Development of Sponge Microspicule Cream as a Transdermal Delivery System for Protein and Growth Factors from Deer Antler Velvet Extract

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Sponge spicules are needle-like structures and used for dermabrasive treatment of the skin. This research aimed to develop an effective delivery system by using sponge spicules for enhancing skin permeation of bioactive proteins and growth factors from deer antler velvet (DAV). DAV was extracted by sonication and bioactivity studies were evaluated. The size of microspicules (MSs) was reduced and mixed with DAV extract cream. In vitro skin permeation was analyzed by using bovine serum albumin–fluorescein isothiocyanate conjugate (BSA–FITC) as a model macromolecular compound. For in vivo study, DAV extract formulations were applied on the skin of healthy humans, and effects were evaluated. Results showed that DAV extract containing proteins and growth factors increased the proliferation and migration of skin fibroblast cells. This extract was homogeneously mixed with spicule cream. Without blending, MS was 11.89 µm wide and 176.77 µm long; blending time exhibited short and broken MSs (MBs) for short blending (30 s) and fine powder (MF) for long blending (10 min). MS cream showed the highest permeation of BSA–FITC through the skin (2.26-fold enhancement), but it resulted in skin irritation. Therefore, MB cream that increased the permeation of BSA–FITC by 1.94-fold was not significantly different from MS formulations chosen for in vivo study. Applying DAV-containing MB cream on the skin for 14 d decreased the melanin content and erythema value but increased elasticity and hydration. Therefore, the MB-containing cream can enhance the macromolecule delivery through the skin, improve the skin properties, and avoid skin irritation.

Key words microspicule; deer antler velvet; protein; growth factor; skin delivery

INTRODUCTION

Deer antler velvets (DAVs) have an annual cycle of full regeneration in mammals such that the constructive tissues (cartilage, bone, nerves, skin, and blood vessel) grow during this period. Some growth factors such as insulin-like growth factor-1 (IGF-1), epidermal growth factor (EGF), transforming growth factor-β1 (TGF-β1), vascular endothelial growth factor, and fibroblast growth factor have been discovered. Growth factors are proteins that regulate cellular functions, such as survival, proliferation, migration, and differentiation. Many exogenous growth factors have been extracted from natural products, and they are used as a potential regenerative medicine for replacing or repairing damaged cells, tissues and organs.

For skin regenerative medicine, EGF exhibits important roles for growth and regeneration of keratinocytes and fibroblasts; moreover, it functions as a therapeutic and cosmeceutical compound for damaged skin and injuries, such as wound healing, anti-wrinkles, and anti-aging. IGF-1 is strongly expressed at the area of injury and plays an important role in epidermal and dermal regeneration. In a previous study, exogenous IGF-1 alone and combined with a binding protein or other growth factors was applied to a wound, and it presented beneficial effects of the wound healing process. This result indicated the stimulating effect on collagen synthesis in fibroblasts, fibroblast and keratinocyte proliferation, and promotion of angiogenesis. TGF-β1 is also involved in healing and regenerating processes by stimulating collagen production and tissue inhibitor of metalloproteinase-1 production. Therefore, growth factors have been used in several skin rejuvenation products, which can promote collagen synthesis and prevent skin aging.

Transdermal delivery of macromolecules is effective in therapeutic administration. It can avoid the side effect and hepatic first pass metabolism by a gastrointestinal enzymatic system that is a problem of oral drug delivery. Additionally, transdermal delivery systems have several advantages over hypodermic injections. A non-invasive route for drug delivery and patient self-administration leads to improved patient acceptance and compliance. Nevertheless, the major limitation of transdermal drug delivery is the permeability barrier of the skin. To overcome this barrier, microneedles are efficient physical enhancers for the skin delivery of macromolecules by bypassing the stratum corneum barrier. Microdermabrasion has also been used to increase skin permeability by damaging or removing the outermost layer of the skin stratum corneum. The sponge spicules are a natural material and have been used as carriers of the transdermal delivery system. Microneedle-shaped spicules containing EGF can enhance skin permeability and function as an effective anti-wrinkle agent. Sponges are multicellular marine animals whose structure consists of soft tissue suspended in jelly-like proteinaceous matrix supported by a hard skeleton. The skeleton structure is controlled by the precipitation of calcium carbonate or siliccon dioxide. Monactines are needle-like structures of sponge...
spicules with the tip growing out from two opposite sites known as “spicules.” The use of *Spongillia* as a dermabration has been reported to be a natural alternative approach for treating skin problems, including hyperpigmentation of different etiology, fine wrinkles, sun-damaged skin, superficial scars, comedones, enlarged facial pores, and dull skin. Upon massaging *Spongillia* spicules onto the skin, they can mechanically separate the epidermal layers and reduce keratinocyte cohesion but increase stratum corneum sloughing and remove loosened keratinocyte. However, the skin erythema and tingling sensation gradually disappear within 12–24 h.

In Thailand, fresh DAV was collected from three species of male deer [*sika deer* (*Cervus Nippon Temminck*), *rusa deer* (*Rusa timorensis*), and *sambar deer* (*Rusa unicolor*)]. This research aimed to develop an effective delivery system from *Spongillia* spicules for enhancing skin permeation of proteins and growth factors from DAV extract. IGF-1, EGF, and TGF-β1 were used as growth factor markers in crude extract. The bioactivities of DAV extract on the cell proliferation and migration of normal human fibroblast (NHF) cells were evaluated. To develop drug delivery systems, microspicules (MSs) were prepared and used for enhancing the skin permeation of macromolecules. In *vitro* skin permeation and *in vivo* studies were also conducted.

**MATERIALS AND METHODS**

**Materials** Fresh DAV was a gift from Karakada 2011 Co., Ltd., Ratchaburi, Thailand. Bovine serum albumin–fluorescein isothiocyanate conjugate (BSA–FITC) and silver stain (ProteoSilver™ Silver Stain Kit) were purchased from Sigma-Aldrich, Co. (St. Louis, MO, U.S.A.). Normal human foreskin fibroblast (NHF) cells were obtained from the American-Type Culture Collection (ATCC) (Rockville, MD, U.S.A.). Dulbecco’s modified Eagle’s medium (DMEM), fetal bovine serum (FBS), trypsin–ethylenediaminetetraacetic acid (EDTA), and penicillin–streptomycin were purchased from Gibco BRL (Rockville, MD, U.S.A.). Sponge spicule extract power (Microspicule extract powder) was blended by using a porcelain mortar and pestle at various blending times. The blended powder was then transported through sieve mesh No. 200 (two times) and pestle at various blending times. The blended powder was then mounted and weighed 1 g of samples to soak in 20 mL of distilled water. A probe sonicator (Vibracell™, VCX 130 PB; Sonics and Materials, Inc., Newtown, CT, U.S.A.) with a frequency of 40 kHz at 40% amplitude was applied for 30 min under an ice bath. The result was determined from the width of the scratch area following the manufacturer’s protocol.

**Bioactivity Studies of DAV Extract** For the proliferation of skin cells, NHF cells (5 × 10⁵ cells/well) were seeded in 100 μL of DMEM supplemented with 10% FBS, 1% penicillin/streptomycin, and 1% non-essential amino acid in 96-well plates and incubated under a humidified atmosphere (5% CO₂, 95% air, 37°C) for 24 h. DAV extract was diluted with serum-free medium (DMEM plus 1% penicillin/streptomycin) in various concentrations (0, 1, 10, 50, 100, 2000, and 4000 μg/mL), followed by removing the cell medium from cell plates. Each concentration of diluted extract was added 100 μL per well and incubated for 24 h. Then, the diluted extracts were removed and washed by phosphate buffer saline (PBS pH 7.4). The cell proliferation was evaluated by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The cells were incubated with MTT solution (2.5 mg/mL) for 3 h. After that, the medium was discarded, and added 100 μL of dimethyl sulfoxide (DMSO). The absorbance at 550 nm was measured using a microplate reader (VICTOR Nivo™ Multimode Plate Reader, PerkinElmer, Inc., Germany).

The percentage of cell proliferation was calculated by the following equation:

\[
\text{% Cell Proliferation} = \frac{\text{Absorbance of treated cells}}{\text{Absorbance of untreated cells}} \times 100 \quad (2)
\]

Skin fibroblasts migration was also evaluated in *in vitro* scratch assay. NHF cells (2 × 10⁵ cells/well) were cultured in 6-well plate and incubated at humidified atmosphere (5% CO₂, 95% air, 37°C) for 48 h. Marking lines were drawn from one side to the other side about 3 lines per well using 200 μL pipette tip. Then, the cell medium was removed and the cells were washed with PBS pH 7.4. The 3 mL of sample was added in each well. Negative control was serum-free DMEM (DMEM supplement with 1% penicillin/streptomycin) and positive control was TGF-β1 (53.03 pg/mL). The images of cells in each well were taken by inverted microscope (Nikon® T-DH, Nikon, Tokyo, Japan) at 0, 24 and 48 h of incubations. The result was determined from the width of the scratch area before and after treatment using image J analysis software. The percentage of migration enhancement was calculated (Eq. 3) and compared between sample and control.

\[
\text{Migration enhancement} (%) = \frac{\text{Gap area at start time} - \text{Gap area at end time}}{\text{Gap area at start time}} \times 100 \quad (3)
\]

**Preparation and Characterization of MSs** Sponge spicule extract powder was blended by using a porcelain mortar and pestle at various blending times. The blended powder was then transported through sieve mesh No. 200 (two times) to remove unwanted sizes. The powder was then mounted on glass slides and observed using an inverted microscope (Nikon ECLIPSE TE2000-S, Nikon) equipped with a Moticam.
20 MP camera system (Motic®, Fujian, China).

Preparation of Cream Containing DAV Extract and MSs  The DAV extract (0.2% (w/w)) was mixed with cream to prepare a cream-based formulation. Subsequently, 2% (w/w) of MSs was added. The appearance of cream and optical microscope images were observed. For in vitro skin permeation study, 0.2% (w/w) of BSA–FITC was used as a model high-molecular-weight protein and mixed with MS cream.

In Vitro Skin Permeation Study Abdominal porcine skin was taken from intrapartum stillborn animals from a farm in Nakhon Pathom. Subcutaneous fat was carefully removed by medical scissors. The skin thickness was approximately 600–700 µm. The skins were stored at −20°C until use and thawed at room temperature in PBS before use.

Permeation of macromolecular proteins through porcine skin was performed using Franz-type diffusion cells. The 1 g of each formulation was applied and gently massaged onto the skin for 2 min (approximately 160 rubbing times/1.96 cm² of skin area) by using forefinger with medical grove before putting in the donor chamber. Approximately 6 mL of PBS as a receptor medium was continuously stirred by using a magnetic stirrer, and the temperature was maintained at 32°C. At 1, 2, 4, 6, 8, and 24 h, 0.5 mL of receiver medium was withdrawn for fluorescence analysis. The same volume of PBS was added to the receiver compartment to maintain a constant volume. Each sample was analyzed in triplicate.

The skin permeation parameters were calculated using the mathematical model based on Fick’s law of diffusion. Cumulative amount versus time profiles were plotted. The steady-state flux was obtained from the slope of the linear portion. The permeability coefficient (Kₚ) was calculated by using the steady-state flux (J) and donor concentration (C₅) of the formulations (Eq. 4).

\[
K_p = \frac{J}{C_d}
\]  (4)

The enhancement ratio (ER) was calculated by following equation:

\[
ER = \frac{\text{Flux of skin permeation enhancing system}}{\text{Flux of solution}}
\]  (5)

Fluorescence Analysis The BSA–FITC concentration was determined using a fluorescence spectrophotometer (PerkinElmer, Inc. Multimode Plate Reader, VICTOR Nivo™, U.K.) with the excitation wavelength at 485 nm and the emission wavelength at 535 nm. About 100 µL of sample was pipetted into a black 96-well plate, and fluorescence was measured for three replicates of each sample.

In Vivo Human Skin Study The study involved 12 healthy human volunteers (between 25 and 35 years old) who agreed to participate in a clinical trial. This study was approved by an Investigational Review Board (Human Studies Ethics Committee, Faculty of Pharmacy, Silpakorn University). For at least 12 h before the measurements, the skin was not treated with moisturizer products. All measurements were performed on the left arm, and three different points in the forearm were marked (3 × 3 cm) and applied each formulation twice a day at the same marked area. Appropriately amount of each formulation was applied and gently massaged onto the skin for 2 min (approximately 120 rubbing times/marked skin area) by using forefinger. After applying these formulations for 14 d, the DermaLab® series (SkinLab Combo; Cortex Technology, Hadsund, Denmark) was used to evaluate the skin (melanin content, erythema, elasticity, and hydration).

The percentage change in all parameters was calculated from untreated skin. The effect of formulations on melanin content (pigmentation) and erythema (vascularity) were determined by generating the value of melanin index % (%
MI) and erythema index % (% EI), which both normal skin pigmentation and vascularity equated to 100% as shown in Eqs. 6 and 7. For skin elasticity, the probe was based on vertical suction applied to the skin surface that the diameter of the circular opening was 10 mm. The Young’s modulus was determined as a ratio of the stress applied to the skin over the skin deformation, which the decreasing of the percentage change of Young’s modulus value represents the increasing of skin elasticity (% elasticity; Eq. 8). Moreover, the percent change of skin hydration was calculated (% hydration increase; Eq. 9).

\[
\% \text{ MI} = 100 + \left(\frac{M_t - M}{M}\right) \times 100
\]  
(6)

Where \(M_t\) is the melanin content of treated skin and \(M\) is the melanin content of untreated skin.

\[
\% \text{ EI} = 100 + \left(\frac{E_t - E}{E}\right) \times 100
\]  
(7)

Where \(E_t\) is the erythema value of treated skin and \(E\) is the erythema value of untreated skin.

\[
\% \text{ Elastic modulus} = \left(\frac{Y_t - Y}{Y}\right) \times 100
\]  
(8)

Where \(Y_t\) is the Young’s modulus of treated skin (mPa) and \(Y\) is the Young’s modulus of untreated skin (mPa).

\[
\% \text{ Hydration} = \left(\frac{H_t - H}{H}\right) \times 100
\]  
(9)

Where \(H_t\) is the hydration value of treated skin and \(H\) is the hydration value of untreated skin.

**Data Analysis** Each data represents mean ± standard deviation (S.D.). Statistical significance was analyzed by one-way ANOVA, followed by a least significant difference (LSD) post hoc test. The significance level was set at \(p < 0.05\).

**RESULTS**

**DAV Extract** DAV extract showed a light red fibrous texture (Fig. 1) and 5.57% of yield. SDS-PAGE showed bands of water-soluble proteins with molecular weights ranging from to 6.5 kDa. For DAV extract, the bands at 66 and 6.5 kDa were found, representing the bands of high-molecular-weight proteins (i.e., BSA) and polypeptides (i.e., growth factor), respectively. The total protein content and growth factors (IGF-1, EGF, and TGF-β1) are shown in Table 1.

**Bioactivity of DAV Extract on Skin Fibroblast Cells** DAV extract significantly enhanced NHF cells growth at concentration more than 500 µg/mL \((p < 0.05)\) compared with control (Fig. 2). The highest cell proliferation was found at 4000 µg/mL of DAV extract concentration, but DAV extract was not completely dissolved, and a fraction remained in the bottom of the cell plate. Therefore, the concentration of DAV extract at 2000 µg/mL was used for in vitro scratch assay to detect migration of skin fibroblast. For the results as shown in Fig. 3, the width of scratch area was narrow at 24 h and closed at 48 h. DAV extract highly induced the migration of NHF cells.
cell at 24 h compared with TGF-β1 and negative control, respectively. As shown in Fig. 4, both DAV extract and TGF-β1 showed 100% of the migration enhancement at 48 h. However, the percent migration enhancement of DAV extract at 24 h was significantly higher than TGF-β1 and control, respectively (p < 0.05). This result represented that DAV extract provided fast and complete healing of the scratch area.

**MS Size Reduction**  Sponge spicules, also called MSs without blending (MS), exhibited needle-like structures with the tip growing out from two opposite sites. The middle-wide and length of spicules were 11.89 ± 0.11 and 176.77 ± 10.52 µm, respectively (Fig. 5). After blending for 30 s, this process broke the length of spicule structure that was called MSs with short blending (MB). Various sizes and shapes of spicules were found, such as needles with one tip (approx. 85 µm) and cylindrical needles (approx. 62 µm). At a blending time of 10 min, microfine sponge powder (MF) was found with a small short part from spicules.

**DAV Extract-Loaded MS Cream**  Three types of MSs were added into cream-based containing DAV extract, in which various shapes of MSs were completely mixed with a general hydrophobic cosmetic paste (Fig. 6). All formulations exhibited a good appearance and smooth texture upon application onto the skin. MS- and MB-containing DAV cream presented a rough texture when applied onto the skin, so MS caused pain and redness. For the optical microscope images, the shape and size of spicules in DAV cream were similar to the spicules without cream (Fig. 5). BSA–FITC, which was used as a model macromolecular protein in this study, was also homogeneously mixed with MS cream (data not shown).

**Skin Permeation Study**  In general, skin has endogenous proteins and peptides that may interfere with the measured amount of exogenous protein and growth factor permeated through the skin. In this study, BSA–FITC was used as a model macromolecular protein. As shown in Fig. 7, the amount of BSA–FITC through the skin at 24 h was in the following order: MS cream > MB cream > MF cream > cream based > extract in solution form.

For the skin permeation parameters as shown in Table 2, the permeated flux of BSA–FITC showed that MS without blending (MS) significantly increased the BSA–FITC flux by 2.26-fold in permeation over solution form. MB also increased the BSA–FITC flux by 1.94-fold in permeation over solution form. MS and MB did not show a significant difference. By contrast, MF-containing cream and cream without MS presented no difference in permeated flux from solutions. Although longer needle of MS was inserted into the deeper skin to create a penetration route for drugs than that MB did, the skin irritation was found. Therefore, MB cream was used for the in vivo study.

**In Vivo Human Skin Study**  After 14 d of three formulations (MB creams, cream-based, and solution containing DAV extract) applications, all subjects were monitored for skin changes in melanin content, erythema, elasticity, and hydration. No unwanted symptoms were observed by visual appearances.
Change in the skin chromophore concentrations (melanin and hemoglobin) induced alterations in both MI and EI. Melanin index values decreased slightly after 14 d of application of all formulations; skin treated with DAV extract-containing MB cream exhibited a decrease in pigmentation that was greater than that with cream and solution (Fig. 8(A)). For skin erythema that generally represents the redness of the skin or mucous membranes, resulting from the hyperemia of superficial capillaries. All formulations exhibited the mean of erythema index near 100% (Fig. 8(B)), representing no significant change in treated skin. Although MB cream showed a high variation in any value that may be caused by different levels of MS insertion in the skin or superficial layer of the skin, no pain or severe redness skin was found.

Skin elasticity and hydration were also measured as shown in Fig. 8(C, D). The values of skin elasticity were basically between 2 to 15 MPa, which the higher MPa value represents a higher vacuum strength needed to lift the skin, indicating a better firmness of skin. The percent change of skin elastic modulus after treated with DAV extract-containing MB cream was lower than that with cream and solution, respectively, indicating the highest elasticity of skin treated with DAV extract-containing MB cream. For skin hydration, skin treated with DAV extract-containing MB cream showed a higher skin hydration percentage than that with cream and solution. These results indicated that the increase in elasticity led to increased hydration of the skin.

**DISCUSSION**

Velvet antlers have many bioactive compounds such as mineral elements, amino acids, polypeptides, proteins, polysaccharides, fatty acid, phospholipids, and biological base. Efficient protein extraction for velvet antlers has been developed; water extraction and probe-type sonication method provide high contents of water-soluble proteins. Apart from high molecular weight proteins, various growth factors are expressed in the growing tip of antlers. In the present study, high content of total proteins and growth factors were measured by using the extraction and probe-type sonication method. Three types of growth factors were used as markers; IGF-1 exhibited higher contents than TGF-β1 and EGF. DAV extract dissolved in water (2000 µg/mL) significantly enhanced the proliferation of NHF cells, indicating the cell growth and no toxicity. To detect the migration of skin fibroblast, in vitro scratch assay is simple and inexpensive method. Starting with marking line in a cell monolayer was done before applying DAV extract. The images at the beginning and interval times were observed the cell migration to close the scratch area. DAV extract induced the migration of NHF cells more than TGF-β1 and control (untreated cells) at 24 h and exhibited the complete healing of the scratch area at 48 h. These results suggested that DAV extract have the ability to repair the skin cells. Enhancement of fibroblasts proliferation indicates the bioactivity of growth factors. Growth factors generally stimulate the proliferation of various cell types, and they are extensively used in skin wound healing and aging. The skin repair capability decline with age due to structural and functional changes by reducing proliferation and migration of fibroblasts and degrading collagen and elastin in the extracellular matrix. For skin rejuvenation, EGF plays a significant role in the growth and regeneration of skin cells (the interaction between keratinocytes and fibroblasts). High levels of serum IGF-1 have also been reported to influence fibroblast functions, such as the inhibition of collagenses and the induction of collagen expression. Therefore, growth factors and cytokines are used in several cosmetic products intended for skin rejuvenation.

Large molecular size of protein and polypeptides (generally 6–66 kDa) limits their ability to passively penetrate the tightly packed stratum corneum. Microneedling or laser resurfacing has been used to create a possible route to transport macromolecules (i.e., protein and growth factors) through the skin. The main component of sponge spicule is silicon dioxide, but other recognized elements, such as iron, manganese and aluminum, were also found. Appreciable amounts of heavy alkaline, such as cesium (2.00% dry weight) and rubidium (0.26% dry weight), in their structures play a considerable role in spicule construction. Therefore, the high physical stability of these spicules has been reported in terms of size distribu-

**Table 2. The Skin Penetration Parameter of the Different Formulations**

<table>
<thead>
<tr>
<th>Formulations</th>
<th>Flux (µg·cm⁻²·h⁻¹)</th>
<th>K₅ (×10⁻⁷) (cm·h⁻¹)</th>
<th>ER</th>
</tr>
</thead>
<tbody>
<tr>
<td>Solution</td>
<td>0.0372 ± 0.02</td>
<td>1.86 ± 0.88</td>
<td>—</td>
</tr>
<tr>
<td>Cream</td>
<td>0.0404 ± 0.01</td>
<td>2.02 ± 0.47</td>
<td>1.09</td>
</tr>
<tr>
<td>MF cream</td>
<td>0.0426 ± 0.01</td>
<td>2.29 ± 0.27</td>
<td>1.15</td>
</tr>
<tr>
<td>MB cream</td>
<td>0.0720 ± 0.04</td>
<td>3.60 ± 2.04</td>
<td>1.94</td>
</tr>
<tr>
<td>MS cream</td>
<td>0.0840 ± 0.01*</td>
<td>4.20 ± 0.61*</td>
<td>2.26</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. (n = 3). *indicates significant difference from solution (p < 0.05).
tion and integrity at 25°C in the air, PBS solution, and pure ethanol (99.9%). Moreover, MSs in hydrophobic cream could not be broken or dissolved, and they presented the same size and shape.

The mechanism of actions of MSs may be either microablative technique or puncture-resembling mesotherapy. Udompataikul et al. reported that the small and sharp thorn-like nature of spicules (nearly the size of aluminum hydroxide crystals used in microdermabrasion) causes deep punctures into the skin, thereby facilitating the permeation of hydrogen peroxide to deeper skin layers. Moreover, Ha et al. found that pyramid-shaped MSs (0.25 µm) can effectively remove the stratum corneum layer and uniformly distribute EGF into the skin. In this study, no significant different between MS and MB to deliver macromolecular protein through the skin. Various shapes and sizes of MB formulations may provide both mechanisms to improve the skin permeation of macromolecules. One tip of MS fractions was inserted into the skin to create a penetration route for drugs, whereas cylindrical shape fractions disrupted the stratum corneum barrier. Although MS was 2-fold higher length than MB, the length of MB (approx. 85 µm) was enough to create pores passing through the skin barrier. The thickness of the stratum corneum barrier is approximately 10–20 µm, and the viable epidermis is 50–100 mm thick. Moreover, the dermis consists of a network of blood vessels to exchange or deliver drugs to the body, and nerves also play an important role in pain regulation.

Therefore, long needles of MS (approx. 176.77 µm) were inserted into the deep skin and affected the pain regulation. Sharp-edged and rod-shaped silicious spicules can physically disrupt skin in a dose-dependent manner and accumulate in the skin for over at least 72 h, allowing the skin penetration of hydrophilic macromolecules. Although increasing the applied dose, massage time, and intensity increased the amount of spicules penetrating into the skin and improved skin permeabilization, skin irritation and pain should be considered. High doses of MS (4%, (w/w)) in a cream formulation caused skin redness and irritation (data not shown). In this study, 2% (w/w) of MS formulation exhibited the highest BSA–FITC permeated through the skin, but this formulation still caused skin irritation. To minimize pain, small MSs (MB formulation) were used for in vivo study.

Zhang et al. reported that sponge spicules offer safe, effective, and sustained skin permeation of hydrophilic macromolecules. In their study, the spicules embedded in the skin were gradually eliminated with desquamation, and the disrupted skin was efficiently repaired with time. To balance the efficacy and safety of sponge spicule-based drug delivery systems, application strategies, including dose, massage time, and intensity, should be adjusted in accordance with the target disease and treatment requirements.

Growth factors are polypeptides that can imitate a peptide
sequence of collagen or elastin molecules, stimulate collagen synthesis, and activate dermal metabolism. Moreover, the activation of hyaluronan synthase-2 by EGF led to produce hyaluronic acid and then restore skin hydration. Growth factors have been used as potentially compounds to improve the clinical appearance of fine and coarse wrinkles visible in aging skin. For microdermabrasion or skin resurfacing, which is used for removing the damaged epidermis and replacing tissue with remodeled skin layers or the potential growth factors. According to Ha et al., pyramid-shaped MSs containing EGF can enhance skin permeability and function as an effective anti-wrinkle agent. Therefore, the synergistic effect of growth factors and MSs in hydrophobic cosmetic paste led to the visible improvement in skin elasticity and moisture to rejuvenate aging skin.

CONCLUSION

In this study, DAV extract containing high total protein content and growth factors promoted skin fibroblast proliferation and migration. The extract was successfully mixed with MS cream, whose small size and various shapes of MSs (MB formulation) provided high skin permeability of macromolecular protein without skin irritation. Moreover, skin treated with DAV extract in MB cream showed the improvement of the skin elasticity and hydration than that treated with DAV extract in cream and solution forms. Therefore, MB cream plays an important role in enhancing macromolecule delivery through the skin, leading to the improvement in skin properties.

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Conflict of Interest The authors declare no conflict of interest.

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