The safety, immunogenicity, and acceptability of inactivated influenza vaccine delivered by microneedle patch (TIV-MNP 2015): a randomised, partly blinded, placebo-controlled, phase 1 trial

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Summary

Background Microneedle patches provide an alternative to conventional needle-and-syringe immunisation, and potentially offer improved immunogenicity, simplicity, cost-effectiveness, acceptability, and safety. We describe safety, immunogenicity, and acceptability of the first-in-man study on single, dissolvable microneedle patch vaccination against influenza.

Methods The TIV-MNP 2015 study was a randomised, partly blinded, placebo-controlled, phase 1, clinical trial at Emory University that enrolled non-pregnant, immunocompetent adults from Atlanta, GA, USA, who were aged 18–49 years, naive to the 2014–15 influenza vaccine, and did not have any significant dermatological disorders. Participants were randomly assigned (1:1:1:1) to four groups and received a single dose of inactivated influenza vaccine (fluvirin: 18 µg of haemagglutinin per H1N1 vaccine strain, 17 µg of haemagglutinin per H3N2 vaccine strain, and 15 µg of haemagglutinin per B vaccine strain) (1) by microneedle patch or (2) by intramuscular injection, or received (3) placebo by microneedle patch, all administered by an unmasked health-care worker; or received a single dose of (4) inactivated influenza vaccine by microneedle patch self-administered by study participants. A research pharmacist prepared the randomisation code using a computer-generated randomisation schedule with a block size of 4. Because of the nature of the study, participants were not masked to the type of vaccination method (ie, microneedle patch *vs* intramuscular injection). Primary safety outcome measures are the incidence of study product-related serious adverse events within 180 days, grade 3 solicited or unsolicited adverse events within 28 days, and solicited injection site and systemic reactogenicity on the day of study product administration through 7 days after administration, and secondary safety outcomes are new-onset chronic illnesses within 180 days and unsolicited adverse events within 28 days, all analysed by intention to treat. Secondary immunogenicity outcomes are antibody titres at day 28 and percentages of seroconversion and seroprotection, all determined by haemagglutination inhibition antibody assay. The trial is completed and registered with ClinicalTrials.gov, number NCT02438423.

Findings Between June 23, 2015, and Sept 25, 2015, 100 participants were enrolled and randomly assigned to a group. There were no treatment-related serious adverse events, no treatment-related unsolicited grade 3 or higher adverse events, and no new-onset chronic illnesses. Among vaccinated groups (vaccine via health-care worker administered microneedle patch or intramuscular injection, or self-administered microneedle patch), overall incidence of solicited adverse events (n=89 *vs* n=73 *vs* n=73) and unsolicited adverse events (n=18 *vs* n=12 *vs* n=14) were similar. Reactogenicity was mild, transient, and most commonly reported as tenderness (15 [60%] of 25 participants [95% CI 39–79]) and pain (11 [44%] of 25 [24–65]) after intramuscular injection; and as tenderness (33 [66%] of 50 [51–79]), erythema (20 [40%] of 50 [26–55]), and pruritus (41 [82%] of 50 [69–91]) after vaccination by microneedle patch application. The geometric mean titres were similar at day 28 between the microneedle patch administered by a health-care worker versus the intramuscular route for the H1N1 strain (1197 [95% CI 855–1675] *vs* 997 [703–1415]; p=0·5), the H3N2 strain (287 [192–430] *vs* 223 [160–312]; p=0·4), and the B strain (126 [86–184] *vs* 94 [73–122]; p=0·06). Similar geometric mean titres were reported in participants who self-administered the microneedle patch (all p>0·05). The seroconversion percentages were significantly higher at day 28 after microneedle patch vaccination compared with placebo (all p<0·0001) and were similar to intramuscular injection (all p>0·01).

Interpretation Use of dissolvable microneedle patches for influenza vaccination was well tolerated and generated robust antibody responses.

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Panel: Research in context

Evidence before this study

Several studies on intradermal influenza vaccination with a hollow microneedle injection have been published, and an approved product using this approach exists (Fluzone Intradermal Quadrivalent Influenza Vaccine, Sanofi Pasteur). Although safe and effective, this microneedle device is not dissolvable (ie, does not eliminate sharps waste), is not thermostable outside the cold chain, and requires administration by trained health-care providers. A dissolvable microneedle patch for influenza vaccination was assessed in a small clinical study published in 2015. This study did not include self-administration or a negative control group, it required multiple vaccinations, and data from 63% of the study participants were discarded because of product failure. We found no additional human studies after searching PubMed for reports published in any language between Jan 1, 2000, and Feb 1, 2017, with the terms "influenza vaccine" and "microneedle".

Added value of this study

This first-in-man study shows that the use of a single dissolvable microneedle patch for influenza vaccination was well tolerated, resulted in robust antibody responses, and was preferred over conventional influenza vaccination with needles and syringes. It also shows that the microneedle patches were reliably self-administered by study participants, were stable for at least 1 year at 40°C, and generated no sharps waste.

Implications of all the available evidence

This study suggests that dissolvable microneedle patches could simplify delivery of influenza vaccines, thereby enabling distribution and storage outside the cold chain (eg, on the pharmacy shelf), disposal as non-sharps waste, and possible self-administration under medical supervision or possibly at home. These advances could reduce the cost of influenza vaccination and increase patient access to influenza vaccine, thereby increasing vaccination coverage and protection from influenza morbidity and mortality. Animal studies have shown improved immunogenicity of influenza vaccination by microneedle patch compared with intramuscular injection; although the present study was not powered or designed to show an effect on immune response, future clinical studies might similarly show that influenza vaccination by microneedle patch also enhances immune response. Once confirmed by larger trials, the use of microneedle patches for influenza vaccination could have major public health implications on vaccination coverage and protection from disease.

Introduction

Even with the recommendation for universal vaccination,[1] influenza illness continues to be a major cause of morbidity and mortality, resulting in up to 48 000 deaths per year in the USA.[2] Influenza prevention through immunisation in adults is hindered by low vaccination coverage,[3] high immunisation costs,[4] and suboptimum vaccine effectiveness.[5,6] Although many types of influenza vaccines are currently licensed, improved delivery methods are needed to address these limitations.

In this study, we examine influenza vaccination with microneedle patches, which are micron-scale solid conical structures made of dissolvable excipients on a patch backing that deliver vaccine antigens across the stratum corneum barrier into the viable epidermis and dermis of the skin. The intradermal route for immunisation offers several immunological advantages due to the presence of large numbers of antigen-presenting cells (eg, Langerhans cells and other dendritic cells) in the skin.[7,8] In mice, influenza antigens delivered by microneedle patch resulted in a more robust immune response with greater longevity, increased breadth of immunity, and potential for dose sparing when compared with the intramuscular route.[9,10]

Microneedle patch immunisation also has the potential to overcome many factors affecting influenza vaccine uptake in adults such as needle phobia,[11] insufficient time, cost, and vaccine access.[12,13] Microneedle patches for vaccine delivery are economically advantageous for several reasons: an expected low manufacturing cost; elimination of costs associated with disposal of sharps waste; reduction or elimination of cold chain requirements through increased thermostability; decreased storage, transport, and disposal costs through smaller packaging volume; and reduced health-care-associated administration costs through self-administration by patients.[14] Microneedle patches for vaccine administration have also been shown to have greater acceptability when compared with traditional intramuscular hypodermic injection.[15]

Dissolvable microneedle patches are used in several cosmetic products[16] and other microneedle patches have been in human trials, most notably for administration of parathyroid hormone drugs.[17–19] However, vaccination with microneedle patches has been studied mostly in animals (eg, for the delivery of polio, measles, human papilloma virus, and influenza antigens). We did a first-in-man clinical trial comparing the safety, reactogenicity, immunogenicity, and acceptability of inactivated influenza vaccine delivered with a dissolvable microneedle patch applied by a health-care worker or through self-administration, with that of traditional intramuscular delivery by hypodermic needle.

Methods

Study design and participants

This partly blinded, randomised, placebo-controlled, phase 1 study at Emory University recruited non-pregnant, immunocompetent adults aged 18–49 years from the local community in Atlanta, GA, USA. Enrolled participants

were healthy, had not previously received the influenza vaccine during the 2014–15 influenza season, and did not have any significant dermatological disorders. Additional inclusion and exclusion criteria are detailed on ClinicalTrials.gov (NCT02438423). All participants provided written informed consent for study participation before enrolment. The study was approved by Emory University and Georgia Institute of Technology institutional review boards and was done in accordance with the Declaration of Helsinki and the International Conference on Harmonization Guidelines for Good Clinical Practice.

Randomisation and masking

Participants were randomly assigned (1:1:1:1) to one of four groups receiving: inactivated influenza vaccine by microneedle patch ($MNP_{IIV\text{-}HCW}$), inactivated influenza vaccine by intramuscular injection (IM_{IIV}), or placebo by microneedle patch ($MNP_{placebo}$), all applied by an unmasked health-care worker; or inactivated influenza vaccine by microneedle patch self-administered by study participants ($MNP_{IIV\text{-}self}$).

A research pharmacist prepared the randomisation code using a computer-generated randomisation schedule (Research Randomizer Form V4.0) with a block size of 4, and provided it to an unmasked health-care worker. Once the study products were administered, the unmasked health-care worker and the research pharmacists were not involved in subsequent study procedures. Participants were unaware if the microneedle patch applied by the unmasked health-care worker contained inactivated influenza vaccine or placebo, which were identical in appearance, and investigators were unaware if microneedle patches were applied by unmasked health-care workers or by participants. Because of the nature of the study, participants and study staff were not masked to the type of vaccination method (ie, microneedle patch *vs* intramuscular injection). Laboratory staff doing the haemagglutination inhibition antibody assays were masked to the group assignment.

Procedures

The licensed 2014–15 seasonal trivalent influenza vaccine (fluvirin) was provided by Seqirus (formerly NVS Influenza Vaccines, Cambridge, MA, USA) in single-dose, prefilled syringes for intramuscular injection containing 15 µg of each the following three influenza vaccine strains: A/Christchurch/16/2010, NIB-74 (H1N1); A/Texas/50/2012, NYMC X-223 (H3N2); and B/Massachusetts/2/2012, NYMC BX-51(B). The dose reported by the manufacturer was 15 µg of haemagglutinin per vaccine strain. Because the single radial immunodiffusion (SRID) assay for quantitating antigen is known to be variable and laboratory-dependent, to ensure consistency and comparability within our study, we quantified haemagglutinin antigen in this vaccine with our own SRID assay, which indicated each dose had 18 µg of haemagglutinin per H1N1 vaccine strain, 17 µg of haemagglutinin per H3N2 vaccine strain, and 15 µg of haemagglutinin per B vaccine strain.

The microneedle patches were designed at the Georgia Institute of Technology and manufactured by the Global Center for Medical Innovation (Atlanta, GA, USA) using Phase 1 Good Manufacturing Practice (figure 1). The formulation and fabrication methods have been previously described.[20] Seqirus also provided concentrated monobulks of each antigen, which were formulated into microneedle patches (MNP_{IIV}) to contain 18 µg (as measured by our own SRID assay) of each of the three influenza vaccine strains in the microneedles. We determined vaccine potency by single radial immunodiffusion assay.[21] Placebo patches contained the same formulation excipients, but without addition of the vaccine monobulks ($MNP_{placebo}$). We assessed MNP_{IIV} stability for 12 months at 5°C, 25°C, and 40°C by single radial immunodiffusion. We also measured residual vaccine content in MNP_{IIV} by single radial immunodiffusion in used patches to determine the actual dose delivered.

IM_{IIV} was administered by hypodermic needle in the deltoid muscle of the arm preferred by the participant and the microneedle patches were applied for 20 min to the dorsal aspect of the wrist of the non-dominant arm.

For the $MNP_{IIV\text{-}self}$ group, instructions were provided with a brief audiovisual presentation, and participants applied the patch under the unmasked health-care worker's supervision, but without physical intervention. Snap components were incorporated into the back of microneedle patches to guide microneedle patch

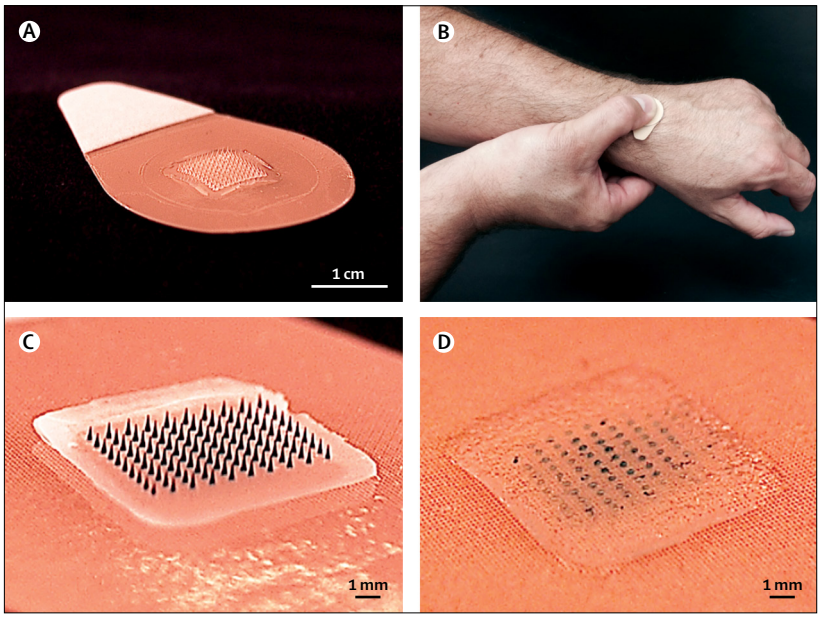

Figure 1: **Microneedle patch for influenza vaccination**
(A) The microneedle patch contains an array of 100 microneedles measuring 650 µm tall that is mounted on an adhesive backing. (B) The microneedle patch is manually administered to the wrist, enabling self-administration by study participants. (C) Microneedles encapsulate influenza vaccine (represented here by blue dye) within a water-soluble matrix. (D) After application to the skin, the microneedles dissolve, thereby depositing vaccine in the skin and leaving behind a patch backing that can be discarded as non-sharps waste.

application by providing audible and tactile feedback to the user when sufficient force was applied.

After receiving the assigned treatment on day 0, study staff assessed participants on days 2, 8, 28, 56, and 180. Solicited injection-site and systemic reactogenicity events were recorded for 7 days after receiving the assigned treatment with a participant's diary and by participant interview and examination by study staff. We recorded unsolicited adverse events for 28 days. We recorded serious adverse events, new onset of chronic medical disorders, and concomitant drug use for the duration of the study. Grading of adverse events was based on Food and Drug Administration toxicity grading.[22] Study staff obtained blood samples at all six clinic visits for safety and immunogenicity testing.

To generate antibody titres, masked staff at Hope Clinic Laboratory did haemagglutination inhibition assays on samples from day 0, 28, and 180 for all three influenza strains. For these assays, we obtained the H1N1 virus reference strain from the National Institute for Biological Standards and Control (Potters Bar, Hertfordshire, UK). We obtained the H3N2 and B virus reference strains from the Influenza Reagent Resource of the Centers for Disease Control and Prevention (Atlanta, GA, USA). These reference strains are the ones contained in the 2014–15 trivalent influenza vaccine.

Influenza viruses were propagated in MDCK.2 cells and MDCK.2 SIAT1 cells in the presence of TPCK trypsin (Sigma Aldrich, Saint Louis, MO, USA). Haemagglutination inhibition assays were performed as described in the WHO Influenza Surveillance Network laboratory manual.[23] Serum samples were treated overnight with receptor destroying enzyme (Sigma Aldrich) at 37°C, inactivated at 56°C for 30 min, and diluted in phosphate-buffered saline for an initial dilution of 1:10. Staff at the Hope Clinic Lab prepared a 0·5% solution of turkey red blood cells (Fisher, Hampton, NH) in haemagglutination buffer (Becton Dickinson, Franklin Lakes, NJ). Staff then tested receptor destroying enzymes and diluted serum samples for non-specific agglutination, and if activity was detected, they were pre-absorbed with red blood cells. Haemagglutination titres of H1, H3, and B viruses were determined with turkey red blood cells, diluted to 8 haemagglutination units per 50 µL, and back titrated to confirm the dilution titre. Treated and diluted serum samples were serially diluted two-fold in haemagglutination buffer, mixed with 4 haemagglutination units of virus, and incubated at room temperature for 30 min. After incubation, turkey red blood cells were added, mixed, and incubated at room temperature for 30 min. We then recorded haemagglutination inhibition for each serum dilution and virus mixture. Haemagglutination titres were summarised as geometric mean titres, percentage of seroprotection, and percentage of seroconversion.

Participants completed questionnaires at 0, 8, and 28 days after enrolment to assess vaccination acceptability outcomes via continuous scales (0–10 likelihood). We also measured vaccination knowledge, attitudes, perceptions, and beliefs using Likert-type scales (1–5 agreement levels).

Outcomes

Primary safety outcome measures are the incidence of study product-related serious adverse events within 180 days, grade 3 solicited or unsolicited adverse events within 28 days, and solicited injection site and systemic reactogenicity on the day of study product administration through 7 days after administration.

Secondary safety outcome measures are the incidence of new-onset chronic illnesses within 180 days and unsolicited adverse events within 28 days from enrolment. Secondary immunogenicity outcome measures are the geometric mean titre of haemagglutination inhibition antibody, percentage of participants achieving seroprotection (defined as a haemagglutination inhibition antibody titre of 1:40 or greater), and percentage of participants achieving seroconversion (defined as either a prevaccination haemagglutination inhibition titre of less than 1:10 and a postvaccination haemagglutination inhibition titre of 1:40 or higher, or a prevaccination haemagglutination inhibition titre of 1:10 or higher and a minimum four-fold rise in postvaccination haemagglutination inhibition antibody titre) at about 28 days after receipt of study products in the $MNP_{IIV\text{-}HCW}$ and IM_{IIV} groups.

Exploratory immunogenicity outcome measures are the geometric mean titre of haemagglutination inhibition antibody, seroprotection, and seroconversion within 28 days and 180 days between each of the MNP_{IIV} groups, and between the MNP_{IIV} groups and the IM_{IIV} group.

Another exploratory outcome measure is the preference for administration method of future influenza vaccination as determined by written survey by study participants on days 0, 8, and 28.

Statistical analysis

For the primary safety endpoint, with 25 participants per group, if the true frequency of participants with adverse events was 5% (or 10%), we would have a 34% (or 38%) chance to observe one adverse event and a 12% (or 34%) chance to observe more than one. For secondary immunogenicity endpoints, the sample size of 25 participants per group allowed 80% power to detect a difference of 1·2 (Cohen's d) in the geometric mean titre between groups at the α level of 0·05 with a two-sided *t* test. The sample size confers 80% power to detect difference in percentage of seroconversion between a vaccine group and the placebo group when the difference in proportions is 0·42 or higher. These effect sizes are powered for the comparison between a vaccinated group and the placebo group, but not for the comparison between the vaccinated groups (eg, non-inferiority test between MNP_{IIV} and IM_{IIV}), which are not the primary aims of this study.

Descriptive data are presented for reactogenicity, safety, acceptability, and immunogenicity. The reactogenicity,

safety, and acceptability populations included all participants who received a study product. The immunogenicity population included all participants who provided serum samples at baseline and at least 28 days or 180 days after receiving the assigned treatment. We calculated the 95% CI of the geometric mean titre on the basis of the normal distribution of log-transformed data, and calculated the Clopper-Pearson exact CI for seroprotection, seroconversion, adverse events, and acceptability percentage in each group. We used the Wilcoxon test to compare the geometric mean titres of each vaccinated group with that of the placebo group, and used Fisher's exact test to compare the frequencies of seroprotection, seroconversion, and frequency of participants with adverse events between each vaccinated group and the placebo group. We compared the frequencies of adverse events between the four groups using Fisher's exact test. We did the analyses with the R statistical software version 3.2.2. We used ANOVA and correlational analyses for the acceptability assessments (SAS 9.2, Cary, NC, USA). An independent safety monitor oversaw the safety of the study. This trial was registered with ClinicalTrials.gov, number NCT02438423.

Role of the funding source

The funder of the study had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The microneedle patch developer provided microneedle patches and was involved in discussions about study design, in study monitoring, and in the training of unmasked clinic staff on microneedle patch application. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Between June 23, 2015, and Sept 25, 2015, 100 participants were enrolled, randomly assigned to a group, and received the assigned treatment (figure 2). The demographics of the four groups were similar (table). Five participants (with three in the placebo group) missed either the day 28 or the day 180 visit.

No serious adverse events related to the study products were reported during the study. Stopping rules were not triggered and there were no withdrawals because of adverse events.

Reactogenicity events observed in the $MNP_{IIV\text{-}HCW}$ and the $MNP_{IIV\text{-}self}$ groups were similar and mostly mild (p=0·2). The IM_{IIV} group had a higher incidence of grade 2 and 3 reactogenicity events (three [12%] of 25 participants [95% CI 26–31]) than either of the MNP_{IIV} groups (one [2%] of 50 participants in the MNP_{IIV} groups combined [95% CI 0–11]; p=0·02). Significantly more local reactogenicity events were reported in the MNP_{IIV} groups than in the IM_{IIV} group: pruritus (41 [82%] of 50 participants [95% CI 69–91] *vs* four [16%] of 25 [5–36]; p<0·0001) and erythema (20 [40%] of 50 [26–55] *vs* zero of

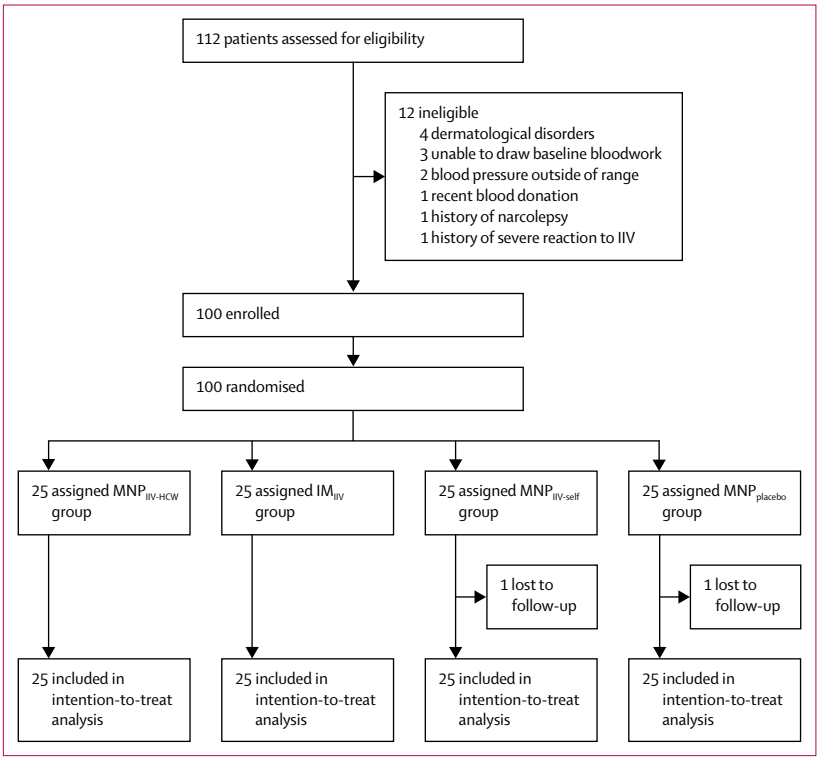

Figure 2: **Trial profile**
IIV=inactivated influenza vaccine. MNP=microneedle patch. HCW=health-care worker. IM=intramuscular.

	IM_{IIV} (n=25)	$MNP_{IIV\text{-}HCW}$ (n=25)	$MNP_{IIV\text{-}self}$ (n=25)	$MNP_{placebo}$ (n=25)	All (n=100)
Age (years)					
Mean (SD)	29·6 (6·9)	27·4 (5·9)	31·4 (8·4)	29·3 (8·4)	29·4 (7·5)
Median (IQR)	29 (25–32)	26 (23–29)	26 (25–38)	26 (24–29)	26 (24–33)
Sex					
Male	14 (56%)	13 (52%)	13 (52%)	13 (52%)	53 (53%)
Female	11 (44%)	12 (48%)	12 (48%)	12 (48%)	47 (47%)
Ethnic origin†					
White	12 (48%)	11 (44%)	14 (56%)	12 (48%)	49 (49%)
Black	8 (32%)	8 (32%)	8 (32%)	7 (28%)	31 (31%)
Other	5 (20%)	6 (24%)	3 (12%)	6 (24%)	20 (20%)
BMI (kg/m²)					
Mean (SD)	24·9 (4·3)	25·0 (4·5)	25·7 (4·0)	24·6 (4·6)	25·0 (4·3)
Median (IQR)	24·8 (21·3–28·2)	24·5 (21·1–26·5)	25·7 (22·3–28·6)	23·8 (21·5–27·0)	24·6 (21·7–27·4)
Previous IIV					
2013–2014 season	4 (16%)	6 (24%)	7 (28%)	6 (24%)	23 (23%)
2012–2013 season	6 (24%)	3 (12%)	6 (24%)	4 (16%)	19 (19%)
Any of these two seasons	8 (32%)	7 (28%)	9 (36%)	9 (36%)	33 (33%)

Data are n (%) unless stated otherwise. IIV=inactivated influenza vaccine. MNP=microneedle patch. HCW=health-care worker. IM=intramuscular. BMI=body-mass index. *All participants were vaccinated between June 23, 2015, and Sept 25, 2015. †Ethnic origin was self-reported.

Table: **Baseline characteristics***

For **R statistical software** see https://www.R-project.org/

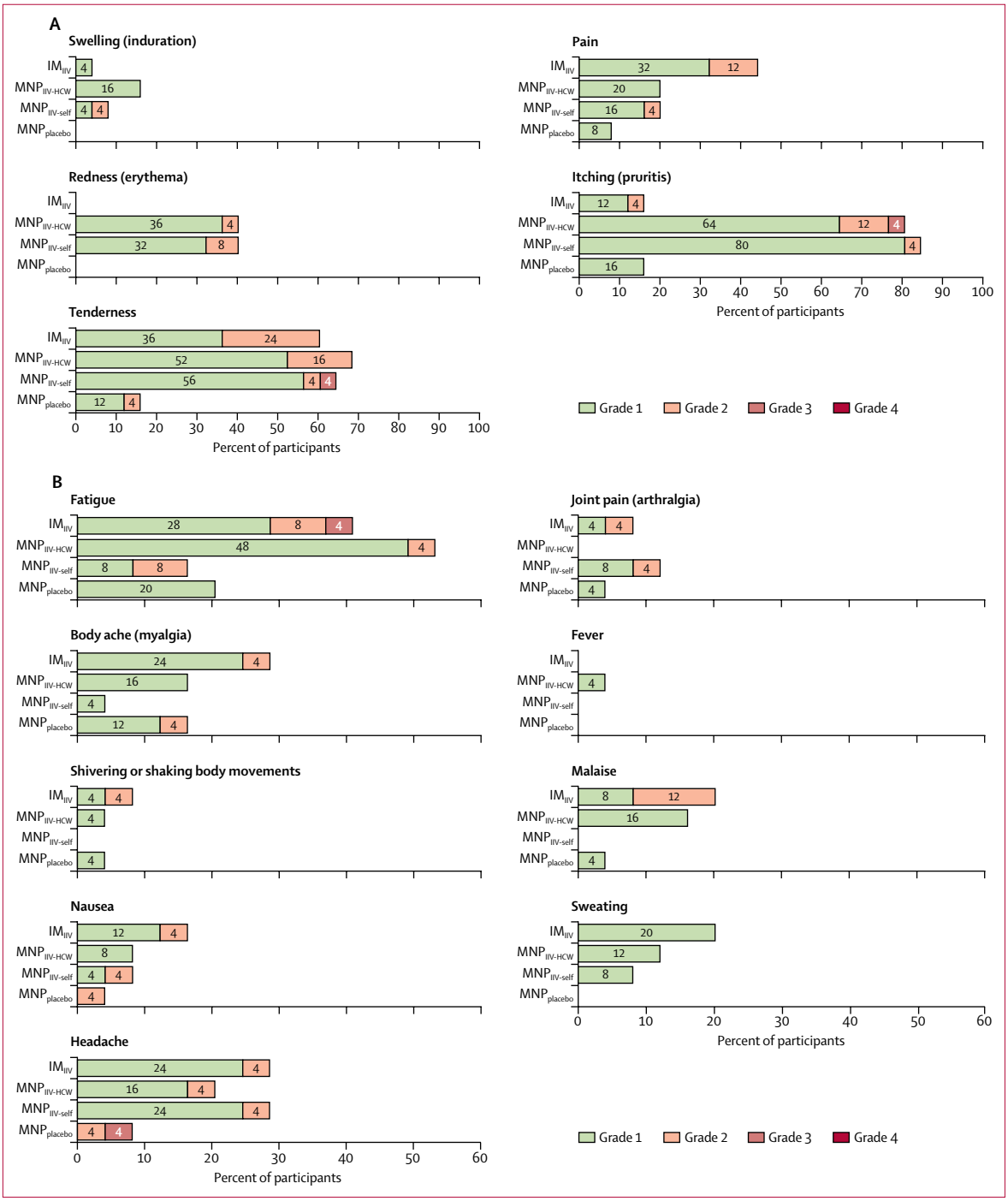

Figure 3: **Solicited reports of adverse events 7 days after vaccination**
(A) Local and (B) systemic adverse events associated with vaccination are shown in different groups. IIV=inactivated influenza vaccine. MNP=microneedle patch. HCW=health-care worker. IM=intramuscular.

25 [0–14]; p=0·0002; figures 3, 4). The most common vaccination site reaction for the two MNP_{IIV} groups was pruritus; 36 (88%) of these reactions in 41 participants were mild and self-limited, lasting 2–3 days on average. In the IM_{IIV} group, injection site pain reported over the days after vaccination was twice as frequent (11 [44%] of 25 participants [24–65] *vs* ten [20%] of 50 [10–34]; p=0·05) and more severe (grade 2 or higher; three [12%] of 25 [3–31] *vs* one [2%] 50 [0–10]; p=0·1) compared with the MNP_{IIV} groups combined. The rate and severity of

systemic reactogenicity events (figure 3) did not differ among the groups receiving inactivated influenza vaccine. Among vaccinated groups ($MNP_{IIV\text{-}HCW}$, IM_{IIV}, and $MNP_{IIV\text{-}self}$), overall incidence of solicited adverse events (n=89 *vs* n=73 *vs* n=73) and unsolicited adverse events (n=18 *vs* n=12 *vs* n=14) were similar.

No new chronic medical illnesses or influenza-like illnesses were reported. 61 unsolicited adverse events were reported by 41 (41%) of 100 participants after receiving the assigned treatment. Few treatment-unrelated grade 3 events were reported. One participant in the $MNP_{IIV\text{-}self}$ group developed acute enteritis requiring hospital treatment, and another participant in the $MNP_{placebo}$ group developed grade 3 hypertension while off her hypertensive drugs. One participant in the $MNP_{IIV\text{-}self}$ group had rhabdomyolysis due to strenuous exercise at baseline before receipt of study product and another participant in the IM_{IIV} group had a grade 3 elevation in liver function test due to exercise and excessive alcohol and paracetamol consumption 30 days after vaccination. These laboratory abnormalities resolved spontaneously. There were 13 treatment-related adverse events (seven in the $MNP_{placebo}$ group, three in the IM_{IIV} group, and three in the $MNP_{IIV\text{-}HCW}$ group) reported in eight participants. These adverse events were mostly grade 1 laboratory events (thrombocytopenia, leucopenia, and neutropenia), all of which resolved during study follow-up. No grade 3 or higher treatment-related laboratory adverse events occurred.

The geometric mean titres determined by haemagglutination inhibition antibody assay were similar at day 28 between the $MNP_{IIV\text{-}HCW}$ group and the IM_{IIV} groups for all virus strains: H1N1 strain (1197 [95% CI 855–1675] *vs* 997 [703–1415]; p=0·5), H3N2 strain (287 [192–430] *vs* 223 [160–312]; p=0·4), and B strain (126 [86–184] *vs* 94 [73–122]; p=0·06). Geometric mean titres similar to these were seen in the $MNP_{IIV\text{-}self}$ group (appendix).

When comparing immune response in the $MNP_{IIV\text{-}HCW}$ and the IM_{IIV} groups, seroprotection and seroconversion percentages at day 28 were similar and significantly higher for all three strains contained in the influenza vaccine groups compared with placebo (all p<0·01; appendix, figure 5), with the exception of a similar day 28 seroprotection percentage against the H3N2 influenza strain between the three groups. There was a higher seroconversion percentage against the B strain for the $MNP_{IIV\text{-}HCW}$ and $MNP_{IIV\text{-}self}$ groups combined (31 [65%] of 48 participants [95% CI 60–78]) compared with the IM_{IIV} group (eight [32%] of 25 [15–54]; p=0·01). Seroprotection against the three influenza strains 6 months after vaccination was seen in 20–24 (83–100%) of 24 participants in the $MNP_{IIV\text{-}HCW}$ group and in 20–25 (80–100%) of 25 participants in the IM_{IIV} group. The $MNP_{IIV\text{-}self}$ group had similar seroprotection, with 18–24 (75–100%) of 24 participants having a haemagglutination inhibition titre of 1:40 or higher at 180 days later (appendix).

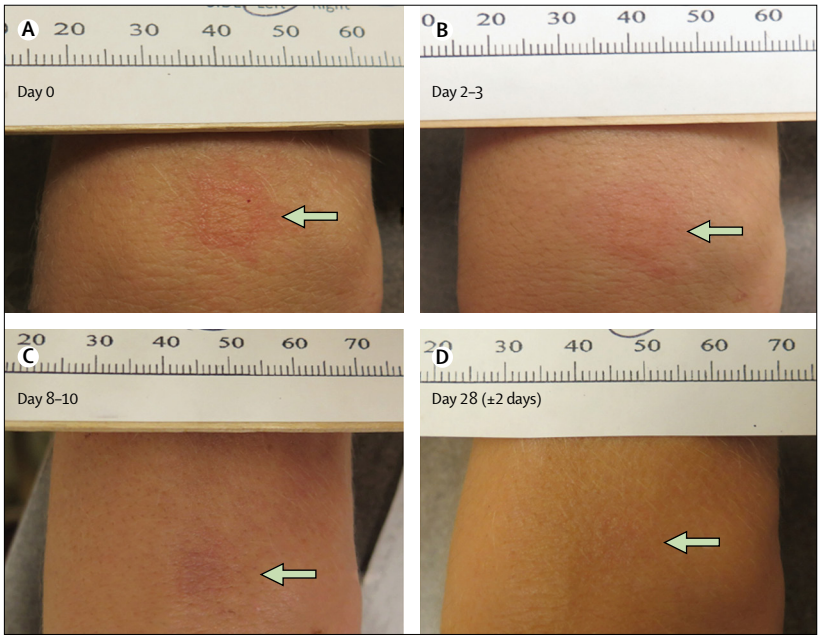

Figure 4: **Typical local reactions seen over time after administration and removal of a microneedle patch delivering inactivated influenza vaccine**
(A) Day 0, (B) days 2–3, (C) days 8–10, and (D) day 28 after vaccination.

Intramuscular vaccination delivered at least 15 µg of each influenza antigen. Measurement of residual antigens in the 50 MNP_{IIV} patches used in the study showed that the mean dose delivered by MNP_{IIV} was 11·3 µg (SE 0·5) for the H1N1 strain, 14·4 µg (0·5) for the H3N2 strain, and 13·1 µg (0·4) for the B strain. No significant difference was reported between the dose of each strain delivered by the $MNP_{IIV\text{-}HCW}$ and $MNP_{IIV\text{-}self}$ groups (p>0·60), suggesting that the participants were able to correctly self-administer microneedle patches. After vaccination, imaging of used microneedle patches showed that the microneedles had dissolved in the skin (figure 1), suggesting that the used patches could be discarded as non-sharps waste. After storage in desiccated packaging at 5°C, 25°C, and 40°C for 12 months, inactivated influenza vaccine potency for all three strains in the MNP_{IIV} remained within product specifications in the Investigational New Drug Application (appendix), which supports the storage of patches without refrigeration.

See Online for appendix

Right after vaccination, 48 (96%) of 50 participants (95% CI 86–100) who received MNP_{IIV} reported no pain during microneedle patch application, but only 18 (82%) of 22 participants (60–95) reported that intramuscular injection was painless (p=0·04). On a scale of 1 (negative experience) to 5 (positive experience), participants in the microneedle patch groups reported high acceptability for microneedle patch vaccination, with mean scores between 4·5 and 4·8 across the three microneedle patch groups. Participants receiving IM_{IIV} reported a mean score of 4·4, which was not significantly different between the

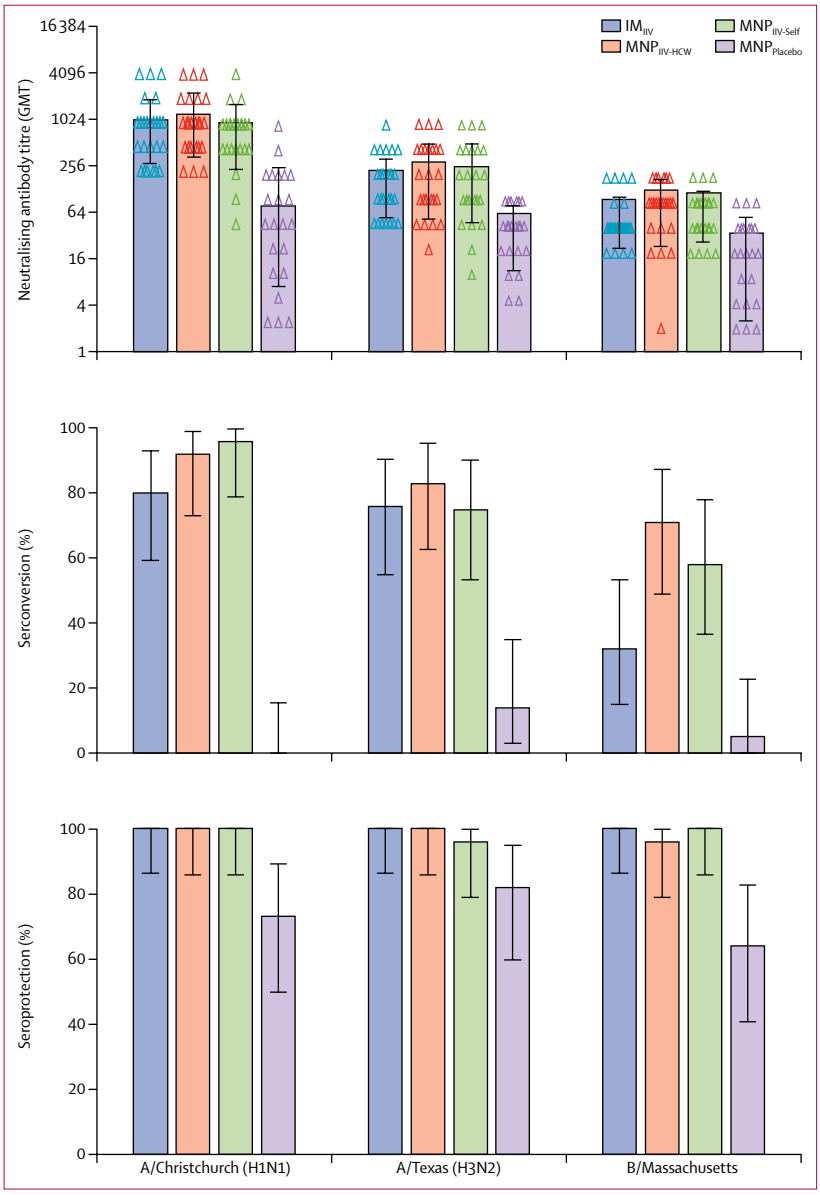

Figure 5: **Serological response to study drug administration**
(A) Haemagglutination inhibition GMTs (log 2), (B) seroconversion, and (C) seroprotection against A/Christchurch/16/2010 (NIB-74 [H1N1]), A/Texas/50/2012 (NYMC X-223 [H3N2]), and B/Massachusetts/2/2012 (NYMC BX-51[B]) strains for MNP$_{IIV-HCW}$, MNP$_{IIV-self}$, MNP$_{placebo}$, and IM$_{IIV}$ 28 days after vaccination. Bars show 95% CI. GMT=geometric mean titres. IIV=inactivated influenza vaccine. MNP=microneedle patches. HCW=health-care worker. IM=intramuscular.

intramuscular and microneedle patch groups (p=0·07; appendix). When asked on day 28 (thereby assessing the complete vaccination and post-vaccination experience), 33 (70%) of 47 MNP$_{IIV}$ recipients (55–83) preferred microneedle patch vaccination over intramuscular or intranasal vaccination (nine [19%] of 47 [9–33]) as a delivery method for future influenza vaccination (p<0·0001; appendix), suggesting a positive experience with microneedle patch vaccination; five stated that they preferred to have no vaccine.

Discussion

This study shows for the first time in a human clinical trial that influenza vaccination with a microneedle patch was well tolerated, immunogenic, and preferred after a single-dose vaccination administered by a health-care worker or by the participants themselves.

Microneedle patches were well tolerated, without any safety issues detected in this phase 1 study, although specific local, mild, self-limited reactions were more commonly reported with MNP$_{IIV}$ compared with IM$_{IIV}$. The higher rates of these local events are consistent with previous research with intradermal influenza vaccination.[24,25] The reported skin reactions could be due to a local immune response that is visible on the skin surface. Pain was more commonly reported in muscle after IM$_{IIV}$ vaccination compared with MNP$_{IIV}$ at frequencies consistent with previous clinical experience.[26]

Both MNP$_{IIV}$ groups met all US Food and Drug Administration immunogenicity criteria for licensure[27] for all strains, except the B strain lower bound CI criterion for seroconversion for the MNP$_{IIV-self}$ group (appendix). This weaker response to the B strain was also seen in the IM$_{IIV}$ group (which did not meet lower bound CI criteria for seroconversion and seroprotection), similar to previous studies of influenza vaccination.[24] These findings are consistent with previous animal studies showing strong immune responses to skin vaccination with microneedle patches.[9,10] Although the preclinical studies in naive animals have in some cases shown superior immunogenicity and efficacy after microneedle patch vaccination (eg, due to targeting of antigen-presenting cells in the skin), this human trial was not powered to show such differences.

In our population, microneedle patches were well accepted and strongly preferred over traditional intramuscular injection for influenza vaccination, consistent with previous results.[15] This finding could be significant because increased acceptability could enable increased rates of influenza vaccination, which are currently less than 50% in adult populations.[3] Moreover, because participants were able to self-vaccinate and 70% or more preferred it, significant cost savings could be enabled by microneedle patches due to a reduction in health-care worker time devoted to vaccination.

Scarce previous research with microneedle patches in human participants exists in the published scientific literature.[18] Parathyroid hormone has been administered in clinical trials with non-dissolvable, metal microneedle patches, and has shown good safety and efficacy.[17] A previous study examined influenza vaccination with a dissolving microneedle patch, but did not include self-administration or a negative control group.[28] That study also differed from the present study in that microneedle patches were worn for 6 h, microneedles were difficult to insert into the skin (such that only 37% of microneedle patches delivered at least half of the vaccine on the first patch application), local skin reactions were more

pronounced (eg, purpura, pigmentation, and longer-lasting erythema), and microneedle patches were not stable during extended storage at elevated temperatures.

Our study showed several advantages compared with other studies on self-administration of influenza vaccine by intradermal injection or nasal spray (currently not recommended by the Advisory Committee on Immunization Practices for the 2016–17 influenza season in the USA).[29,30] In our study, self-immunisation by microneedle patch was achieved in all participants with only brief training with audiovisual materials and without health-care worker intervention. The participants also had no specific medical background and therefore reflected the general adult population. Self-administration with microneedle patches might be further facilitated by the strong patient acceptance and preference of microneedle patches; the absence of sharps waste; the ability to store without refrigeration; generally painless vaccination and only infrequent, minor pain afterwards; small package size; and expected cost-savings. In the future, self-vaccination with microneedle patches could occur in a clinical or workplace vaccination setting with health-care worker supervision, at home after purchasing at the pharmacy, or after distribution by mail in a pandemic scenario.

There are several limitations of the study. The participants enrolled were probably less inclined to receive influenza vaccination by hypodermic injection because only those who did not receive the 2014–15 influenza vaccine were included in the study. Other comparative groups were considered for the study (such as intranasal and intradermal injection); however, we elected, for logistical reasons, to focus on comparing delivery by microneedle patch to the most widely approved administration method (ie, intramuscular injection). The study population had very high titres at baseline before vaccination, which makes differentiating the effects of the different routes of administration difficult (eg, only the B strain showed a significant difference between the IM_{IIV} and MNP_{IIV} immune response, possibly because it had the lowest prevaccination titres). A detailed analysis of the immunological mechanisms of MNP_{IIV} is needed. In our study, additional blood samples were collected in a subset of participants for exploratory outcome measures of immune response; their analysis will be the subject of a future publication. Additional studies testing the acceptability and reliability of microneedle patch self-application in larger populations are also warranted. The next generation of microneedle patch formulation could be optimised to further reduce local reactogenicity and increase delivery efficiency. Larger human trials are needed to confirm the findings of this study with greater power.

We conclude that influenza vaccination with microneedle patches is well tolerated, well accepted, and results in robust immunological responses, whether administered by health-care workers or by the participants themselves. These results provide evidence that microneedle patch vaccination is an innovative new approach with the potential to improve present vaccination coverage and reduce immunisation costs.

Contributors

All authors contributed to the study concept and design, and approved the final version of the Article. NGR wrote the manuscript with significant contributions from MRP and MJM. NGR, MP, PMF, and TY analysed and interpreted the data. NGR, MP, RM, SK, MJM, and the TIV-MNP 2015 Study Group collected clinical data. DVM, HK, and WP prepared and analysed microneedle patches. NJT, LL, and EVV optimised and performed immunological assays. NGR and MJM supervised the study. MP and TY provided statistical analyses. NGR and MRP searched the scientific literature.

The TIV-MNP 2015 Study Group

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Declaration of interests

SH, DVM, and MRP are inventors on licensed patents and have ownership interest in companies developing microneedle products (Micron Biomedical). SH, DVM, and WP are currently employed by Micron Biomedical. These potential conflicts of interest have been disclosed and are overseen by Georgia Institute of Technology and Emory University. All other authors declare no competing interests.

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