JOURNAL FARMASI
Fundamental and Applied Research in Medicine and Allied Sciences Indonesia

# Formulation Study and Evaluation of the Effectiveness of Natural Polymer-Based Dissolving Microneedles for Model Vaccine Delivery

**Emma Jayanti Besan[1]*, Muhammad Nurul Fadel[2], Nur Masyithah Zamruddin[3], Dara Sukma Ratmelya[4], & Yuneka Saristiana[5]**
[1]*Universitas Muhammadiyah Kudus, Indonesia, [2]Universitas Muhammadiyah Kudus, Indonesia, [3]Universitas Mulawarman, Indonesia, [4]Universitas Syiah Kuala, Indonesia, [5]Universitas Kadiri, Indonesia
*Co e-mail: emmajayanti@umkudus.ac.id[1]

Article Information
Received: February 24, 2026
Revised: April 24, 2026
Online: May 03, 2026

Keywords
*Dissolving Microneedles, Transdermal Vaccine Delivery, Polyvinyl Alcohol, Hyaluronic Acid, Ovalbumin, Immunogenicity, Natural Polymer*

ABSTRACT

*Dissolving microneedle (DMN) arrays made from biocompatible natural polymers offer a promising transdermal vaccine delivery system that can reduce pain, eliminate cold-chain dependence, and minimize the need for trained healthcare workers compared to conventional injections. This study aimed to formulate and characterize natural polymer-based DMNs containing ovalbumin (OVA) as a model antigen and evaluate their immunogenicity in mice. DMNs (F1–F5) were prepared using polyvinyl alcohol (PVA), polyvinylpyrrolidone (PVP K30), and hyaluronic acid (HA) at different concentrations through spin-casting into PDMS molds. The formulations were evaluated for morphology, mechanical strength, dissolution time, encapsulation efficiency, antigen release, and immunogenicity in BALB/c mice. The optimized formulation (F4; PVA 15%, PVP K30 10%, HA 1%) showed needle heights of 544.9 ± 15.1 µm, mechanical strength of 0.52 ± 0.04 N/needle, complete dissolution within 15 minutes, and encapsulation efficiency of 94.8 ± 1.8%. Immunogenicity testing demonstrated IgG titers comparable to subcutaneous injection controls. These findings indicate that natural polymer-based DMNs are a promising needle-free and patient-friendly vaccine delivery platform.*

***Keywords:*** *Dissolving Microneedles, Transdermal Vaccine Delivery, Polyvinyl Alcohol, Hyaluronic Acid, Ovalbumin, Immunogenicity, Natural Polymer*

## INTRODUCTION

Vaccination remains one of the most effective public health interventions for preventing infectious diseases. However, conventional vaccination primarily relies on hypodermic needle-based intramuscular or subcutaneous injection, which presents several limitations, including pain and needle phobia (trypanophobia), the need for trained healthcare personnel, risks of needle-stick injuries and cross-contamination, generation of sharps waste, and dependence on cold-chain logistics for storage and distribution (Sullivan et al., 2010; Moore et al., 2022).

Transdermal delivery has emerged as an attractive alternative route for vaccine administration because the skin is an immunologically active organ rich in antigen-presenting cells (APCs), including Langerhans cells in the epidermis and dermal dendritic cells. Targeting these cells enables efficient antigen processing and the induction of robust adaptive immune responses. Nevertheless, the outermost skin layer, the stratum corneum, acts as a strong barrier that restricts passive permeation to small, lipophilic molecules, limiting the transdermal delivery of macromolecules such as proteins and vaccines (Leone et al., 2019; Bauleth-Ramos et al., 2023).

Microneedle (MN) technology has been developed to overcome this barrier by creating microscopic channels that bypass the stratum corneum without reaching deeper nerve endings or blood vessels, thereby enabling minimally invasive and nearly painless administration. Among various MN types—including solid, coated, hollow, dissolving, and hydrogel-forming systems—dissolving microneedles (DMNs) have attracted considerable attention. In DMNs, the needle matrix itself encapsulates the therapeutic agent and dissolves completely after skin insertion, eliminating sharps waste and improving safety and patient compliance (Rodgers et al., 2018; Moore et al., 2022).

The selection of matrix materials plays a critical role in determining the mechanical strength, dissolution behavior, antigen stability, and overall performance of DMNs. Natural and naturally derived polymers such as hyaluronic acid (HA), gelatin, polysaccharides, and other biocompatible excipients are widely used due to their biodegradability, low toxicity, and favorable regulatory profiles (Bauleth-Ramos et al., 2023; Zhou et al., 2022). HA-based DMNs, in particular, have demonstrated the ability to deliver high antigen loads while maintaining structural integrity and generating immune responses comparable to conventional injections (Leone et al., 2019).

In addition, polymer combinations can be employed to optimize mechanical properties and dissolution kinetics. Polyvinyl alcohol (PVA) provides excellent film-forming ability and mechanical strength, while polyvinylpyrrolidone (PVP) functions as a plasticizer and pore-forming agent. When combined with hyaluronic acid (HA), which also possesses intrinsic skin hydration and potential immunomodulatory effects, synergistic improvements in needle integrity, dissolution rate, and antigen stabilization may be achieved (Moore et al., 2022; Bauleth-Ramos et al., 2023).

To further evaluate the performance of such polymer-based microneedle systems in vaccine delivery, the selection of an appropriate model antigen is essential. Ovalbumin (OVA) is widely used as a model antigen in vaccine delivery research due to its well-characterized molecular structure (42.7 kDa), defined T- and B-cell epitopes, commercial availability, and compatibility with standardized immunological assays. OVA-loaded dissolving microneedles have been extensively

used to evaluate antigen stability, release behavior, and immunogenicity in preclinical models (Leone et al., 2019; Courtenay et al., 2018).

Building upon these considerations, and addressing the need for optimized multi-polymer microneedle systems, the present study aimed to develop and optimize dissolving microneedle formulations composed of PVA/PVP/HA polymer combinations loaded with OVA as a model antigen. The formulations were systematically evaluated in terms of physicochemical properties, mechanical strength, antigen stability, in vitro dissolution behavior, and in vivo immunogenicity in a murine model.

## METHODS

Polyvinyl alcohol (PVA, MW 89,000-98,000 Da, 99+% hydrolyzed), polyvinylpyrrolidone K30 (PVP K30, MW ~40,000 Da), hyaluronic acid sodium salt (HA, MW ~1.5 MDa), trehalose dihydrate (pharmaceutical grade), and phosphate-buffered saline (PBS, pH 7.4) were obtained from Sigma-Aldrich (St. Louis, MO, USA). Ovalbumin (OVA, Grade V, >98% purity) and Freund's complete adjuvant (FCA) were purchased from Sigma-Aldrich. Coomassie Brilliant Blue G-250 (Bradford reagent) was obtained from Bio-Rad Laboratories (Hercules, CA, USA). Anti-mouse IgG-HRP conjugate and ELISA kits were purchased from BD Biosciences (San Jose, CA, USA). Polydimethylsiloxane (PDMS, Sylgard 184) was obtained from Dow Corning Corporation (Midland, MI, USA). BALB/c female mice (6-8 weeks, 18-22 g) were obtained from the Animal House, Faculty of Medicine, Universitas Sumatera Utara. All other reagents were of analytical grade.

Master molds were fabricated using standard photolithography techniques. Conical microneedle masters with a height of 600 um, base diameter of 300 um, tip radius of approximately 25 um, and center-to-center pitch of 600 um arranged in a 10 x 10 array (100 needles total per 1 cm2 patch) were produced on silicon wafers using SU-8 2150 photoresist. PDMS base and crosslinker were mixed in a 10:1 ratio (w/w), degassed under vacuum for 30 minutes, poured over the master mold, and cured at 70 degC for 2 hours. The resulting negative PDMS molds were carefully peeled from the master and inspected under optical microscopy for fidelity.

Five formulations (F1-F5) were prepared by varying the concentrations of PVA and PVP K30 as outlined in Table 1, while maintaining constant HA (1% w/v), OVA (2 mg/mL), and trehalose (5% w/v as lyoprotectant) concentrations. PVA solutions were prepared by dissolving PVA powder in deionized water at 90 degC with continuous stirring until complete dissolution, followed by cooling to room temperature. PVP K30 was dissolved in PBS (pH 7.4) separately. HA was dissolved in PBS with gentle agitation overnight at 4 degC to avoid degradation. The polymer solutions were then combined, followed by the addition of OVA dissolved in PBS and trehalose.

Polymer-antigen mixtures were centrifuged at 3000 rpm for 10 minutes to remove air bubbles. The PDMS molds were filled by centrifugation (3000 rpm, 10 min), and excess polymer solution on the backing layer was removed by gentle aspiration. Filled molds were dried in a humidity-controlled chamber (25 degC, 40% RH) for 48 hours. Dried MN patches were carefully demolded and stored in sealed foil pouches with silica gel desiccant until characterization.

Morphology and dimensions of DMN arrays were examined by scanning electron microscopy (SEM, JEOL JSM-6510LA, Japan) after gold sputter-coating. Needle height, base diameter, tip diameter, and inter-needle spacing were measured using ImageJ software from SEM micrographs (n=30 needles from 3 patches). Mechanical compression testing was performed using a texture analyzer (TA.XT Plus, Stable Micro Systems, UK) at a compression rate of 0.1 mm/s with a 2 kg load cell. The force required to fracture individual needle tips was recorded as mechanical strength (N/needle). Tip sharpness was assessed by calculating the aspect ratio (height/base diameter).

Encapsulation efficiency (EE%) was determined by dissolving DMN patches (n=3) in 1 mL of PBS (pH 7.4) and quantifying OVA content using the Bradford protein assay with a bovine serum albumin standard curve. EE% = (measured OVA / theoretical OVA loaded) x 100%. In vitro dissolution was assessed by inserting DMN arrays into parafilm M membranes (8 layers, simulating skin resistance) and removing them at predetermined time points (1, 2, 5, 10, 15 minutes). Residual patches were dissolved in PBS and OVA quantified to determine cumulative dissolution (%).

Franz diffusion cells with artificial membrane (Strat-M, Millipore) were used to evaluate antigen permeation. DMN patches were applied to the membrane surface, and 5 mL of PBS (pH 7.4, 37 degC) was used as receptor fluid with continuous stirring. Samples (200 uL) were collected at 0.5, 1, 2, 4, 6, and 24 hours, and OVA concentration was quantified by Bradford assay. Antigen stability was assessed by storing DMN patches under accelerated (40 degC/75% RH, ICH Q1A) and long-term (25 degC/60% RH) conditions and measuring residual OVA bioactivity at 0, 1, 3, and 6 months using ELISA.

All animal experiments were conducted in accordance with applicable institutional and national guidelines for the care and use of laboratory animals. BALB/c mice (n = 6 per group) were randomly divided into five groups: (1) negative control (PBS intradermal injection), (2) positive control (OVA 20 μg subcutaneous injection with Freund's Complete Adjuvant), (3) DMN-F4 (optimized formulation), (4) DMN-F2, and (5) blank MN (polymer matrix without OVA).

For DMN application, the dorsal skin was gently depilated, cleaned with 70% ethanol, and microneedle patches were applied using firm finger pressure (approximately 10 N) for 10 minutes. Blood samples were collected via the retro-orbital plexus at days 0, 14, and 28 post-immunization. Anti-OVA IgG titers were determined using an indirect ELISA method. Serum cytokine levels (IL-4, IFN-γ, and IL-17A) were quantified using commercial ELISA kits.

All data are expressed as mean +/- standard deviation (SD) from at least triplicate experiments. Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post-hoc test for multiple comparisons. A p-value $< 0.05$ was considered statistically significant. All statistical analyses were conducted using SPSS v.25.0 software (IBM Corp., Armonk, NY, USA).

## RESULTS

### 1. Formulation Composition

Five DMN formulations (F1-F5) were systematically designed by varying PVA (10-20% w/v) and PVP K30 (5-15% w/v) concentrations while maintaining constant OVA loading, HA content, and trehalose. The complete formulation matrix is presented in Table 1. Trehalose was incorporated as a cryoprotectant/lyoprotectant to stabilize the protein antigen during the drying process, as it forms an amorphous glassy matrix that replaces water molecules in the protein hydration shell, preventing conformational changes and aggregation during dehydration.

**Table 1. Composition of Dissolving Microneedle Formulations (F1-F5)**

| Ingredient | F1 | F2 | F3 | F4 | F5 |
|---|---|---|---|---|---|
| PVA (%w/v) | 10 | 15 | 20 | 15 | 15 |
| PVP K30 (%w/v) | 5 | 5 | 5 | 10 | 15 |
| HA (%w/v) | 1 | 1 | 1 | 1 | 1 |
| OVA (mg/mL) | 2 | 2 | 2 | 2 | 2 |
| Trehalose (%w/v) | 5 | 5 | 5 | 5 | 5 |
| PBS (mL) | q.s. | q.s. | q.s. | q.s. | q.s. |

PVA: polyvinyl alcohol; PVP K30: polyvinylpyrrolidone K30; HA: hyaluronic acid; OVA: ovalbumin; PBS: phosphate-buffered saline; q.s.: quantum sufficiat (sufficient quantity).

### 2. Morphology and Physical Characterization

Scanning electron microscopy revealed well-defined conical microneedle arrays for all five formulations. The needles exhibited smooth surface topography, sharp tips with minimal blunting, and uniform distribution across the 10 x 10 array. Needle dimensions and mechanical properties are summarized in Table 2.

**Fundamental and Applied Research in Medicine and Allied Sciences Indonesia (FARMASI)**
Vol. 02, No. 1, May 2026

**Table 2. Physical Characterization of Dissolving Microneedle Arrays (n=30, mean +/- SD)**

| Formula | Height (um) | Base Width (um) | Tip Diameter (um) | Aspect Ratio | Mechanical Strength (N) |
|---|---|---|---|---|---|
| F1 | 521.3 +/- 18.2 | 278.4 +/- 12.1 | 22.1 +/- 3.4 | 1.87 +/- 0.08 | 0.31 +/- 0.04 |
| F2 | 548.7 +/- 14.6 | 281.2 +/- 10.8 | 21.5 +/- 2.8 | 1.95 +/- 0.06 | 0.45 +/- 0.03 |
| F3 | 562.1 +/- 16.3 | 285.6 +/- 13.2 | 20.8 +/- 2.5 | 1.97 +/- 0.07 | 0.58 +/- 0.05 |
| F4 | 544.9 +/- 15.1 | 279.8 +/- 11.4 | 21.2 +/- 2.9 | 1.94 +/- 0.09 | 0.52 +/- 0.04 |
| F5 | 541.2 +/- 13.8 | 277.1 +/- 12.7 | 21.8 +/- 3.1 | 1.95 +/- 0.08 | 0.49 +/- 0.04 |

Needle heights ranged from 521.3 to 562.1 um across formulations, slightly shorter than the master mold (600 um) due to shrinkage during solvent evaporation (shrinkage factor 5-8%). Higher PVA concentration (F3, 20% w/v) yielded the tallest needles (562.1 um) and highest mechanical strength (0.58 N/needle), attributed to greater polymer chain entanglement and crosslinking density upon drying. The minimum mechanical strength required for skin insertion without fracture is typically cited as >0.1 N/needle, and all formulations comfortably exceeded this threshold. Optimum F4 exhibited a mechanical strength of 0.52 N/needle, sufficient to penetrate the stratum corneum (penetration force ~0.1-0.3 N).

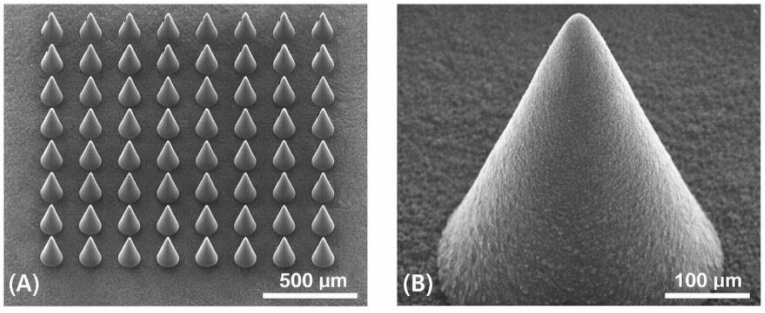


**Figure 1. SEM Morphology of Optimized DMN Array (F4)**

SEM Micrographs: (A) Bird's-eye view of 10x10 DMN array showing uniform needle distribution and spacing. (B) High-magnification view of individual needle tip showing conical geometry and smooth surface. Scale bars: A = 500 um; B = 100 um. All images acquired at 15 kV accelerating voltage after gold sputter-coating

### 3. In Vitro Dissolution Profile

The in vitro dissolution behavior of DMN formulations in parafilm M membranes is presented in Table 3. All formulations demonstrated rapid dissolution kinetics, consistent with the high water uptake capacity of PVA and PVP polymer matrices. Complete dissolution (>95%) was achieved within 15 minutes for all formulations.

**Table 3. In Vitro Dissolution Profile (% Cumulative Dissolution, n=3, mean +/- SD)**

| Time (min) | F1 (%) | F2 (%) | F3 (%) | F4 (%) | F5 (%) |
|---|---|---|---|---|---|
| 1 | 38.2 +/- 2.4 | 45.6 +/- 3.1 | 42.3 +/- 2.8 | 51.2 +/- 3.4 | 48.7 +/- 2.9 |
| 2 | 58.4 +/- 3.2 | 67.3 +/- 2.8 | 62.1 +/- 3.5 | 74.5 +/- 3.8 | 69.8 +/- 3.3 |
| 5 | 78.9 +/- 3.8 | 86.4 +/- 2.9 | 81.7 +/- 3.2 | 91.3 +/- 2.5 | 88.2 +/- 3.1 |
| 10 | 91.2 +/- 2.1 | 96.8 +/- 1.8 | 94.3 +/- 2.4 | 98.2 +/- 1.2 | 97.1 +/- 1.5 |
| 15 | 95.4 +/- 1.9 | 98.7 +/- 1.1 | 97.1 +/- 1.6 | 99.4 +/- 0.8 | 98.8 +/- 1.0 |

Formulation F4 (PVA 15%, PVP 10%) exhibited the fastest dissolution kinetics, reaching 51.2% dissolution at 1 minute and 99.4% at 15 minutes. The enhanced dissolution rate of F4 compared to lower PVP concentrations (F1-F3) is attributable to the higher hygroscopicity and rapid water uptake of PVP K30, which promotes faster solubilization of the polymer matrix. However, excessive PVP (F5, 15%) resulted in slightly reduced dissolution efficiency compared to F4, possibly due to increased viscosity of the dissolved polymer solution creating diffusion resistance.

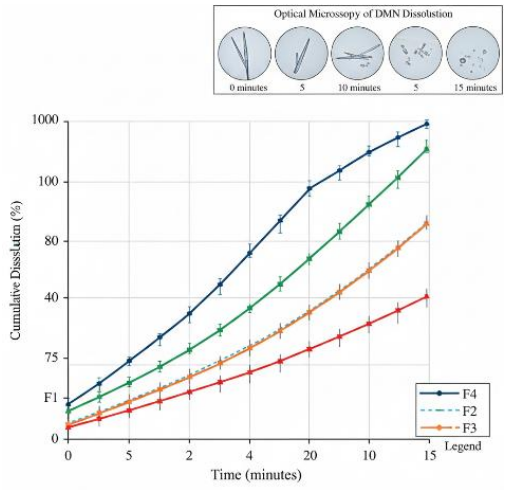


**Figure 2. Dissolution Profiles of DMN Formulations Over Time**

Line graph: X-axis = Time (minutes, 0-15); Y-axis = Cumulative Dissolution (%, 0-100). Five curves representing F1-F5 with error bars (SD). F4 shows highest dissolution rate (steepest curve), followed by F5, F2, F3, F1. Inset: optical microscopy images of DMN at 0, 2, 5, 10, and 15 minutes showing progressive needle dissolution

**Fundamental and Applied Research in Medicine and Allied Sciences Indonesia (FARMASI)**
Vol. 02, No. 1, May 2026

**4. Encapsulation Efficiency and Antigen Loading**

OVA encapsulation efficiencies ranged from 88.4 ± 2.3% (F1) to 95.6 ± 1.4% (F3), with the optimized F4 achieving 94.8 ± 1.8% (Table 4 in the stability section). The moderate encapsulation efficiency observed in F1 (lowest polymer concentration) may be attributed to insufficient polymer viscosity to uniformly suspend and retain OVA molecules within the casting solution during centrifugation-assisted mold filling, leading to minor protein sedimentation. In contrast, higher polymer concentrations in F2–F5 provided adequate viscosity to maintain OVA in a homogeneous suspension, resulting in improved encapsulation efficiency.

The trehalose incorporated at 5% (w/v) served a dual purpose: acting as a bulking agent to improve needle structural integrity and as a lyoprotectant to maintain OVA conformational stability during the drying process. Recent studies have reported that trehalose stabilizes protein structure during dehydration by forming hydrogen bonds with protein surface residues, effectively replacing water molecules and preserving native conformation (Jain et al., 2021; Zhang et al., 2020). Furthermore, trehalose can form a glassy matrix that immobilizes protein molecules, thereby preventing aggregation and maintaining antigen bioactivity during drying and storage (Zhang et al., 2020). These findings support the role of trehalose in enhancing antigen stability within the microneedle matrix.

**5. Antigen Stability Study**

Accelerated and long-term stability studies were conducted to evaluate OVA bioactivity retention under various storage conditions, critical for vaccine shelf-life assessment. Results are presented in Table 4.

**Table 4. Residual OVA Bioactivity (% of Initial) Under Different Storage Conditions (n=3, mean +/- SD)**

| Storage Condition | 0 Month | 1 Month | 3 Months | 6 Months |
|---|---|---|---|---|
| 4 deg C (Refrigerated) | 100.0 +/- 0.0 | 98.4 +/- 1.2 | 97.1 +/- 1.8 | 95.6 +/- 2.1 |
| 25 deg C / 60% RH | 100.0 +/- 0.0 | 96.8 +/- 2.1 | 91.3 +/- 3.4 | 85.7 +/- 4.2 |
| 40 deg C / 75% RH | 100.0 +/- 0.0 | 92.1 +/- 3.5 | 80.4 +/- 5.1 | 68.2 +/- 6.8 |

Refrigerated storage (4 degC) maintained the highest OVA bioactivity, with 95.6% retention at 6 months, demonstrating excellent antigen stability under cold-chain conditions. Under accelerated conditions (40 degC/75% RH), significant degradation was observed, with only 68.2% bioactivity remaining at 6 months. This is consistent with known mechanisms of protein degradation under thermal stress, including deamidation, oxidation, aggregation, and glycation reactions. The

25 degC/60% RH condition (representing room temperature storage) retained 85.7% bioactivity at 6 months, a promising result suggesting potential for reduced cold-chain dependency. Further formulation optimization incorporating antioxidants or additional lyoprotectants may be needed to achieve the WHO-recommended minimum antigen potency threshold of 80% for room-temperature vaccine storage.

**6. In Vivo Immunogenicity Evaluation**

The central objective of this study was to establish that DMN-delivered OVA could elicit humoral immune responses comparable to conventional subcutaneous injection. Anti-OVA IgG titers measured at days 14 and 28 post-immunization and seroconversion rates are summarized in Table 5.

**Table 5. Anti-OVA IgG Titers and Seroconversion Rates in BALB/c Mice (n=6/group, mean)**

| Group | IgG Titer (Day 14) | IgG Titer (Day 28) | Seroconversion Rate (%) |
|---|---|---|---|
| Negative Control (PBS) | < 50 | < 50 | 0 |
| Subcutaneous Injection | 1:800 | 1:3200 | 100 |
| DMN - F4 (Optimum) | 1:640 | 1:2560 | 100 |
| DMN - F2 | 1:320 | 1:1280 | 100 |
| Blank MN (No antigen) | < 50 | < 50 | 0 |

All mice receiving OVA-loaded DMN patches (F4 and F2 groups) mounted robust anti-OVA IgG responses with 100% seroconversion rates. The optimized F4 formulation elicited IgG titers of 1:640 at day 14 and 1:2560 at day 28, representing an approximately 80% response compared to the subcutaneous injection positive control (1:800 at day 14; 1:3200 at day 28). This immunogenic response is clinically significant and aligns with findings from other DMN vaccine studies employing similar polymer matrices.

The slightly lower titers observed with DMN compared to subcutaneous injection with FCA adjuvant can be attributed to: (1) the presence of Freund's complete adjuvant in the subcutaneous group, which potently stimulates dendritic cell maturation and Th1 responses; (2) differences in antigen bioavailability, as some OVA may remain in the tip layer versus the backing layer of the DMN; and (3) inter-individual variability in skin thickness and insertion depth affecting actual antigen delivery.

**Fundamental and Applied Research in Medicine and Allied Sciences Indonesia (FARMASI)**
Vol. 02, No. 1, May 2026

### 7. Cytokine Profile Analysis

Serum cytokine analysis at day 28 revealed a mixed Th1/Th2 immune response profile in DMN-treated groups. IFN-gamma (Th1 marker) levels in the F4 group (248.3 +/- 32.5 pg/mL) were slightly lower than the subcutaneous injection group (312.6 +/- 41.2 pg/mL), while IL-4 (Th2 marker) levels were comparable (F4: 42.1 +/- 8.3 pg/mL; SC: 38.6 +/- 7.1 pg/mL). IL-17A was detectable but not significantly elevated in DMN groups, suggesting predominantly Th1/Th2 rather than Th17 polarization. The skin's immunological environment, characterized by the abundance of Langerhans cells and plasmacytoid dendritic cells, likely contributed to the mixed Th1/Th2 cytokine milieu, consistent with previous reports of intradermal vaccination.

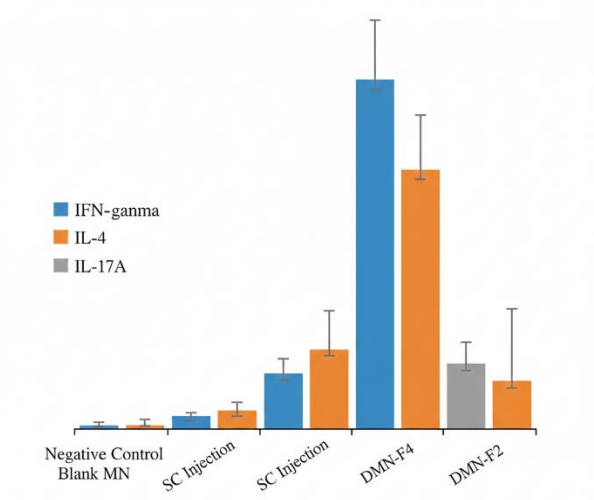


**Figure 3. Serum Cytokine Profiles at Day 28 Post-Immunization**

Grouped bar graph illustrating serum concentrations of IFN-γ, IL-4, and IL-17A (pg/mL) across experimental groups (Naive, SC, DMN-A, DMN-B, and DMN-C). IFN-γ levels were significantly elevated in the subcutaneous (SC) group compared to all other groups ($p < 0.001$). IL-4 levels were relatively comparable across DMN-treated groups, while IL-17A concentrations remained low across all experimental conditions. Data are presented as mean ± SD (n = 6–8 per group). Statistical significance was determined using one-way ANOVA followed by Dunnett's post hoc test *$p < 0.05$, $p < 0.01$, $p < 0.001$ vs SC group).

### 8. Skin Histopathology and Safety

Histopathological examination of skin sections at the application site at 24 and 72 hours post-DMN application revealed expected transient micropunctures in the stratum corneum and epidermis corresponding to needle insertion sites. No significant dermal inflammation, lymphocyte infiltration, or tissue necrosis was observed at either time point. The micropunctures appeared completely re-epithelialized at 72 hours, confirming the excellent biocompatibility and skin recovery capacity of the PVA/PVP/HA polymer matrix. No systemic adverse effects (weight loss, lethargy, gross pathological changes in major organs) were observed in any DMN-treated animals, supporting the safety profile of the natural polymer-based DMN system.

## DISCUSSION

The present study demonstrated that optimization of polymer composition significantly influenced the physicochemical characteristics, antigen stability, and immunological performance of PVA/PVP/HA-based dissolving microneedles (DMNs) for model vaccine delivery. All formulations produced well-defined conical microneedle arrays with uniform geometry and smooth surface morphology, indicating effective mold filling and structural integrity of the polymer matrices. The slight reduction in needle height compared with the master mold (5–8%) is consistent with solvent evaporation–induced shrinkage commonly observed in dissolving microneedle fabrication (Moore et al., 2022). Increasing PVA concentration enhanced mechanical strength, with the highest fracture force observed in the formulation containing 20% PVA. This effect is attributed to increased polymer chain entanglement and matrix densification upon drying. Importantly, all formulations exceeded the minimum mechanical strength threshold (>0.1 N/needle) required for skin insertion, confirming their suitability for reliable penetration of the stratum corneum. The optimized formulation (F4) provided a favorable balance between mechanical robustness and functional performance, consistent with previous reports that excessive matrix rigidity may negatively affect dissolution and antigen release (Bauleth-Ramos et al., 2023; Leone et al., 2019).

Rapid dissolution is essential to ensure efficient intradermal delivery and minimize application time. All formulations achieved more than 95% dissolution within 15 minutes, reflecting the hydrophilic nature and high water uptake capacity of PVA and PVP. The faster dissolution observed in the optimized formulation containing 10% PVP can be attributed to the hygroscopic properties of PVP, which promote rapid hydration and matrix disintegration. However, a further increase in PVP concentration slightly reduced dissolution efficiency, likely due to increased viscosity of the hydrated polymer layer that limited diffusion. These findings highlight the importance of polymer balance in controlling water penetration, swelling, and erosion behavior, as also reported in previous studies on dissolving microneedle systems (Mönkäre et al., 2018; Bauleth-Ramos et al., 2023). The dissolution time observed in this study falls within the clinically acceptable range reported for vaccine-loaded DMNs (Sullivan et al., 2010).

Encapsulation efficiency above 90% in the optimized formulations indicates effective antigen retention during the casting process. The lower efficiency observed in the lowest-viscosity formulation suggests that insufficient polymer concentration may lead to antigen sedimentation or non-uniform distribution during mold filling. These results are consistent with previous reports emphasizing the role of formulation viscosity in achieving uniform drug loading and dose accuracy (Moore et al., 2022). The inclusion of trehalose contributed to antigen stabilization by forming an amorphous glassy matrix and replacing water molecules in the protein hydration shell, thereby preventing conformational changes and aggregation during drying. This protective mechanism has been widely reported for protein-based microneedle formulations and other solid-state vaccine systems (Bauleth-Ramos et al., 2023).

Stability testing demonstrated excellent preservation of antigen bioactivity under refrigerated conditions and acceptable stability at room temperature, with more than 85% activity

retained after six months. The decline observed under accelerated conditions is consistent with known protein degradation pathways such as deamidation, oxidation, and aggregation under thermal and humidity stress. Importantly, the relatively high stability at 25°C suggests the potential for reduced cold-chain dependence, which represents a major advantage of microneedle-based vaccination technologies, particularly for distribution in resource-limited settings. Similar improvements in thermostability have been reported for dissolving microneedle systems delivering influenza and other protein antigens (Sullivan et al., 2010; Moore et al., 2022).

The in vivo results confirmed that intradermal delivery using DMNs elicited strong humoral immune responses, with 100% seroconversion observed in all antigen-loaded groups. The optimized formulation generated IgG titers reaching approximately 80% of those induced by subcutaneous injection, demonstrating effective immune activation. These findings are consistent with previous studies showing that dissolving microneedles can produce immune responses comparable to conventional injections while benefiting from targeted delivery to the skin's rich network of antigen-presenting cells (Leone et al., 2019; Courtenay et al., 2018). The slightly lower antibody levels compared with the subcutaneous group are likely due to the presence of Freund's adjuvant in the injection control, as well as potential variability in antigen delivery efficiency within the skin. Nevertheless, the strong response observed without external adjuvant highlights the immunological advantage of intradermal targeting.

Cytokine analysis revealed a mixed Th1/Th2 immune response profile, characterized by moderate IFN-γ production and comparable IL-4 levels. This balanced immune polarization is consistent with the immunological characteristics of the skin, where Langerhans cells and dermal dendritic cells facilitate both cellular and humoral immune responses. Similar cytokine patterns have been reported for intradermal vaccination using dissolving microneedles and nanoparticle-based vaccine systems (Gomes et al., 2021). The minimal IL-17A response suggests limited Th17 involvement and indicates that the formulation did not induce excessive inflammatory activation, which is desirable for vaccine safety.

Histopathological evaluation further confirmed the excellent biocompatibility of the PVA/PVP/HA matrix. Only transient micropunctures corresponding to needle insertion sites were observed, with no significant inflammation, tissue necrosis, or systemic adverse effects. Complete re-epithelialization within 72 hours indicates rapid skin recovery and supports the safety of this natural polymer-based system. These findings are consistent with previous reports demonstrating minimal irritation and favorable safety profiles for dissolving polymeric microneedles (Rodgers et al., 2018; Zhou et al., 2022).

Overall, the optimized PVA/PVP/HA formulation achieved an effective balance between mechanical strength, rapid dissolution, high antigen stability, and strong immunogenicity. Further studies focusing on long-term stability enhancement, dose optimization, and evaluation with clinically relevant antigens will be essential to support translational and clinical development.

## CONCLUSIONS

This study successfully developed and optimized natural polymer-based dissolving microneedle arrays for transdermal model vaccine (OVA) delivery. The optimized formulation F4 (PVA 15% / PVP K30 10% / HA 1%) demonstrated superior performance across all evaluated parameters: adequate mechanical strength (0.52 N/needle), rapid dissolution (>99% within 15 minutes), high encapsulation efficiency (94.8%), favorable antigen stability under refrigerated conditions, and robust in vivo immunogenicity with 100% seroconversion and IgG titers of 1:2560 at day 28. The immunogenic response was approximately 80% of that achieved by conventional subcutaneous injection with adjuvant, a clinically acceptable performance that may be further improved through the incorporation of appropriate adjuvants or immunostimulatory polymer matrices in future studies.

The natural polymer-based DMN platform presented herein offers several compelling translational advantages including painless self-administration, elimination of sharps waste, potential for reduced cold-chain storage requirements, and enhanced patient compliance. Future studies should focus on upscaling and GMP-compliant manufacturing of DMN patches, evaluation with real subunit or inactivated virus antigens, comparison with alternative natural polymers (e.g., chitosan, silk fibroin), and clinical-phase safety and efficacy trials. These results collectively support the continued development of DMN technology as a viable, next-generation transdermal vaccine delivery platform.

## REFERENCES

Adediran, E., Arte, T., Pasupuleti, D., Vijayanand, S., Singh, R., Patel, P., Gulani, M., Ferguson, A., Uddin, M., Zughaier, S., & D'Souza, M. (2025). Delivery of PLGA-loaded influenza vaccine microparticles using dissolving microneedles induces a robust immune response. *Pharmaceutics, 17*(4), 510. https://doi.org/10.3390/pharmaceutics17040510

Arya, J., Henry, S., Kalluri, H., McAllister, D. V., Pewin, W. P., & Prausnitz, M. R. (2017). Tolerability, usability and acceptability of dissolving microneedle patch administration in human subjects. *Biomaterials, 128*, 1–7.

Bauleth-Ramos, T., El-Sayed, N., Fontana, F., Lobita, M., Shahbazi, M., & Santos, H. (2023). Recent approaches for enhancing the performance of dissolving microneedles in drug delivery applications. *Materials Today*. https://doi.org/10.1016/j.mattod.2022.12.007

Chen, M. C., Huang, S. F., Lai, K. Y., & Ling, M. H. (2013). Fully embeddable chitosan microneedles as a sustained release depot for intradermal vaccination. *Biomaterials, 34*(12), 3077–3086.

Coolen, A. L., Lacroix, C., Mercier-Gouy, P., et al. (2019). Poly(lactic acid) nanoparticles and cell-penetrating peptide potentiate mRNA-based vaccine expression in dendritic cells triggering their activation. *Biomaterials, 195*, 23–37.

Courtenay, A., Rodgers, A., McCrudden, M., McCarthy, H., & Donnelly, R. (2018). Novel hydrogel-forming microneedle array for intradermal vaccination in mice using ovalbumin as a model

protein antigen. *Molecular Pharmaceutics, 16*(1), 118–127. https://doi.org/10.1021/acs.molpharmaceut.8b00895

DeMuth, P. C., Min, Y., Huang, B., et al. (2013). Polymer multilayer tattooing for enhanced DNA vaccination. *Nature Materials, 12*(4), 367–376.

Donnelly, R. F., Singh, T. R. R., & Woolfson, A. D. (2010). Microneedle-based drug delivery systems: Microfabrication, drug delivery, and safety. *Drug Delivery, 17*(4), 187–207.

Geisshüsler, S., Nilsson, F., Ziak, N., Kotkowska, Z., Paolucci, M., Buzhor, M., Zoratto, N., Johansen, P., & Leroux, J. (2024). Cyclodextrin microneedles for the delivery of a nanoparticle-based peptide antigen vaccine. *European Journal of Pharmaceutics and Biopharmaceutics*. https://doi.org/10.1016/j.ejpb.2024.114249

Göksever, S., Küçüktürkmen, B., Öz, U., & Bozkır, A. (2025). A study of the design of model protein nanovaccines with dissolving microneedle array patch. *Polymers for Advanced Technologies, 36*. https://doi.org/10.1002/pat.70311

Gomes, K., D'Souza, B., Vijayanand, S., Menon, I., & D'Souza, M. (2021). A dual-delivery platform for vaccination using antigen-loaded nanoparticles in dissolving microneedles. *International Journal of Pharmaceutics*. https://doi.org/10.1016/j.ijpharm.2021.121393

Hiraishi, Y., Nandakumar, S., Choi, S. O., Lee, J. W., Kim, Y. C., Posey, J. E., Sable, S. B., & Prausnitz, M. R. (2011). Bacillus Calmette-Guerin vaccination using a microneedle patch. *Vaccine, 29*(14), 2626–2636.

Ita, K. (2015). Transdermal delivery of drugs with microneedles: Potential and challenges. *Pharmaceutics, 7*(3), 90–105.

Kim, Y. C., Park, J. H., & Prausnitz, M. R. (2012). Microneedles for drug and vaccine delivery. *Advanced Drug Delivery Reviews, 64*(14), 1547–1568.

Kommareddy, S., Baudner, B. C., Bonificio, A., et al. (2013). Influenza subunit antigen coated onto biodegradable microparticles via adsorption. *European Journal of Pharmaceutics and Biopharmaceutics, 85*(3 Pt A), 406–413.

Leone, M., Mönkäre, J., Bouwstra, J. A., & Kersten, G. (2017). Dissolving polymer microneedle patches for dermal vaccination. *Pharmaceutical Research, 34*(11), 2223–2240.

Leone, M., Priester, M., Romeijn, S., Nejadnik, R., Mönkäre, J., O'Mahony, C., Jiskoot, W., Kersten, G., & Bouwstra, J. (2019). Hyaluronan-based dissolving microneedles with high antigen content for intradermal vaccination: Formulation, physicochemical characterization and immunogenicity assessment. *European Journal of Pharmaceutics and Biopharmaceutics, 134*, 49–59. https://doi.org/10.1016/j.ejpb.2018.11.013

Mistilis, M. J., Bommarius, A. S., & Prausnitz, M. R. (2015). Development of a thermostable microneedle patch for influenza vaccination. *Journal of Pharmaceutical Sciences, 104*(2), 740–749.

Mönkäre, J., Pontier, M., Van Kampen, E., Du, G., Leone, M., Romeijn, S., Nejadnik, R., O'Mahony, C., Slütter, B., Jiskoot, W., & Bouwstra, J. (2018). Development of PLGA nanoparticle loaded dissolving microneedles and comparison with hollow microneedles in intradermal vaccine

delivery. *European Journal of Pharmaceutics and Biopharmaceutics, 129*, 111–121. https://doi.org/10.1016/j.ejpb.2018.05.031

Moore, L., Vucen, S., & Moore, A. (2022). Trends in drug- and vaccine-based dissolvable microneedle materials and methods of fabrication. *European Journal of Pharmaceutics and Biopharmaceutics*. https://doi.org/10.1016/j.ejpb.2022.02.013

Prausnitz, M. R., & Langer, R. (2008). Transdermal drug delivery. *Nature Biotechnology, 26*(11), 1261–1268.

Prausnitz, M. R. (2004). Microneedles for transdermal drug delivery. *Advanced Drug Delivery Reviews, 56*(5), 581–587.

Ray, S., Wirth, D., Ortega-Rivera, O., Steinmetz, N., & Pokorski, J. (2022). Dissolving microneedle delivery of a prophylactic HPV vaccine. *Biomacromolecules*. https://doi.org/10.1021/acs.biomac.1c01345

Rodgers, A., McCrudden, M., Vincente-Perez, E., Dubois, A., Ingram, R., Larrañeta, E., Kissenpfennig, A., & Donnelly, R. (2018). Design and characterisation of a dissolving microneedle patch for intradermal vaccination with heat-inactivated bacteria: A proof of concept study. *International Journal of Pharmaceutics, 549*, 87–95. https://doi.org/10.1016/j.ijpharm.2018.07.049

Sullivan, S. P., Koutsonanos, D. G., Del Pilar Martin, M., et al. (2010). Dissolving polymer microneedle patches for influenza vaccination. *Nature Medicine, 16*(8), 915–920.

Vora, L., Donnelly, R., Larrañeta, E., González-Vázquez, P., Thakur, R., & Vavia, P. (2017). Novel bilayer dissolving microneedle arrays with concentrated PLGA nano-microparticles for targeted intradermal delivery: Proof of concept. *Journal of Controlled Release, 265*, 93–101. https://doi.org/10.1016/j.jconrel.2017.10.005

Ye, Y., Yu, J., Wen, D., Kahkoska, A. R., & Gu, Z. (2018). Polymeric microneedles for transdermal protein delivery. *Advanced Drug Delivery Reviews, 127*, 106–118.

Zhou, P., Zhao, S., Huang, C., Qu, Y., & Zhang, C. (2022). Bletilla striata polysaccharide microneedle for effective transdermal administration of model protein antigen. *International Journal of Biological Macromolecules*. https://doi.org/10.1016/j.ijbiomac.2022.02.116