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PORCINE PLACENTA EXTRACT-LOADED MATRIX POLYMERIC-BASED DISSOLVING MICRONEEDLES FOR IMPROVING SKIN HYDRATION

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ABSTRACT

Porcine placenta extract (PPE) is a macromolecular substance, that affects the limitation of permeability through the skin. The aim of this study was to fabricate matrix polymeric-based dissolving microneedles (DMNs) loaded with PPE for improving skin conditions. DMNs were developed by a mold-based method using polyvinylpyrrolidone K90, polyvinyl alcohol, and hyaluronic acid at a weight ratio of 2:0.5:1. The DMNs properties such as the morphology, PPE loading efficiency and capacity, the mechanical strength, skin insertion, *in vitro* dissolution, *in vitro* skin permeation, and stability were analyzed. Then, the *in vivo* human study was also evaluated to reveal the safety and effectiveness of PPE-loaded DMNs. The results showed that 3% w/w of PPE-loaded DMNs had a suitable morphology, good mechanical properties, complete insertion, and rapid dissolution into the skin. These DMNs exhibited high skin permeability of entrapped macromolecular protein. In the stability test, the DMNs showed a total protein content of over 80% at storage for a month in all temperatures. For *in vivo* human study, no skin irritation was found. Transepidermal water loss values increased, referring to the micro-holes created by DMNs for delivering PPE. Additionally, the PPE-loaded DMNs significantly increased skin hydration. Therefore, the good physical properties of 3% w/w PPE-loaded DMNs play an important role to enhance the PPE penetration through the skin and provide effective skin hydration without skin irritation.

Keywords: dissolving microneedle, porcine placenta, polyvinylpyrrolidone, polyvinyl alcohol, hyaluronic acid, skin hydration

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Introduction

The placenta has many bioactive substances such as proteins, minerals, amino acids, and steroid hormones, which can improve many aspects of cellular functions. According to previous publications, the placental extracts provided pharmacological activities: anti-inflammatory, antioxidant, cytoprotective, and stimulating proliferation.[1] Porcine placenta extract (PPE) improved skin conditions by increasing skin hydration and presented anti-inflammatory and antioxidant activities.[2,3] The main bioactive compounds in PPE were proteins, peptides, amino acids, and minerals.[4,5] Proteins, which are long-chain amino acids, are connected with the covalent peptide bonds, while peptides are short chains of amino acid sequences linked by peptide bonds. These compounds are hydrophilic and have a high molecular weight between 300 Da and 1,000 kDa.[6,7] Protein and peptide-based therapeutic compounds have limited skin permeability because the hydrophilic macromolecules have poor permeability across the stratum corneum (SC).[8]

Overcoming the limitation of PPE transportation through the skin, microneedles (MNs) have been developed continuously over the past decade to deliver these bioactive macromolecules.[9] MNs are the third generation of transdermal drug delivery to gain limitation of the skin barrier by using a mechanical force-based enhancement. The needle-like microscopic holes can create micro-channels in the skin after insertion into the dermal layer.[10] For ideal characteristics of MNs, the length of MNs should be between 50 and 1,000 μm, which can be inserted deeply into the skin depth without breaking and bending, rapid onset of action, efficient drug delivery, self-administer, minimally invasive, and painless.[9] The structure and release behaviors of MNs can be classified into five categories: solid MNs, coated MNs, hollow MNs, dissolving MNs, and hydrogel-forming MNs.[11]

Dissolving microneedles (DMNs) have obtained attention to deliver macromolecules such as proteins and peptides. The method of preparation and some materials are cost-effective, widely available, and high temperature inappropriate for the fabrication process.[12] The DMNs could be produced from the dissolvable polymers by loading the drugs into the matrix polymers. The mechanism of drug release of the DMNs can use the principle of poke and release. In this principle, the DMNs penetrate through the skin by thumb pressure, and the matrix polymers dissolve with the release of the drug, respectively.[13,14] Polyvinyl pyrrolidone (PVP) is water-soluble, biodegradable, biocompatible, and has low cytotoxicity, indicating that it can be used in pharmaceutical and cosmetic industries.[15,16] PVP exhibited different degrees of polymerization, presenting in having various molecular weights. The PVP has different viscosities in aqueous solutions, and the K-value indicates the viscosities of PVP (10 to 120).[17] Polyvinyl alcohol (PVA) is water-soluble, biocompatibility, and extensively used as the material, which affects the mechanical strength by influencing the hardness of the MNs. It also affects the dissolution rate of MNs and the controlled release of the drugs.[18] Hyaluronic acid (HA), as a natural polymer, is a non-sulfate glycosaminoglycan comprising of a repeating residue of N-acetyl-D-glucosamine and D-glucuronic acid. The properties of HA are suitable for DMNs fabrication because the evidence of its pharmacological activities for skin rejuvenation is the stimulation of epithelial, dermal, and extracellular matrix regeneration. Moreover, HA is a biodegradable polymer, that avoids biohazardous sharp waste, and provides HA-drug conjugation-based release for drug delivery.[19,20]

In the previous study, PVP-K90, PVA, and HA were successfully fabricated in the DMNs, which the polymer mixture of PVP-K90 and HA, and the polymer mixture of PVP-K90 and PVA showed good mechanical strength of DMNs.[21,22] However, the polymer matrix of PVP-K90, PVA, and HA has never been formulated to load PPE. The aim of this study was to fabricate PPE-loaded matrix polymeric-based

DMNs for improving skin conditions. The characterization of PPE-loaded DMNs such as the morphology, mechanical strength, *in vitro* skin insertion, *in vitro* dissolution, *in vitro* skin permeation, and stability were evaluated. Besides, *in vivo* of the human study was evaluated to assess the effectiveness of the PPE-loaded polymeric DMNs such as skin hydration, transepidermal water loss (TEWL), and skin irritation.

Materials and methods

Materials

PVP-K90 (MW = 360 kDa), PVA (MW = 85,000-124,000 kDa, >99% hydrolyzed), and Bovine serum albumin-fluorescein isothiocyanate conjugate (BSA-FITC) were purchased from Sigma-Aldrich, St. Louis, MO, USA. HA (MW = 1,200-1,800 kDa) was obtained from P.C. Drug Center, Bangkok, Thailand. The raw material of porcine placenta was gifted from Charnchai Farm, Ratchaburi, Thailand. All chemical agents used were analytical grade.

Methods

Preparation of the polymer solutions

The polymer solutions were prepared prior to fabricating the DMNs. The 40% w/w of PVP-K90 solution was prepared by dissolving PVP-K90 in distilled water. The 10% w/w of PVA solution was formulated by dissolving PVA in preheated distilled water at 80°C at a continuous stirring until a homogenous solution for 4 h. The 5% w/w of HA was made by dissolving HA in distilled water at a continued stirring until transparent.

Fabrication of PPE-loaded DMNs

In this study, each PVP (40%, w/w), PVA (10%, w/w), and HA (5%, w/w) was prepared and mixed to a homogeneous consistency at a weight ratio of 2:0.5:1 in a beaker. The various concentrations of PPE (1, 3, 5, and 7% w/w) were added. Then, the polymer mixture was weighed at 0.67 grams per mold to fill in the laser-engineered silicone micro-mold templates. Each micro-mold template had a total number of 121 (11 × 11) conical needles with a base diameter of 300 µm, a height of 600 µm, and a distance between the base of each microneedle of 300 µm. Afterward, all micro-molds were centrifuged at a speed of 3,500 rpm for 30 min at 25°C to fill the polymer in the holes, then placed to complete the DMNs form for 12 hours at 25°C. The excess polymer was removed using a spatula and cleaned the outer micro-mold surface with damp paper. After that, the cellulose membrane was attached to the base of the micro-mold and placed in a desiccator to dry completely for 72 hours. Finally, the DMNs were removed from the micro-mold template.

Determination of PPE content in the DMNs

In our study, the marker of an active compound in PPE was total protein content. The analysis of protein content was determined using the modified Lowry method,[23] in which phosphate buffer saline (PBS) pH 7.4 acts as a medium due to its resemblance to interstitial fluid in the skin.

In the Lowry method, the copper solution (solution A) containing 0.05 g of $CuSO_4$ and 0.1 g of potassium sodium tartrate dissolved in 20 ml of water was mixed with 10 ml of Na_2CO_3 solution (0.1 g/ml), 1 ml of 5M NaOH, and then adjusted to an approximate volume of 50 ml in the volumetric flask. In solution B, the Folin & Ciocalteu's phenol reagent was diluted with water at a ratio of 1:1. For the measurement process, PPE-loaded DMNs dissolved in 10 ml of PBS pH 7.4. Then, 40 µl of the test sample was mixed with 200 µl of solution A and incubated in the dark for 10 min at 25°C. After that, 20 µl of solution B was mixed and centrifuged at a speed of 5,000 rpm for 5 min at 25°C. The supernatant was collected to measure the total protein content by a microplate reader (VICTOR Nivo™ Multimode Plate Reader, PerkinElmer, Pontyclun, CF72 8YW, UK) at a wavelength of 550 nm. The loading efficiency (%LE) and the loading capacity (%LC) were calculated according to the equation (1) and equation (2).

$$\%LE = \frac{\text{Loaded amount of drug}}{\text{Initial amount of drug}} \times 100 \quad \text{.....(1)}$$

$$\%LC = \frac{\text{Loaded amount of drug}}{\text{Total amount of polymer}} \times 100 \quad \text{.....(2)}$$

The morphology of PPE-loaded DMN patches

The morphology of PPE-loaded DMNs was taken by a digital microscope (Dino-Lite Edge 5 MP, AM7915 series, Hsinchu, Taiwan). The dimension of each picture was evaluated using the Dino-capture 2.0 software.

Mechanical strength determination of DMN patches

The mechanical strength of PPE-loaded DMNs was evaluated by a Texture Analyzer (TA.XTexpressC, Stable Micro Systems, Godalming, GU7 1YL, UK). The PPE-loaded DMNs and blank DMNs were mounted on the flat probe by the double-coated tissue tape. The probe moved down in the vertical direction, then the force was applied at 3.38 N – 13.31 N with a triggered force of 0.049 N/121 needles, a speed of 0.5 mm/s, and maintained the force for 30 s. The percentage reduction height was calculated using an equation (3).

$$\%\text{Reduction height} = \left[\frac{H1-H2}{H1}\right] \times 100 \quad \text{.....(3)}$$

Where H1 is the initial height of the needles and H2 is the height of the needles after compression

In vitro skin insertion study of DMNs

In this study, the neonatal abdominal porcine skins were used as an alternative skin model. The newborn piglets naturally died, which were supported by a local farm in Ratchaburi province, Thailand. Briefly, the subcutaneous layer was carefully removed by surgical scissors and cleaned with PBS pH 7.4. The skin (600-700 µm-thick) was kept at -20°C and thawed in PBS pH 7.4 at 25°C before use.

The skins were placed and stretched on a wax sheet underneath the dermis and wiped gently with the delicate task wipers to dry. All DMNs were inserted into the skin using thumb pressure for 30 s and the DMNs were then removed. A concentration of 1% v/v methylene blue was dropped onto the applied area for staining and incubated for 5 min, while excess methylene blue was washed out by PBS pH 7.4. The marked area was photographed by a digital microscope (Dino-Lite Edge 5 MP, AM7915 series, Hsinchu, Taiwan). The percent of the DMNs insertion was calculated from methylene dark blue spots.

In vitro dissolution study of DMNs

The neonatal abdominal porcine skins were placed and stretched onto the wax sheet. Blank DMNs and PPE-loaded DMNs were inserted into the skins using thumb pressure for 30 s. The dissolution of these DMNs was performed by removing them from the skins at different time intervals (10, 15, and 20 min) to evaluate the change in the height by the Dino-Lite microscope.

In vitro skin penetration of BSA-FITC-loaded DMNs

In vitro skin permeation was determined by the vertical Franz diffusion cell. The abdominal neonatal porcine skin was used as a skin model. Normally, endogenous proteins and peptides in the skin can interfere with the measured amounts of exogenous proteins permeated into the skin. In this study, BSA-FITC was used as a model macromolecular protein. In brief, BSA-loaded DMNs were inserted into the skin using thumb pressure for 30 s and fixed these skins between the acceptor compartments and the receptor compartments using clamps. PBS pH 7.4 acts as a medium and was filled in each receptor compartment, then maintained the temperature of the receptor fluid at 32 ± 1°C by water circulating jacket during the experiment. The sample was collected at different time intervals (0.5, 1, 2, 4, 6, 8, and 24 h) and replaced with the same volume of fresh PBS to maintain a constant volume of the receptor fluid. The permeation of BSA-FITC was determined using a fluorescence spectrophotometer (VICTOR Nivo™ Multimode Plate Reader, PerkinElmer, Pontyclun, CF72 8YW, UK) at excitation and emission wavelength of 485 nm and 535 nm, respectively.

Stability test of PPE-loaded DMNs

The stability was performed for a month. The suitable PPE-loaded DMNs were kept in aluminum foil zipper packaging pouch at the different temperatures such as 5 ± 3°C, 25 ± 2°C, and 40 ±

2°C/75 ± 5%RH. The physical stability was evaluated based on physical appearance and mechanical strength using a Dino-Lite microscope. For the chemical stability, the DMNs were dissolved in PBS pH 7.4 at a volume of 10 ml, followed by measuring the total protein content by Lowry protein assay.

Irritation and hydration tests

In vivo, a human study was approved by an ethics committee (COE. 65.0223-032, Human Studies Ethics Committee, Silpakorn University Research, Innovation and Creativity Administration Office, Sanam Chandra Palace Campus). Fifteen healthy volunteers (25-40 years old, males = 4, females = 11) who participated in the study applied the DMNs (blank and 3% w/w of PPE-loaded DMNs) on two areas on their left forearm using thumb pressure for 30 s and covered them with waterproof tape (OPSITE◊ Post-Op, Smith&nephew, HULL HU3 2BN, UK) for 24 h. Afterward, the DMNs were removed, and then measured the skin erythema, TEWL, and skin hydration by a digital skin analyzer (DermaLab® series; Cortex Technology, Hadsund, Denmark) connected with the skin color probe, TEWL probe, and hydration probe, respectively.

The measured values were used to calculate the change in skin condition after treatment with PPE-loaded DMNs compared to blank DMNs as the control group. The skin erythema in terms of erythema index (%EI) was calculated according to the equation (4).

$$\%EI = (E_t - E_0) / E_0 \times 100 \quad \text{.....(4)}$$

Where E_t is the erythema value after treatment with blank DMNs or PPE-loaded DMNs and E_0 is the erythema value before treatment with blank DMNs or PPE-loaded DMNs

The skin hydration was measured by a DermaLab hydration probe. The hydration probe assesses the water binding capacity in the SC layer using the principle of conductance. The software automatically calculates the average results shown in unit μS. Skin hydration can refer to the effect of skin moisturizers and be calculated as the following equation (5).

$$\%\text{Skin hydration} = ((H_t - H_0) / H_0) \times 100 \quad \text{.....(5)}$$

Where H_t is the hydration value after treatment with blank DMNs or PPE-loaded DMNs and H_0 is the hydration value before treatment with blank DMNs or PPE-loaded DMNs

Finally, the TEWL values were measured by a DermaLab TEWL probe. The principle is relative humidity and temperature sensors centered in open chamber. The TEWL measurement determines the change of water vapor density at a fixed area of SC with a fixed time, and the units show as grams of water/square meter/hour ($g \cdot m^{-2} \cdot h^{-1}$).[24]

Statistical analysis

The results were repeated in triplicate and presented as the mean ± standard deviation (S.D). The statistical analysis of data was evaluated by one-way analysis of variance (ANOVA), followed by Tukey's test for post-hoc analysis. For *in vivo* human study, the Wilcoxon signed-rank test was used for statistical analysis. Significant difference in values were shown at p-value < 0.05.

Results

Morphology of blank DMN and PPE-loaded DMN arrays

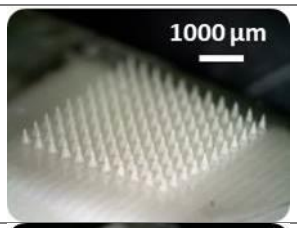
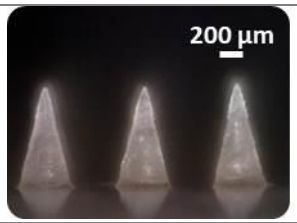
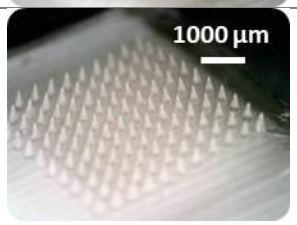
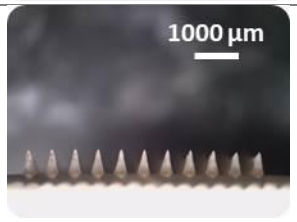
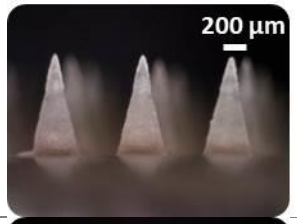
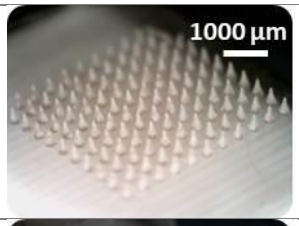
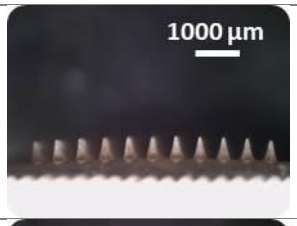
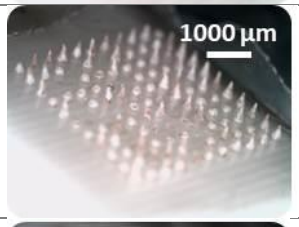
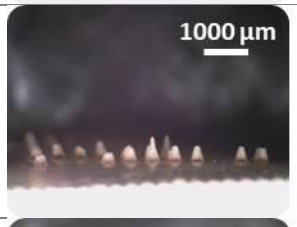
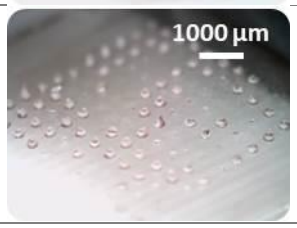
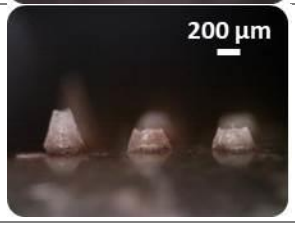
In this study, three different polymers were selected to prepare the DMNs, 40%w/w of PVP-K90, 10% w/w of PVA, and 5% w/w of HA at a weight ratio of 2:0.5:1 by using the micro-molding process. We prepared the various concentrations of PPE-loaded DMNs such as 1% w/w, 3% w/w, 5% w/w, and 7% w/w to find good appearance and the maximum loading. As the results shown in Table 1, the morphology of blank DMNs, 1% w/w and 3% w/w of PPE-loaded DMNs showed to be well-formed with sharp tips and complete array of needles with a baseplate of appropriate strength. In comparison, 5% w/w and 7% w/w of PPE-loaded DMNs were unsuccessfully in fabrication. The complete DMNs consisted of 121

shaped needles with a patch size of 0.5 cm^2. The physical appearance of the blank DMNs was colorless, whereas PPE-loaded DMNs were a light brown color due to the characteristic of the PPE. The results indicated that 1% w/w and 3% w/w of PPE-loaded DMNs were successfully fabricated.

Loading efficiency and loading capacity of PPE-loaded DMNs

The drug content in the DMNs plays a crucial role in the formulation's effectiveness. The percent of the drug loading efficiency (%LE) and loading capacity (%LC) of PPE-loaded DMNs are presented in Table 2. The DMNs can encapsulate more PPE according to a concentration of an extract increase. The 3(% w/w of PPE-loaded DMNs showed higher %LE and %LC than 1% w/w of PPE-loaded DMNs, which the % LC of 3% w/w PPE-loaded DMNs (0.63±0.15%) was significantly higher than the 1% w/w of PPE-loaded DMNs (0.16±0.01%) (p-value < 0.05). The DMNs exhibited the maximum PPE loaded at a concentration of 3% w/w. Therefore, 3% w/w PPE-loaded DMNs were appropriate for use as a transdermal delivery system of PPE.

Table 1 Images of Blank DMNs and various concentrations (1%, 3%, 5%, and 7%, w/w) of PPE-loaded DMNs

DMNs	Top view (45x)	Side view (50x)	Close-up view (200x)
Blank DMNs			
1% w/w of PPE-loaded DMNs			
3% w/w of PPE-loaded DMNs			
5% w/w of PPE-loaded DMNs			
7% w/w of PPE-loaded DMNs			

Mechanical strength of PPE-loaded DMNs

The mechanical strength of the DMNs has an important role in application because the DMNs must have a sufficient strength to resist the compression for insertion into the skin. Recently, the maximum manual insertion force of human-applied MNs into the skin was reported at around 10 N per array 11x11 needles).[25] In this experiment, we studied the percent reduction height of each DMN array at various axial forces such as 3.388 N, 6.655 N, 10.769 N, and 13.31 N per needle against a flat surface as shown in Figure 1. For blank DMNs, they showed the %reduction height in the range of 2.97 ± 0.95 to 5.16 ± 0.69 %. While the % reduction height of 1% w/w of PPE-loaded DMNs presented 3.84 ± 1.02% to 8.32 ± 0.86%, and 3% w/w of PPE-loaded DMNs expressed the % reduction height between 2.97 ± 1.01 and 6.09 ± 1.02%, indicating that the 3% w/w of PPE-loaded DMNs have better mechanical strength against the compression force than the 1% w/w of PPE-loaded DMNs. There was no significant difference in 3% w/w of PPE-loaded DMNs at all compression forces compared to blank DMNs.

Table 2 The %LE and %LC of the various concentrations (1 and 3% w/w) of PPE-loaded DMNs. Data are exhibited as mean ± S.D. Each sample was repeated in triplicate.

DMN arrays	%LE	%LC
1% w/w of PPE-loaded DMNs	15.66±1.24	0.16±0.01
3% w/w of PPE-loaded DMNs	17.10±0.79	0.63±0.15*

* presents the significant difference when compared to the 1% w/w of PPE-loaded DMN array at *p*-value < 0.05.

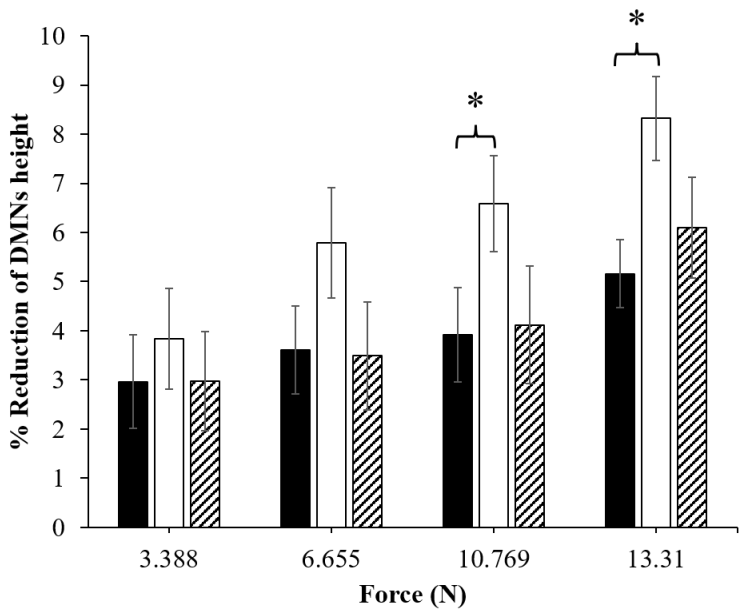


Figure 1 Comparison of % reduction height between blank DMNs (■), 1% w/w of PPE-loaded DMNs (□) and 3% w/w of PPE-loaded DMNs (▨). * indicates the significant difference when compare to blank DMNs at p-value 0.05. Each sample was repeated in triplicate.

Skin insertion study of the DMNs

The assessment of percutaneous insertion of MNs is a crucial factor in transdermal drug delivery. The DMNs were inserted into the methylene blue stained skin using thumb pressure for 30 s. As a result, 100 % skin insertion of DMNs was calculated from the amounts of inserted needles into the skin. The results indicated that all DMNs with or without PPE were successfully inserted and created approximately 100% micro-holes on the porcine skin (Table 3).

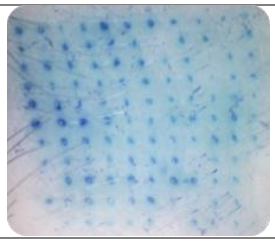
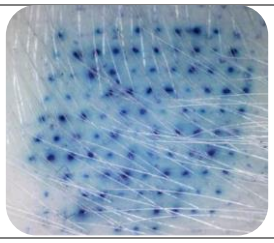
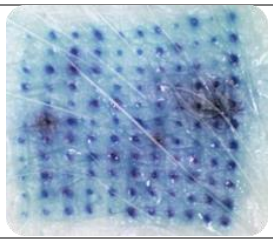
In vitro dissolution study of the DMNs

Solubility of the DMNs has an essential property because the dissolution of MNs can describe the drug release behavior. Moreover, DMNs fabricated from biodegradable polymers affects the dissolving rate of needle arrays. After the DMNs were inserted into the skin and in contact with interstitial fluid, the DMNs were dissolved and the encapsulated drugs were released, resulting in a therapeutic effect.[26,27] As shown in Figure 2, all formulations began to dissolve within the first 5 min and completely dissolved at 20 min. Therefore, the high concentration of PPE content (3% w/w) and rapid dissolution of DMNs exhibited the most suitable formulation for *in vitro* skin permeation study.

In vitro skin permeation

In this study, BSA-FITC was used as a protein model to analyze the ability of DMNs to deliver macromolecular protein through the skin. The *in vitro* skin permeation profile of 3% w/w of BSA-FITC-loaded DMNs and BSA-FITC solution was exhibited in Figure 3. The amount of BSA-FITC in the DMN formulation was significantly higher than BSA-FITC solution throughout the testing period. The mean cumulative permeation of BSA-FITC-loaded DMN arrays at 24 h was 1,800.56 ± 82.17 μg/cm^2, while the BSA-FITC solution presented the value of 15.10 ± 10.62 μg/cm^2. The results exhibited that the DMNs increased the skin penetration of BSA-FITC due to the micro-holes created by the DMNs.

Table 3 Images of neonatal abdominal porcine skin stained with 1% v/v methylene blue after the insertion of the blank DMNs, 1% w/w and 3% w/w PPE-loaded DMNs.

Blank DMNs (17.5x)	1% w/w PPE-loaded DMNs (17.5x)	3% w/w PPE-loaded DMNs (17.5x)
		

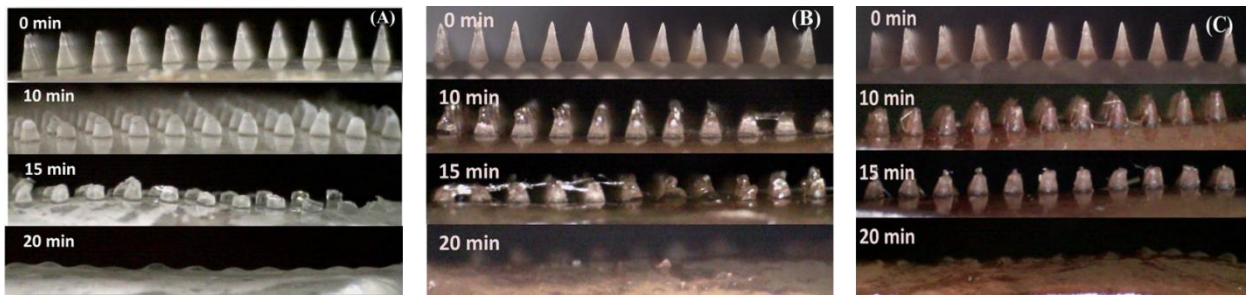


Figure 2 Images of the dissolution behavior of the DMNs:(A) blank-DMNs, (B) 1% w/w of PPE-loaded DMNs, and (C) 3% w/w of PPE-loaded DMNs. The resolutions of the images are 22x.

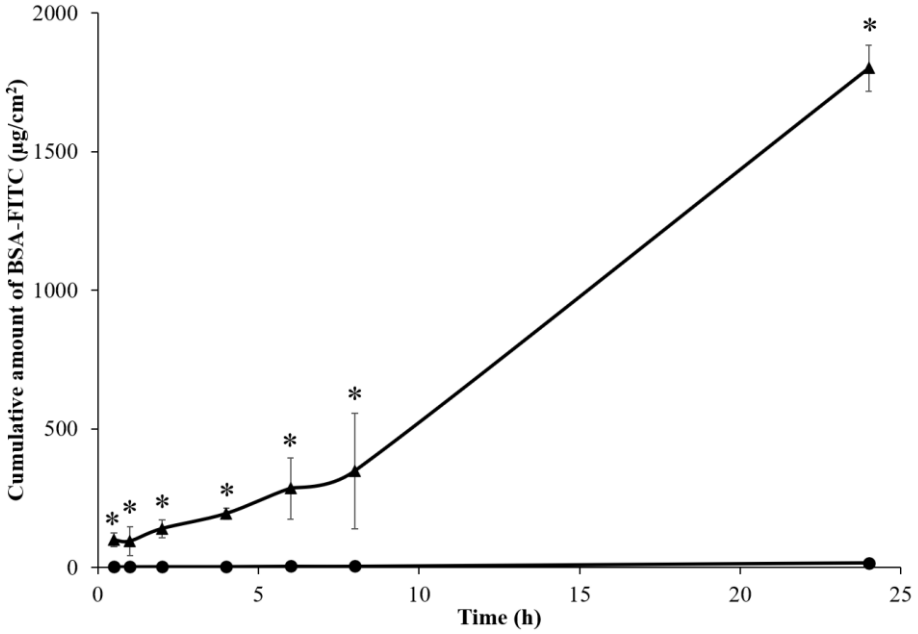


Figure 3 The cumulative permeation profile of BSA-FITC-loaded DMNs (—▲—) compared to BSA-FITC solution (—●—) at different time intervals (0-24 h). * shows the significant difference compared to BSA-FITC solution as a control group at *p*-value less than 0.05.

In vivo human study

For *in vivo* human study, 3% w/w of PPE-loaded DMNs were the treatment group, while blank DMNs were the control group. After the healthy volunteers applied the DMNs for 24 h, the skin hydration of PPE-loaded DMNs slightly increased greater than blank DMNs. The % skin hydration medians of the two formulations were significantly different at a *p*-value less than 0.05. However, the pairing of these two groups was not significantly different (Figure 4A). For the result of skin erythema, the value of % EI can describe skin irritation such as skin redness and blood flow. The median % EI of PPE-loaded DMNs was significantly different from blank DMNs at a *p*-value less than 0.05, but the pairing of both was not significantly effective (Figure 4B). The changes in TEWL values are presented in Figure 4C. After applying blank DMNs and PPE-loaded DMNs, the medians of TEWL values were not significantly different. Therefore, PPE-loaded DMNs could improve skin hydration, and have non-irritation or non-allergic reactions.

Stability study

The PPE-loaded DMNs were contained in a tight container and kept at three temperatures (5°C, 25°C, and 40°C) for a month. As shown in Figure 5, the % total protein content as an active compound marker of PPE-loaded DMNs slightly decreased at all testing temperatures from 100 ± 2.47 % (day 0) to 83.41 ± 2.81 % (5°C), 86.64 ± 0.88 % (25°C), and 83.13 ± 8.47 %(40°C), respectively.

Discussion

The ideal polymeric DMNs offer biodegradable properties with biocompatibility and are strong enough to penetrate through the skin barrier without bending or breaking, avoidance of active compounds degrading in the fabrication process, and the efficiency of drug release. Dissolvable MNs are fabricated from water-soluble polymers, which quickly and completely dissolve after insertion into the skin.[28] PVP, PVA, and HA showed a good appearance for each DMN, including a smooth surface and complete needle tip structure. PVA

showed the best mechanical properties and elastic modulus, but the PVA-fabricated DMNs were poorly flexible and easily brittle to fracture. HA-fabricated DMNs had better flexibility and a stronger transverse loading capacity, but this polymer was soft.[29] PVP-fabricated DMNs had sharp tips and were strong enough to insert into the skin without breaking and bending.[30] Copolymers of PVA and PVP-fabricated DMNs containing insulin could improve the dissolution speed faster than PVA-fabricated DMNs, leading to improvement in efficiency.[31] PVP is widely used to increase the dissolution rate of the DMNs and enhance drug release. The mixtures of PVP and PVA at different ratios affected the dissolution rate, which

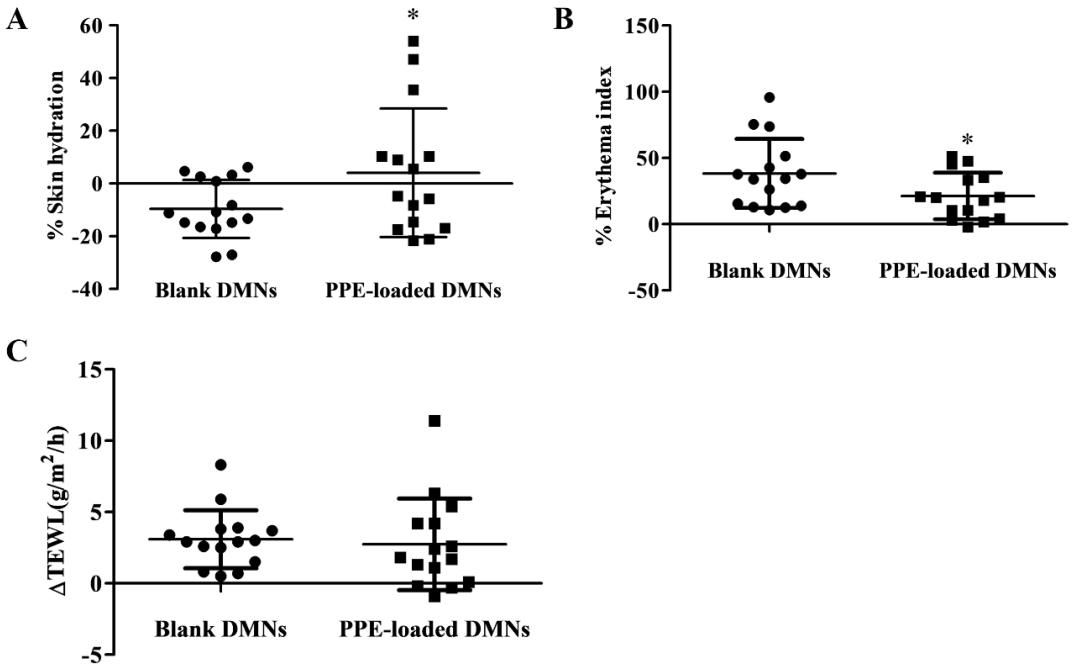


Figure 4 Comparison of the skin condition parameters after application of blank DMNs (control group) and PPE-loaded DMNs (treatment group) on the skin for 24 h. (A) % skin hydration, (B)% erythema index, (C) Δ TEWL values of blank DMNs and PPE-loaded DMNs. * indicates a significant difference in the median values from blank DMNs (control group) at a *p*-value less than 0.05 (n = 15).

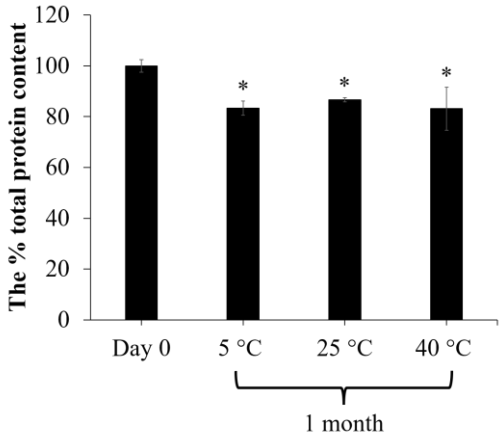


Figure 5 The profile of % total protein content in the PPE-loaded DMNs at different storage temperatures (5°C, 25°C, and 40°C) for a month. * represents the significant difference values compared to day 0 ($p < 0.05$).

increasing PVP content exhibited significantly fast dissolution.[32] A concentration of 10% w/w PVA-formed DMNs had great morphology and good mechanical strength.[33] In a recent report, the concentration of the mixture polymers of PVP (15-30%, w/w) and PVA (7.5-15%, w/w), PVP (20-30%, w/w) and HA (5%, w/w), PVP (30%, w/w) and PVA (7.5-10%, w/w), and PVP (30%, w/w) and HA (5% w/w) presented the best of mechanical strength and fast dissolution profiles.[21]

The active compounds of PPE, as a macromolecular protein, are degraded by heat. The solvent casting technique is the mold-based method without a high-temperature process to fabricate the DMNs. In this fabrication process, the polymer solution was added to the mold, followed by the centrifugation and vacuum steps to fill the polymer in the microcavities.[34] The polymer mixture of 40%w/w of PVP K90, 10% w/w of PVA, and 5% w/w of HA at the weight ratio of 2:0.5:1 were successful in fabricating DMNs for loading PPE because the DMNs showed good appearance and fully needle arrays. When we prepared the various concentrations of PPE loading at 1% w/w, 3% w/w, 5% w/w, and 7% w/w in the DMNs, the concentrations of 1% w/w and 3% w/w of PPE-loaded DMNs showed good morphology, full needle arrays, and good mechanical strength. Because the extract has a suspended solution when dissolved in an aqueous medium, high concentrations of the extract resulted in obstruction of the needle-holes, and the polymer could not flow through the micro-holes. For %LE and %LC, 3% w/w of PPE-loaded DMNs was higher than 1% w/w of PPE-loaded DMNs because the DMNs could contain more extracts at higher concentrations. After compression, % w/w of PPE-loaded DMNs was better maintaining its original shape and no crack than 1% w/w of PPE-loaded DMNs. All formulations were completely inserted into the skin and rapidly dissolved within 5 min and completed in 20 min. These results presented that 3% w/w of PPE-loaded DMNs was an appropriate system of DMNs to deliver macromolecular protein from PPE into and through the skin.

In the previous study, PPE contained water-soluble proteins, polypeptides (i.e., growth factor and amino acids), which these compounds provided high bioactivity on dermal fibroblasts and contributed to skin cell regeneration. However, bioactive macromolecular proteins and growth factors are hydrophilic compounds and have a limited ability to passively pass the skin barrier.[35] This study showed that 3% w/w of PPE-loaded DMNs enhanced the delivery of macromolecular protein through the skin. The morphology DMNs exhibited an acceptable height range for the delivery of entrapped compounds into the skin with minimal invasion and sufficiently sharp and strong for passage through the layer of SC. After DMN insertion into the skin, possible routes to deliver protein through the skin were created, leading to enhancing the ability to deliver the macromolecular protein into the skin.[35] Therefore, DMNs presented a suitable dermal delivery system for PPE.

The effectiveness and safety of PPE-loaded DMNs were measured by *in vivo* human study. After applying DMN for 24 h, the change in the skin chromophore content (hemoglobin) is related to the erythema index (EI).[36] Skin erythema appears when the irritants (e.g., allergens and chemical compounds) are expose to the skin. The values of EI can analyze the dermatological studies in the evaluation of allergy-induced ingredients and level of skin erythema. The EI values can refer to skin irritation (skin redness and blood flow).[37] In the results, the % EI of PPE-loaded DMNs was lower than blank DMNs, suggesting that PPE-loaded DMNs did not cause skin irritation.

The values of TEWL can measure the quantity of water loss that of diffusion via the SC and evaluate the barrier function of the skin.[24] The TEWL measurement is a method to define the boundary of the skin barrier function that broke after the MNs application.[38] In this study, the high values of TEWL

were observed immediately after removing the DMNs from the skin and creating the microchannels, indicating that the DMNs could improve the skin permeability of PPE.[39] However, the TEWL values can decrease after the micro-holes recover over time.[38] In addition, PPE could improve skin conditions by enhancing skin hydration, demonstrating effectiveness for improving skin moisture. Recently, the intake of porcine placenta extract accelerated the expression of moisturizing-related proteins such as ceramide synthase and maintained the skin barrier function, resulting in increasing the water-holding capability and barrier functions.[2,40] Therefore, the 3% w/w of PPE-loaded DMNs play a very useful and efficient delivery of PPE through the skin without serious skin damage, leading to improve the skin hydration.

Conclusion

In this study, 3%w/w of PPE-loaded DMNs had complete needle structure and arrays, good mechanical properties without breaking and bending, complete insertion, and rapid dissolution into the skin. The DMNs could improve skin conditions by increasing skin hydration, and had no irritation to the skin. Therefore, these findings suggested that PVP K90/PVA/HA-fabricated DMNs containing a concentration of 3% w/w PPE could be an alternative technology in cosmeceutical products for improving skin hydration.

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