (0.9% sodium chloride), were provided by Q-Pharm Pty Ltd (Australia, lot 0906-46202) as the IM placebo (for the IM-placebo/D group). The vaccine was within its shelf-life when used in the study.

2.3. Trial subjects and study design

The study was approved by the QIMR-Berghofer Medical Research Institute (Brisbane, Australia) Human Research Ethics Committee, and conducted in accordance with the National Statement of Ethical Conduct in Human Research (2007), the Australian National Health and Medical Research Council guidelines, and Good Clinical Practice (CPMP/ICH/135/95), as adopted by the Therapeutic Goods Administration (2000). Written informed consent was obtained from all participants. The trial was registered with Australian New Zealand Clinical Trials Registry (ANZCTR.org.au), trial ID ACTRN12616000880448. Universal Trial Number U1111-1184-5260.

The study was restricted to fair-skinned subjects (types I, II or III on the Fitzpatrick scale) [19], to facilitate clear observation of the skin response. Exclusion criteria included influenza vaccination or known influenza infection during the previous year (to minimise baseline antibody titres).

The study was a randomized, partly-blinded, placebo-controlled trial. Healthy females and males, aged 18–45 years, non-pregnant and non-nursing (N = 61), with a BMI in the range of 18–30 kg/m^2 were randomly allocated into one of six vaccination groups: (1) NPs coated with inactivated split influenza virus (A/California/07/2009 (H1N1), 15 μg haemagglutinin (HA) per dose), applied to the volar forearm (NP-HA/FA), n = 15; (2) NPs coated with the same inactivated split influenza virus (A/California/07/2009 (H1N1), 15 μg HA per dose), applied to the upper arm, above the acromial prominence of the deltoid muscle, (NP-HA/UA), n = 15; or (3) Fluvax® 2016 containing 15 μg of the same H1 HA antigen

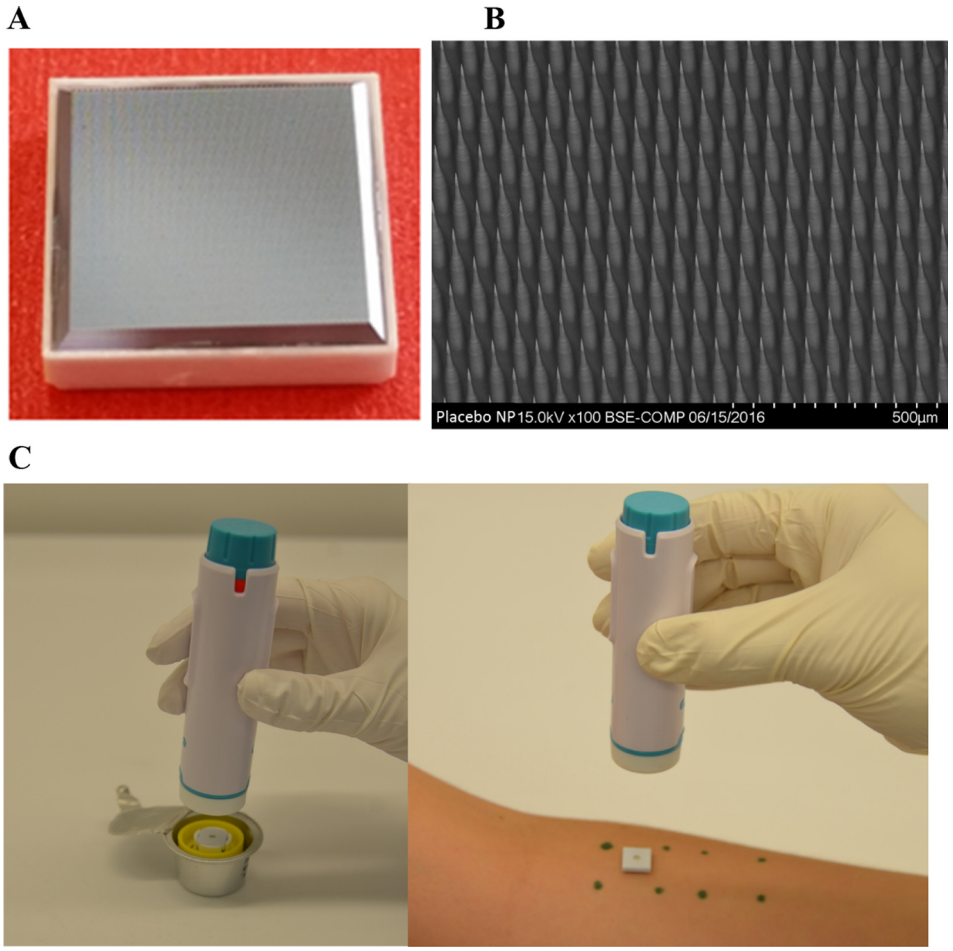

Fig. 1. Images of the Nanopatch (NP). (A). The NP, a high-density array of microprojections (10,000 projections/cm^2, 250 μm in length). The external dimensions of the NP are 1 cm × 1 cm. (B) Scanning electron micrograph (SEM) showing the micro-projections of the NP. (C) NPs were loaded into applicators by pushing the applicator directly into the open can. The NP has a metal insert in the base that attaches to, and is held in place to the applicator via a low-strength magnet in the applicator, allowing 'hands-free' removal of the NP from the MediCan. (D) Attachment of the NP onto forearm skin after application with the spring-loaded applicator. The NP's microprojections are embedded in the skin, which provides sufficient resistance to hold the NP in place until it is removed. No adhesive or patch is required to keep the NP in place. A different applicator was used for each subject in the study.

injected intramuscularly (IM) into the deltoid (IM-HA/D), n = 15; (4) NPs coated with excipients only, applied to the volar forearm (NP-placebo/FA), n = 5; (5) NPs coated with excipients only applied to the upper arm (NP-placebo/UA), n = 5; or (6) Saline injected IM into the deltoid (IM-placebo/D), n = 5 (Fig. 2).

2.4. Study procedures

Subjects received a single vaccination at day 0, given to the non-dominant arm. Application sites were selected to be free from scarring, tattoos, skin conditions, and sunburn. The area for application was marked, swabbed using a 70% ethanol swab and photographed. Two NPs were administered to deliver the 15 μg dose, applied to the same site, at least 4 cm apart. The NP was applied to the skin with the applicator and left on the skin for 2 min before being removed. All NP applications were performed by the same nurse following training.

Subjects were monitored by clinic safety assessment visits at days 0, 3, 7, 21, and 28; and phone calls at days 1, 2, and 14. On day 0, all vaccination sites were assessed at 10 min, 1 and 2 h after NP or IM administration. Photographs of the application sites were taken at every clinic review. Skin reactions were assessed for: erythema (0 = none; 1 = very slight; 2 = well-defined; 3 = moderate to severe; 4 = severe [beet red]) and oedema (0 = none; 1 = very slight; 2 = slight; 3 = moderate [raised approximately 1 mm]; 5 = severe), and a skin irritation index (SII) was calculated, as the sum of the erythema and oedema scores as described previously [7]. Induration, bruising, skin flaking, discolouration, itching and bleeding were also assessed. Pain scores were collected 10 min after the patch removal and at all outpatient-assessments using a visual analogue scale (VAS, QPharm) with 0 = no pain; 5 = moderate pain; 10 = worst pain possible.

2.5. Qualitative acceptability assessment

Acceptability data were collected via an investigator-blinded, semi-structured interview (Supplementary Table 4) with open questions conducted between 1 and 2 h post vaccination and again at the end of study. Interviews were digitally recorded, transcribed verbatim, and de-identified prior to analysis. Potential subject bias against N&S was addressed as part the screening questionnaire [7].

2.6. Immunogenicity evaluation

Serum samples were collected for testing in haemagglutination inhibition (HAI) and microneutralisation (MN) assays (360biolabs Pty Ltd, Australia) on days 0 (pre-vaccination), 7, and 21.

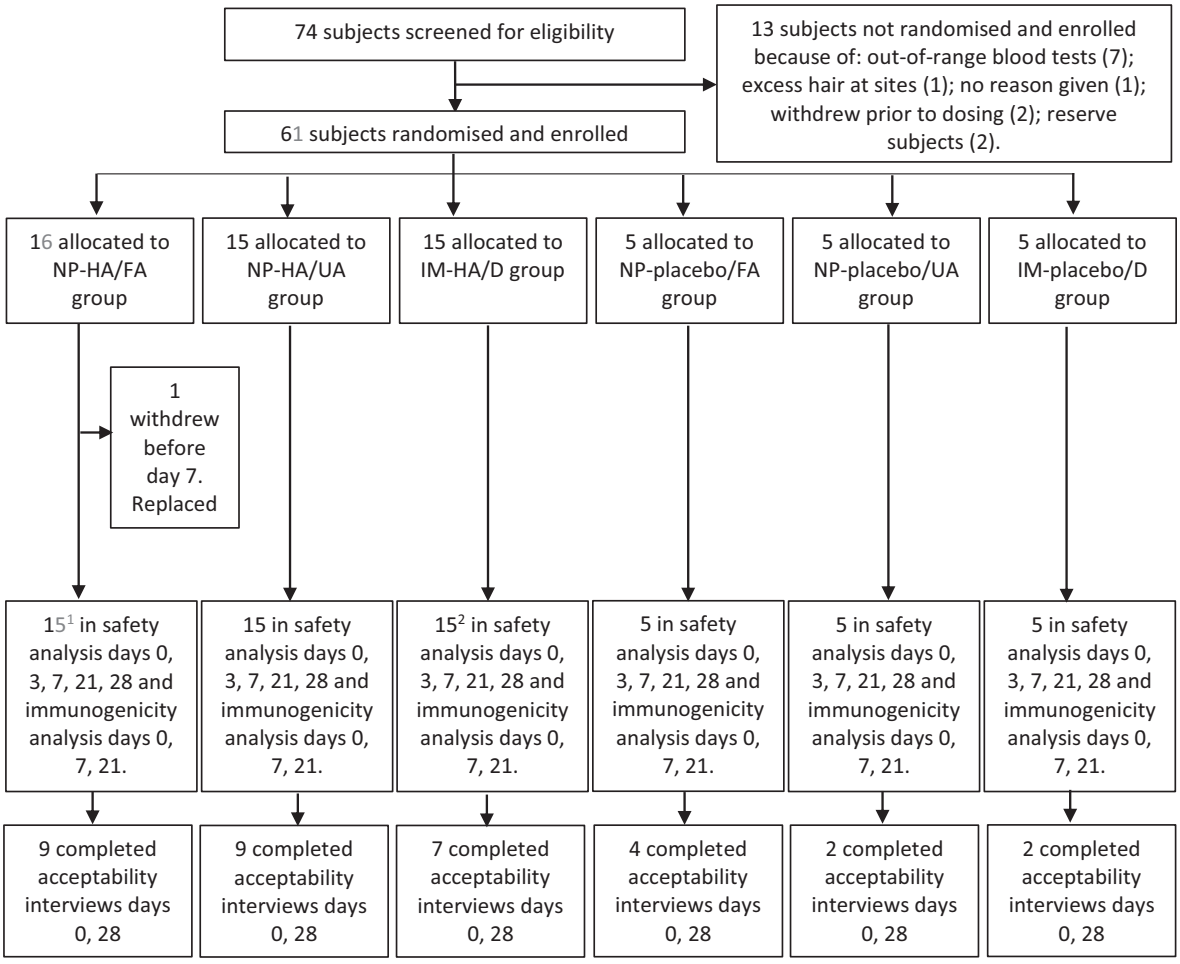

Fig. 2. Trial profile. Treatment group abbreviations: NP-HA/FA – NPs coated with inactivated split influenza virus (A/California/07/2009 (H1N1), 15 μg haemagglutinin (HA) per dose), applied to the volar forearm; NP-HA/UA – NPs coated with inactivated split influenza virus (A/California/07/2009 (H1N1), 15 μg HA per dose, applied to the upper arm; IM-HA/D – Fluvax® 2016 containing 15 μg of the same H1 HA antigen injected intramuscularly (IM) into the deltoid; NP-placebo/FA – NPs coated with excipients only, applied to the volar forearm; NP-placebo/UA – NPs coated with excipients only applied to the upper arm; IM-placebo/D – saline injected IM into the deltoid. Notes: [1]16 subjects in the NP-HA/FA group contributed data to the safety assessment at day 0. [2]14 subjects in the IM-HA/D group contributed safety data at day 0.

Serum samples for HAI were treated with receptor destroying enzyme (Denka Seiken Co Ltd, Japan) and adsorbed to washed, packed turkey red blood cells (TRBC) for 30 min at room temperature (RT). TRBC were diluted to 1% v/v in PBS prior to testing. Twofold serum dilutions starting from 1:10 were prepared and 4 HA Units/25 μL of A/California/07/2009 virus (WHO Collaborating Centre, Australia) were added to each test well and incubated for 45 min at RT. TRBC were added and incubated for a further 30 min at RT. The HAI titre was the reciprocal of the highest dilution of the sera that completely inhibited agglutination of TRBC by the virus.

MN assays were conducted according to published methodology [20]. Briefly, serum samples were heat inactivated at 56 °C for 30 min. Twofold serum dilutions starting from 1:100 were prepared and 100 $TCID_{50}$ of A/California/07/2009 virus (WHO Collaborating Centre, Australia) were added to each test well. Prevention of infection of MDCK cells by A/California/07/2009 virus was tested using ELISA detection of influenza nucleoprotein.

2.7. Statistical analysis

Intragroup comparisons were performed using Wilcoxon signed-rank test to assess the immune responses post treatment, and intergroup comparisons were made by using the non-parametric Kruskal-Wallis test (One-way ANOVA on ranks). Mann-Whitney test was used to compare intergroup skin irritation index scores in response to vaccination or application site. These tests were used to assess statistical differences, where significance is assumed $p < 0.05$. Analyses were performed with GraphPad PRISM version 7.03 (La Jolla, USA).

2.8. Role of the funding source

Vaxxas served as the sponsor and took main responsibility for design and execution of the study. The corresponding author (AHF) had full access to all the data in the study and final responsibility for the decision to submit for publication.

3. Results

Between 29 June and 12 August 2016, 61 subjects were enrolled, randomly assigned to a treatment group and were vaccinated. Thirteen subjects out of 74 volunteers screened were not enrolled into the study (Fig. 2). There were no substantial differences between the three groups in terms of demographic profile (Supplementary Table 1).

Table 1
Number (percent) of adverse events by treatment and group.

Event	NP-HA/FA (N = 16)*	NP-HA/UA (N = 15)	IM-HA/D (N = 15)	Overall, vaccine groups (N = 46)	NP-placebo/FA (N = 5)	NP-placebo/UA (N = 5)	IM-placebo/D (N = 5)	Overall, placebo groups (N = 15)
Any	**8 (50)**	**9 (60)**	**5 (33)**	**22 (48)**	**1 (20)**	**4 (80)**	**3 (60)**	**8 (53)**
Lymph-adenopathy	0 (0)	0 (0)	1 (7)	**1 (2)**	0 (0)	0 (0)	0 (0)	**0 (0)**
Application site pruritus	2 (13)	0 (0)	0 (0)	**2 (4)**	0 (0)	0 (0)	0 (0)	**0 (0)**
Axillary pain	1 (6)	0 (0)	0 (0)	**1 (2)**	0 (0)	0 (0)	0 (0)	**0 (0)**
Fatigue	0 (0)	0 (0)	0 (0)	**0 (0)**	0 (0)	0 (0)	1 (20)	**1 (7)**
Sinusitis	1 (6)	0 (0)	0 (0)	**1 (2)**	0 (0)	0 (0)	0 (0)	**0 (0)**
URTI	0 (0)	0 (0)	0 (0)	**0 (0)**	0 (0)	3 (60)	0 (0)	**3 (20)**
Viral URTI	0 (0)	2(13)	0 (0)	**3 (4)**	0 (0)	0 (0)	1 (20)	**1 (7)**
Joint injury	1 (6)	0 (0)	0 (0)	**1 (2)**	0 (0)	0 (0)	0 (0)	**0 (0)**
Myalgia	0 (0)	1 (6)	0 (0)	**1 (2)**	0 (0)	0 (0)	0 (0)	**0 (0)**
Headache	0 (0)	2 (13)	4 (27)	**6 (13)**	1 (20)	0 (0)	1 (20)	**2 (13)**
Abortion, spontaneous	1 (6)	0 (0)	0 (0)	**1 (2)**	0 (0)	0 (0)	0 (0)	**0 (0)**
Pregnancy	1 (6)	0 (0)	0 (0)	**1 (2)**	0 (0)	0 (0)	0 (0)	**0 (0)**
Oropharyngeal pain	1 (6)	0 (0)	0 (0)	**1 (2)**	0 (0)	1 (20)	0 (0)	**1 (7)**
Throat irritation	0 (0)	0 (0)	0 (0)	**0 (0)**	1 (20)	0 (0)	0 (0)	**1 (7)**

Group abbreviations: NP-HA/FA – NPs coated with inactivated split influenza virus (A/California/07/2009 [H1N1], 15 μg haemagglutinin [HA] per dose), applied to the volar forearm; NP-HA/UA – NPs coated with inactivated split influenza virus (A/California/07/2009 [H1N1], 15 μg HA per dose, applied to the upper arm; IM-HA/D – Fluvax® 2016 containing 15 μg of the same H1N1 HA antigen injected intramuscularly (IM) into the deltoid; NP-placebo/FA – NPs coated with excipients only, applied to the volar forearm; NP-placebo/UA – NPs coated with excipients only applied to the upper arm; IM-placebo/D – saline injected IM into the deltoid.
URTI: upper respiratory tract infection.
* One subject withdrew after day 3 and was replaced, but contributed no data for subsequent assessments.

3.1. Summary of adverse events

A total of 40 adverse events (AEs) were reported by 30 subjects (Table 1). Of the subjects receiving vaccine, 8 of 16 (50%) NP-HA/FA subjects, 9 of 15 (60%) NP-HA/UA subjects, and 5 out of 15 (33%) IM-HA/D subjects reported at least one AE, and a similar proportion of placebo recipients, 8 of 15 (53%), reported at least one AE. Headaches were the most common AE considered to be related to treatment, reported by 6 (13%) subjects receiving vaccine and 2 (13%) subjects in the placebo groups. All AEs were mild or moderate with the exception of one serious AE, a spontaneous abortion that was not considered related to treatment. No clinically significant events were observed with respect to clinical laboratory tests, vital signs or aural temperature. There was also one severe AE, a soft tissue injury due to bicycle accident. This was considered to be unrelated to vaccination, but the subject withdrew from the study.

3.2. NP-application-site reactogenicity and resolution

Examples of application site reactions and resolution following application of a placebo (excipient-coated) NP and vaccine-coated NP are shown in Fig. 3. The initial erythema associated with placebo NP application typically faded to an area of mild discolouration between 2 h and day 3 after removal of the NP. This was consistent with previous observations with placebo NPs [7]. In contrast, the skin response following HA-coated NP application peaked at day 3 and faded between day 7 and day 28.

Analysis of the skin irritation index (SII) scores indicated that the erythema response predominated over oedema and lasted longer (Fig. 4A and B). The erythema seen with the placebo NPs decreased steadily following removal of the NP, but persisted longer with the HA-NPs, with significantly higher erythema scores for the HA-NPs compared with the placebo at all timepoints from day 3 onwards after application (Fig. 4C). Comparing the skin

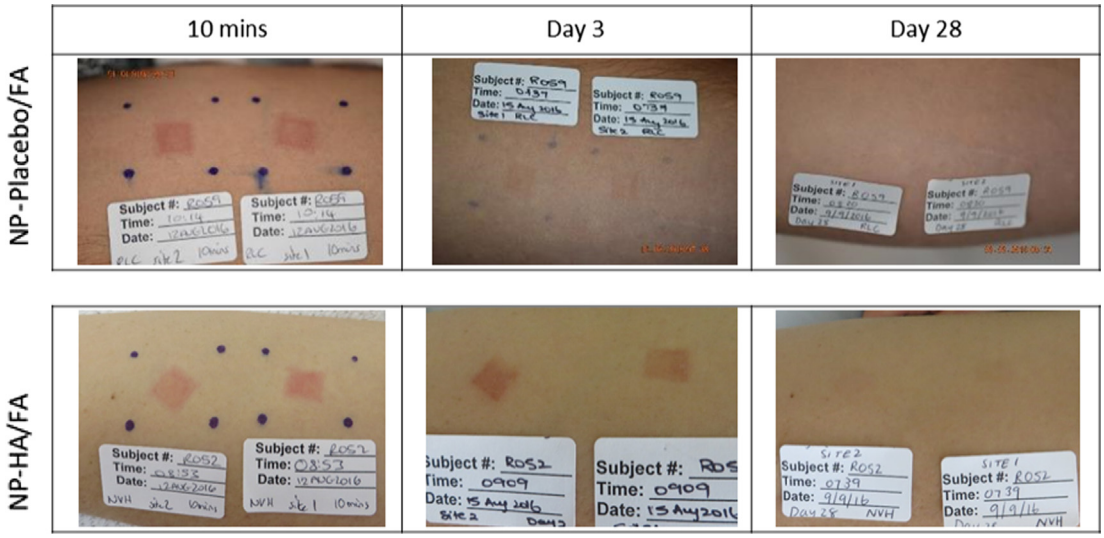

Fig. 3. Representative images for time course of skin reactions at Nanopatch (NP) application site. Photographs of time course of skin reactions observed with two NPs applied at two adjacent sites on the forearm (FA). The upper panels show the skin reaction following application of placebo patches (NP-placebo/FA group). The bottom panels show the skin reaction following application of A/California/07/2009-coated NPs (NP-HA/FA group).

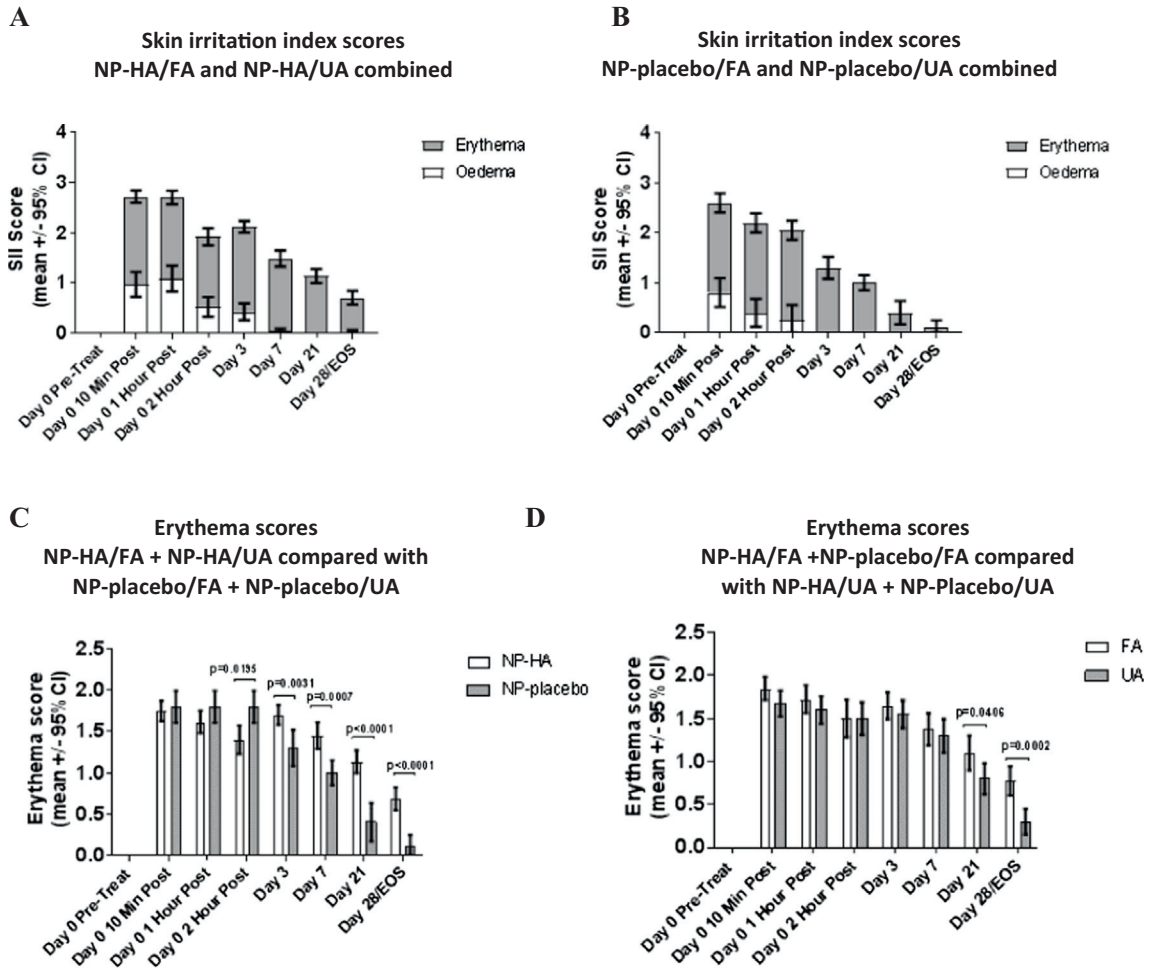

Fig. 4. Skin Response to Vaccination by Nanopatch (NP). Combined Skin Irritation Index Score (SII); erythema (scored 0–5) plus oedema (scored 0–5) for NP applications sites for (A) NP-HA/FA and NP-HA-UA recipients, and (B) NP-placebo/FA and NP-placebo/UA recipients. (C) Erythema scores for active NP applications (NP-HA/FA+NP-HA/UA) compared with placebo (NP-placebo/FA+NP-placebo/UA). (D) Comparison of erythema scores for FA applications (NP-HA/FA+NP-placebo/FA) compared with UA applications (NP-HA/UA+NP-placebo/UA). Columns represent the mean scores; error bars show the standard deviation. p values calculated by the non-parametric two-tailed Mann-Whitney Test.

response at application sites on the forearm or upper arm at each timepoint, there was a greater decrease in erythema at the upper arm sites at days 21 and 28 (Mann-Whitney Test, $p < 0.05$) (Fig. 4D). Mild erythema was observed around the IM injection site on the day of injection, but in general the IM injections were well tolerated.

The application sites were also monitored for spreading redness around the application site and colouration, itching, skin flaking, bleeding, induration, seepage and bruising (Supplementary Table 2). Mild itching was recorded by 13% and 7% of NP-HA/FA and NP-HA/UA recipients, respectively, at day 7. Some subjects (13% and 33% in the NP-HA/FA and NP-HA/UA groups, respectively) experienced mild or moderate skin flaking at the area of skin directly under the NP application site at day 3, and at day 7 (67% and 87% in the NP-HA/FA and NP-HA/UA groups, respectively). Placebo recipients also experienced mild or moderate skin flaking (60% of subjects in the NP-placebo/FA and NP-placebo UA groups). It was not seen at later timepoints. Only one subject (NP-placebo/FA) experienced induration; classified as mild. Only one subject (NP-HA/FA) recorded pin-point bleeding from 10 min to 2 h post-application.

All application site reactions recorded were mild or moderate, with the exception of a single subject with 'severe' colouration at 10 min after application (NP-HA/FA group) graded as 3 on a scale of 0 to 5. All patches and microprojections appeared intact after removal from skin as assessed by scanning electron microscopy (SEM) (data not shown).

3.3. Pain at application site

Self-reported pain scores 10 min after NP application or IM injection were low overall (Fig. 5). The mean scores for the groups receiving vaccine were 1.1 (0.51–1.61 95% CI), 0.9 (0.24–1.50 95% CI). 0.8 (−.04 to 1.64 95% CI) for the NP-HA/FA, NP-HA/UA and IM-HA/D groups respectively. Scores in placebo recipients were similar; 0.8 (−0.16 to 1.76 95% CI), 0.8 (−0.16 to 1.76 95% CI) and 0 (no pain recorded) for the NP-placebo/FA, NP-placebo/UA and IM-placebo/D groups. Overall 55% of NP-HA/FA and NP-HA/UA applications were scored as '0' (no pain), compared to 60% of NP-placebo/FA and NP-placebo/UA applications, 73% of the IM-HA/D vaccinations, and 100% IM-placebo/D injections. The highest pain score recorded was '6', for an IM vaccination, 10-min post administration. Only one subject experienced pain later than 10 min after vaccination, recording a score of '2' at 1 h in the NP-HA/FA group.

3.4. Acceptability questionnaire and interviews

The questionnaire and interview results were consistent with those obtained in the previous study with NPs without antigen

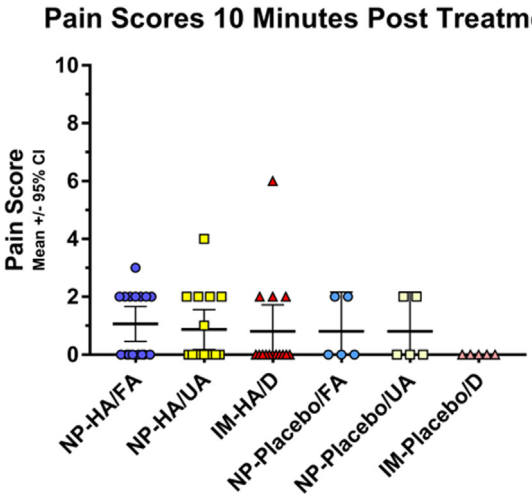

Fig. 5. Pain scores. Pain arising from vaccination with either the intramuscular (IM) injection or the Nanopatch (NP) groups was recorded using a self-assessment analogue scale (from 0 = no pain, 5 = moderate pain, to 10 = worst pain imaginable) at 10 min after application of NPs or IM injection. The coloured symbols represent individual subject scores, the horizontal bar shows the mean score for the group and the error bars shown 95% CI.

[7]. Most subjects (55%) preferred the NP compared to their previous experience of N&S injection, which was preferred by 24%, with the remaining subjects having no preference or not indicating a preference. Reasons for opting for N&S were to avoid the red mark left by the NP, or due to itching at the application site, or because the N&S was more familiar.

3.5. Immunogenicity

HAI titre was used as the primary measure of immunogenicity. All subjects were included in the analysis, irrespective of their baseline HAI titres at day 0. There was an increase in the HAI geometric mean titre (GMT) compared to baseline for the NP-HA/FA, NP-HA/UA and IM-HA/D groups at day 7 ($p \leq 0.0022$ in all groups) and day 21 ($p < 0.0001$ in all groups) post vaccination, while there was no increase in HAI titre in any of the placebo groups following vaccination (Fig. 6A). The HAI GMTs at day 21 for the NP-HA/FA, NP-HA/UA and IM-HA/D groups were: 335 (189–593 95% CI), 160 (74–345 95% CI), and 221 (129–380 95% CI) respectively (Supplementary Table 3). There was no statistical difference in the HAI GMTs at day 21 between the NP-HA/FA, NP-HA/UA and IM-HA/D groups for each binary comparison between groups. The percentage of subjects seroconverting after treatment (i.e. with HAI titre <10 prevaccination and ≥1:40 at day 21, or showing a 4-fold increase in titre following vaccination) were: NP-HA/FA 12 out of 15 (80%); NP-HA/UA 9 (60%); IM-HA/D 12 (80%). There were no seroconversions in any of the placebo groups (Supplementary Table 3).

MN assays were performed to assess levels of functional anti-influenza antibodies (Fig. 6B). As with the HAI results, all groups receiving the vaccine showed an increase in MN titres at days 7 and day 21. The MN GMT at day 21 for the NP-HA/FA, NP-HA/UA and IM-HA/D groups were: 6703 (3534–12,713, 95% CI), 2211 (1057–4624, 95% CI), and 4032 (1854–8767, 95% CI) respectively (Supplementary Table 3) and there was no statistical difference in the GMT between the different treatment groups. There was no increase in MN titre in any of the placebo groups following vaccination. In both the HAI and MN assays, there was a slight trend for the NP-HA/FA group titres to be higher than those for the NP-HA/UA or IM-HA/D groups, but this was not statistically significant.

4. Discussion

This study was the first use of the NP to deliver a vaccine antigen to the skin of human volunteers. Therefore it is encouraging that no clinically significant local or systemic adverse events were observed that were considered to be related to NP vaccinations. It was however expected that reactogenicity at the site of NP application would be more apparent than seen with IM injection due to the vaccine being delivered very close to the skin surface. We decided to study local skin responses in detail to understand their impact on the acceptability of vaccination by NPs. There were more local reactions at the site of NP application than were seen with IM injection as expected, but these were self-limiting and mild or moderate in intensity. It is likely that any erythema and itchiness reflected inflammatory processes in the skin involved in the induction of immune responses, which would be more visible with NP delivery than IM injection as they occur closer to the surface of the skin. Tenderness and pain are generally observed with IM injection of vaccines. Erythema at the NP-application site took longer to resolve with HA-antigen-containing NPs compared with placebo NPs, possibly due to the initial innate inflammatory response to the antigen, and/or activation of antigen specific T cells resident in the skin. Hirobe et al. [8] also reported peak application site redness 2 days after application of DMNs, that lasted for at least 21 days, and then a second patch was applied. It was not reported whether this resulted in a reaction at the original patch application site. Overall, the tolerability and acceptability findings with NPs were similar to those seen with uncoated and placebo NPs [7], and similar to those reported for DMNs without antigen by Arya et al. [6], and with influenza antigen [9].

The scores for pain at the application site appeared to be higher with the HA-antigen-containing NPs than the placebo in our study. However, the majority of subjects did not find NP administration painful, and a slight majority preferred NP to N&S injections, potentially reducing resistance to vaccination. The short wear time for NPs (2 min) could be particularly advantageous in this regard, making it more acceptable to vaccinees and healthcare systems than MAPs with longer wear times (20 min–6 h) [8,9], as it would be less disruptive to standard work patterns. Future studies will investigate whether the NP wear time can be further reduced which might further increase compliance [21].

The HAI and MN titres induced were not significantly different between the different modes of vaccination, indicating that, at the standard dose of 15 μg HA, the antibody responses induced by the NP were similar to those induced by IM injection, with the caveat that the group sizes in this study were small. A single dose level of HA was selected for the study (estimated to be a delivered dose of 15 μg), so it was not possible to determine whether NP administration resulted in dose-sparing, as seen in preclinical models [10–15]. Some of the analyses (GMT and fold-increase in GMT, Supplementary Table 3) suggested higher antibody responses in the forearm NP group than the IM group, and lower titres in the upper arm NP group; these differences were not statistically significant however. Similar results were seen with the MN and HAI assays, although the MN GMTs were higher than the HAI titres, as has been observed by other groups [22]. The overall finding that the MAP-induced antibody responses were similar in magnitude to those following IM injection, was similar to the results reported by Hirobe et al. [8] and Rouphael et al. [9]. These two studies used DMNs and involved longer patch wear times than needed for the NP.

This study had several limitations. For this initial test of the NP in humans, a monovalent influenza vaccine (H1N1) was used for simplicity, rather than the tri-valent formulation administered IM. Thus, immune responses to NP-delivered vaccine were measured to only one influenza subtype. It is also possible that the

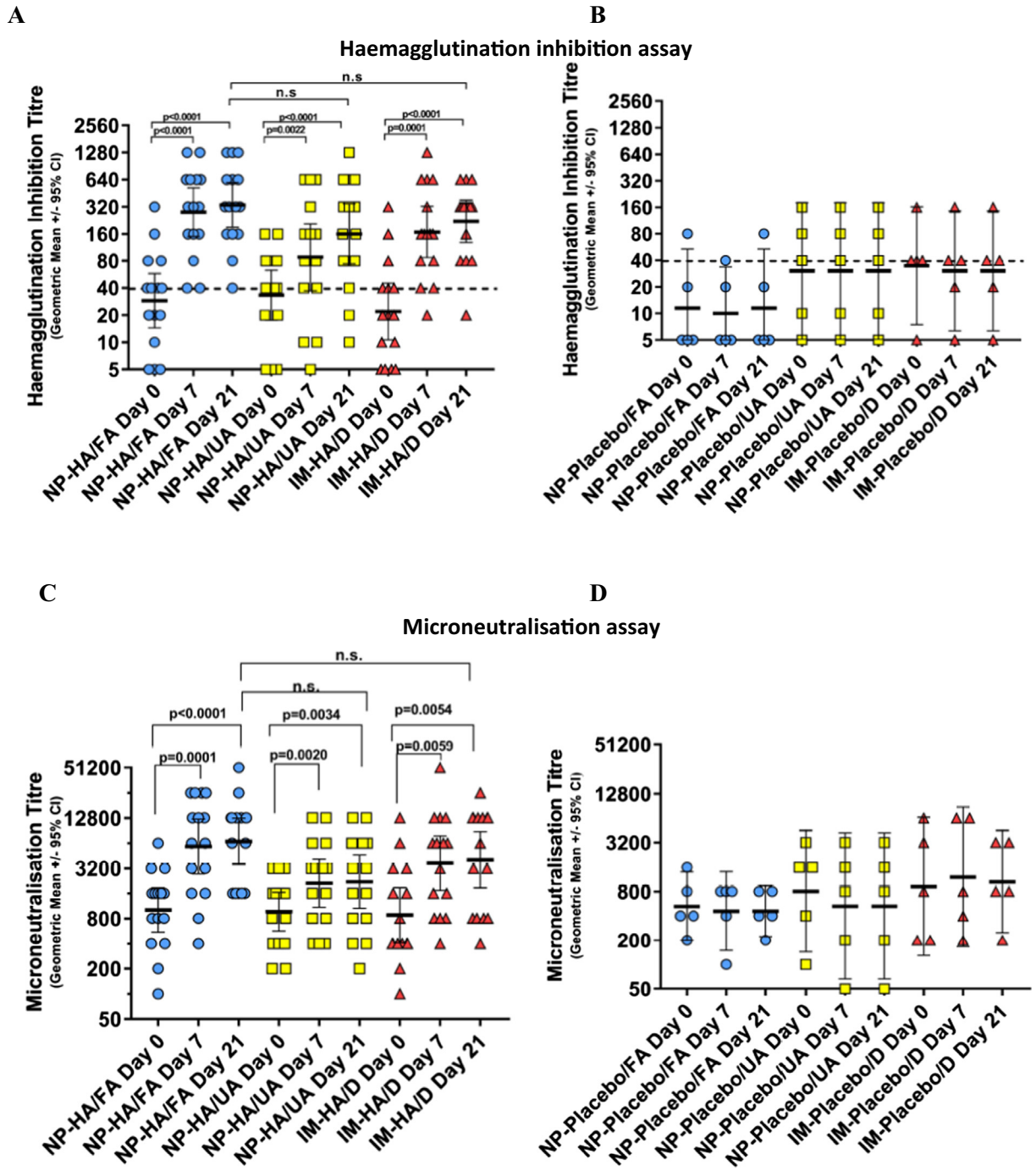

Fig. 6. Haemagglutination inhibition (HAI) and microneutralising (MN) antibody titres following vaccination with either A/California 07/2009 H1N1 vaccine or placebo. (A) and (B) HAI titres induced by A/California 07/2009 H1N1 vaccine or placebo respectively. The seroprotective titre of 1:40 is shown by the broken line. (C) and (D) MN titres induced by A/California 07/2009 H1N1 vaccine or placebo respectively. Symbols represent responses from individual subjects; the heavy solid bars represent the GMT and the error bars show the 95% confidence intervals. Intragroup comparisons by Wilcoxon signed-rank test. Intergroup comparisons by Kruskal-Wallis test.

response to the H1N1 antigen given IM would be different when administered as a monovalent or trivalent formulation, although we are not aware of any data to suggest this might be the case. In addition, assays were not performed to determine whether NPs induced qualitatively different immune responses, as have been reported before with MAPs or transcutaneous delivery [23,24]. It should be stressed that the number of subjects in each group was small, therefore any conclusions regarding the relative immunogenicity of the different treatment groups need to be made in this context of this limitation. These points will be investigated and addressed in future, larger clinical studies, as will the longevity of the immune response. Finally, although MAPs offer the potential for enhanced vaccine thermostability, this was not explored in detail in this study. In a previous study, influenza vaccine coated onto NPs in a similar formulation to the one used in this study, was shown to be stable for at least 6 months at room temperature when stored protected from moisture [25].

Given the encouraging immunogenicity and acceptability data from this and other recent clinical MAP vaccination studies [6,7,9], the challenge now facing all vaccine MAP developers is to

scale-up manufacturing processes to enable production millions of devices at an acceptable cost of goods. To this end, a number of key technological developments have been put in place for NPs since this study was conducted. A new method is used for NP vaccine coating which targets the tips of the microprojections, rather than the whole NP (as in this study), so that >80% of the vaccine payload is delivered to the skin (unpublished data); the current, relatively low delivery efficiencies obtained with the original version of the device as used in this study were an obvious limitation to the technology. In addition, NPs are now produced using medical-grade synthetic polymers rather than silicon, reducing the cost of goods of MAP production significantly. Finally, in this study, a separate applicator was used, whereas in the commercial embodiment of the device, the NP and applicator will be in an integrated, single-use device.

5. Conclusion

In this small first time in human study, the NP vaccine delivery system appeared to be safe, well tolerated by the subjects receiving the vaccination, and induced similar immune responses as IM injection. The results suggest that the NP has the potential to be an effective approach for vaccination with seasonal influenza and other vaccines.

Contributors

All authors contributed to the design of the study and approved the final version of the article. GJF, JH, and AHF wrote the manuscript with significant contributions from KW, CMJ and IHF. CMJ, GJF, JH, KW, MP, RSS, CD, JB, SR, CDA, and AHF analysed and interpreted the data. PG, IHF and AHF supervised the study.

Declaration of interests

CMJ, KW, and AHF are employees of Vaxxas Pty. Ltd. JH and IHF are both members of the Scientific Advisory Board of Vaxxas Pty Ltd. JH, GJF and CDA have received consultancy fees from Vaxxas Pty Ltd. The University of Sydney received funds from Vaxxas Pty Ltd for time spent to undertake training and analysis of qualitative data.

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Appendix A. Supplementary material

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.vaccine.2018.05.053.

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