*Original*

Appl. Cosmetic Sci. & Tech. 1 (1): 56–64

# Enhancing Skin Permeability and Moisturization Using Petroleum Nanoemulsion

Ken Uda* and Toshiyuki Homma

Bio Science & Engineering Laboratories, FUJIFILM Corporation, Kanagawa, Japan

The stratum corneum (SC) acts as a barrier that regulates water loss from the body and prevents various external stimuli. Additionally, it retains water and maintains moisture in the body. These barrier and moisturizing functions deteriorate with age and in dry environmental conditions. In this study, we focused on petroleum (widely used as a moisturizer in creams and other products) as a novel approach to maintain the water retention capacity of the SC, aiming to develop ingredients that further enhance moisturization. Petroleum is a water-insoluble, long-chain hydrocarbon with very low skin permeability. Its moisturizing effect is exclusively achieved by covering the skin surface to prevent moisture loss. In contrast, it is easily eliminated from the skin surface via contact or rubbing, and its effects are short-lived. A newly developed petroleum nanoemulsion (nano-petroleum), achieved by reducing the particle size to approximately ≤80 nm, enhances penetration into the SC. Additionally, it was confirmed that the product had post-penetration moisture retention and swelling effects on the SC. The skin permeability of petroleum is considered to be extremely low; however, nanosizing enhances its texture by stably dissolving it in a water-based formulation, combining its moisture retention effect with enhanced skin permeability. We believe that this research has uncovered novel potential applications for petroleum, enabling its use in various cosmetic formulations.

**Key words:** petroleum, hydrocarbon oil, stratum corneum, moisture retention, permeability, cosmetics, formulation, nanoemulsion, moisturizer, skin, epidermal model

## 1. Introduction

Moisturizing functions are crucial for the development of cosmetics. The main methods to achieve this function are that is retains moisture in the stratum corneum (SC), preventing moisture loss even in low-humidity environments. Substances that retain moisture are called moisturizers. Based on their mechanisms, they can be classified into two categories: emollients—hydrophobic with a high water-blocking capacity and humectants—hydrophilic with a high water-holding capacity.[1)] Emollients include petroleum (hydrocarbon oil) and triglycerides (components of the sebaceous film), whereas humectants include glycerin, amino acids, and urea. Cosmetic strategies for producing moisturizing effects can be broadly divided into these two categories, and various formulation technologies have been developed to address them.[2)] This study aimed to enhance the moisture content of SC over a long period.

Received: July 25, 2024; Accepted: November 8, 2024

*Corresponding author: Ken Uda (E-mail: ken.uda@fujifilm.com)

Bio Science & Engineering Laboratories, FUJIFILM Corporation, 577, Ushijima, Kaisei-machi, Ashigarakami-gun, Kanagawa 258-8577, Japan

DOI: 10.69336/acst.2024-04

Moisturization is crucial for healthy skin because it helps maintain the moisture content of the SC, thereby preventing the deterioration of its barrier function. The barrier function of the SC is crucial for protecting human tissues from various external stimuli. Additionally, this function is closely associated with skin formation and homeostasis. Recent advances in experimental molecular biology and analytical techniques have elucidated these mechanisms in dermatology. Studies on epidermal cells have revealed the mechanisms of stem cell maintenance and keratinocyte differentiation as a sequence of events from basal cells to SC formation. Moreover, the disruption of these homeostatic systems is believed to impair skin barrier function and contribute to the pathogenesis of various skin diseases.[3] In particular, research on SC cells that are denucleated during final differentiation has been progressing. Reports have proposed a mechanism involving differences in the distribution of protein structure, amino acids, pH, and trilayer structure in the central SC associated with the age-related deterioration of barrier function, elucidating the detailed mechanisms underlying skin homeostasis.[4,5]

Specifically, deterioration of the skin barrier function results in its further deterioration because the skin is susceptible to external environmental factors, such as dryness and mechanical stress. Atopic dermatitis is a well-studied disease, and various complex associated factors have been identified, including the exacerbation cycle.[6] The most effective treatment for atopic dermatitis involves combining different therapies, notably the use of humectants in combination with moisturizers and emollients.[7,8] These examples highlight the significance of moisturizing as directly associated with enhancing the barrier function to maintain healthy skin.

To enhance its moisturizing function, we aimed to deliver effective ingredients to the entire SC to generate a barrier function. Therefore, we focused on petroleum, which is widely and commonly used as an emollient because it is highly safe and effective for covering the skin surface. We initiated this research with the belief that developing a technology that can increase petroleum permeability can enhance the moisture retention effect of the skin.

Petroleum is highly effective in treating atopic dermatitis, primarily because it inhibits moisture transpiration and helps maintain high skin moisture. In contrast, it is limited in terms of persistence, and its moisturizing effect following petroleum application reduces over time.[9] Assuming that petroleum may be applied in small amounts or removed owing to physical contact or other causes, it is necessary to apply a high concentration or moderate reapplication to obtain effectiveness. Additionally, when used as a cosmetic, it is often formulated into creams used to obtain the same effect. Even when used for daily use, it is possible that the effects may be reduced for these reasons. From the perspective of cosmetic formulation, because of its sticky wax-like texture, petroleum cannot be used in formulations other than creams. Moreover, the comfort of use is crucial, specifically for water-based formulations, such as lotions, where a strong sticky feel is undesirable and reduces the user's quality of life. Therefore, in this study, we aimed to develop stable petroleum particles with enhanced permeability that provides moisturizing effects at low concentrations similar to those observed at high concentrations.

## 2. Materials and Methods

### 2.1. Preparation of nano-petroleum

High-purity petroleum (SUPER WHITE PROTOPET, Sonneborn LLC), glycerin (GLYCERIN, Kao Co.), sucrose stearate (DK ESTER, DKS Co. Ltd.), lecithin (SLP-WHITE, Tsuji Oil Mills Co., Ltd.), and purified water were mixed and subjected to a micronization process (Star Burst Mini, Sugino Machine Co.). The particle size was measured using an FPAR-1000 (Otsuka Electronics Co., Ltd.). Electron microscopy images were obtained using a transmission electron microscope (JEM-2010, JEOL Ltd.).

### 2.2. Skin penetration verification of nano-petroleum

Three-dimensional reconstructed epidermal models (EpiDerm EPI-200; MatTek) were used. 100 μL of nano-/non-nano-petroleum was added to each skin model. After 3 h of incubation, the remaining formulation on the surface was removed, and the skin model was washed with 500 μL PBS five times. The amount of petroleum that penetrated the epidermal model was measured using gas chromatography-mass spectrometry (GCMS QP2010, Shimadzu Co.). GC-MS analyses were performed on aliquots of tetrahydrofuran extracts that were injected in the split mode. A dimethylpolysiloxane column (DB-1, Agilent Technologies Japan, Ltd.) was used. Two oven temperature programs were used with helium as the carrier gas at a constant pressure control mode at 50 kPa. GC-MS spectra were obtained in the scanning mode in the mass-to-charge ratio (m/z) range of 30–800 and fragment ion at m/z = 57 for quantification. A calibration curve was constructed using the carbon number (20-40) of the hydrocarbon complex peak of petroleum, and representative peaks that provided linearity and were not problematic for quantification were selected. The SC water content of

the skin model was measured using an ASA-MX100 (Nihon Ash Co., Ltd.) instrument to determine the ΔW value (μS) before and after application of the formulation.

### 2.3. Verification of moisture retention effect of nano-petroleum

Keratin powder (Keratin Powder, Tokyo Chemical Industry Co., Ltd.) was dissolved in pure water and solidified through natural drying to create a pseudo-SC model. 50 μL of nano-/non-nano-petroleum was added to each model and the remaining formulation on the surface was removed after 1 h. In this experiment, the weight change is measured from the penetration of the respective applied substance inside the model. Subsequently, the moisture retention capacity was assessed by measuring the weight change owing to water evaporation at 3 and 24 h.

### 2.4. Verification of the effect of nano-petroleum on SC swelling

A sheet of SC (STR002, Biopredic International, Inc.) obtained from human skin tissue and 10 μL of nano-/non-nano-petroleum was added to each SC sheet. Three hours after application, the thickness of the SC was measured from microscopic images. Specifically, the thickness of the SC was measured at three random locations on a skin model (8 mm diameter), and the average of four sections per level was adopted. All measurements were performed using ImageJ software.

## 3. Results

### 3.1. Nano emulsification processing of petroleum

The petroleum nano-formulation was adjusted using a high-pressure emulsifier according to the composition detailed in Table 1, resulting in the production of stabilized nano-petroleum particles approximately ≤80 nm in diameter (Figs. 1 and 2). The average particle size of the nano-petroleum formulation was 74 nm, compared with $6.1 \times 10^3$ nm particles of unstable petroleum in suspension. Transmission electron microscopy (TEM) images were obtained using different methods because of the large differences in particle size observed. Non-nano-petroleum was photographed via negative staining, and the particles were observed as white spheres with low electron density. Their size was approximately several micrometers (the TEM image demonstrates the smallest at approximately 1 μm). Nano-petroleum was photographed using the ice-embedding method, and the lipid particles were observed as black spheres. Their size was <100 nm, comparable to the particle size analyzer measurements.

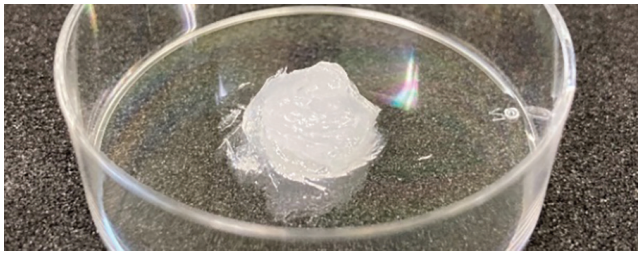

Fig. 1 Photograph of the petroleum raw materials used. The petroleum raw material used is high-purity white petroleum.

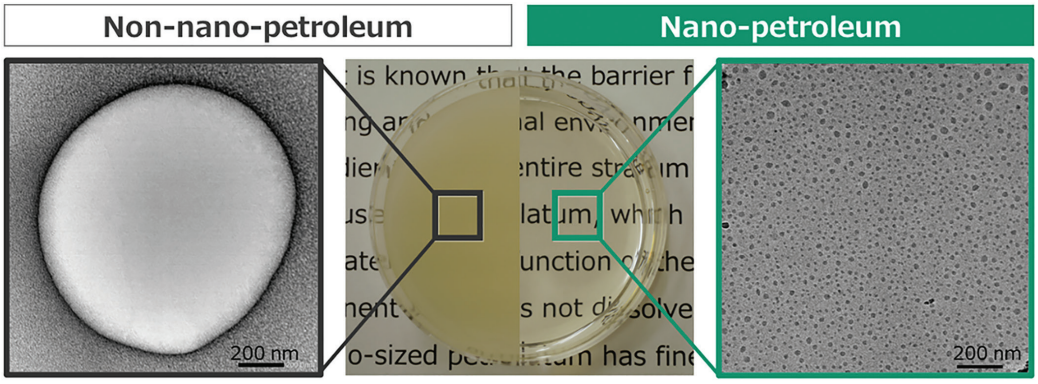


Fig. 2 Photograph and electron microscope image of nano-petroleum. External view and TEM images of nano- and non-nano-petroleum in Petri dishes. Non-nano-petroleum has a large particle size, making it cloudy and obscuring the letters in the background. In contrast, nano-petroleum has a high transparency owing to its extremely small size.

Table 1 Sample composition and particle size.

| Composition (%) | Non-nano-petroleum | Nano-petroleum |
|---|---|---|
| Petroleum | 14.0 | 14.0 |
| Glycerin | 52.3 | 52.3 |
| Sucrose stearate | 10.0 | 10.0 |
| Lecithin | 1.0 | 1.0 |
| Water | 22.7 | 22.7 |
| Average particle size (nm) | $6.1 \times 10^3$ | 74 |

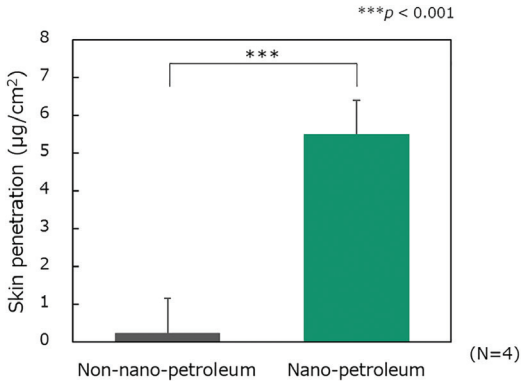


Fig. 3 Skin penetration effect of nano-petroleum. A reconstituted human skin model was used to assess the skin penetration of each formulation. Non-nano-petroleum hardly penetrated the skin model, whereas nano-petroleum demonstrated high skin penetration (N = 4).

### 3.2. Effect of nano-petroleum on skin penetration

An epidermal model containing SC was used to assess the penetration of nano-petroleum into the skin. Nano-petroleum was applied to the epidermal model, and after 3 h, the surface petroleum was thoroughly removed. Non-nano-petroleum was used as the control. The amount of petroleum in the epidermal model was determined using GC-MS. The amount of petroleum that penetrated the nano-petroleum applied epidermal model was approximately 22 times higher than that of the non-nano-petroleum applied (Fig. 3). Additionally, it was observed that non-nano-petroleum hardly penetrated. The appearance and H&E-stained images of the skin model are shown in (Fig. 4A). The thickness measurement results revealed that the thickness of the SC increased with the topical application of nano-petroleum (Fig. 4B).

### 3.3. Effect of nano-petroleum on SC water content in a skin model

Following the results obtained previously, nano-petroleum was applied to an epidermal model. Non-nano-petroleum was used as the control. After 3 h, the petroleum treatments were thoroughly removed from the surface of the skin model, and the SC water content was measured. The nano-petroleum applied epidermal model exhibited a higher water content than the non-nano-petroleum applied (Fig. 5).

### 3.4. Electron microscope image of the skin model SC permeated with nano-petroleum

Using the skin model mentioned in Section 3.2., the SC with increased permeability owing to nano-petroleum was directly observed using TEM. The thickness of the SC layer was measured from the resulting TEM images, and the average value was calculated. The results demonstrated an increased thickness in the SC layer applied with nano-petroleum (Figs. 6A, 6B). A detailed observation revealed particle-like structures within the SC cells, which had approximately the same size as the average petroleum particle, indicating possible penetration through the SC cells (Fig. 6C).

### 3.5. Moisture retention effect of nano-petroleum

The moisture retention effect of nano-petroleum was verified based on the weight permeation of the applied formulation using a pseudo-SC model, in which keratin protein powder was dissolved in water and allowed to solidify. Nano-/non-nano-petroleum was applied to the pseudo-SC model. Subsequently, the weight change in the penetrated petroleum was measured after removing non-penetrated in the remaining application (Fig. 7A). The rate of weight change in the nano-petroleum applied pseudo-SC model was significantly lower than that in the non-nano-petroleum applied at 3 and 24 h (Fig. 7B).

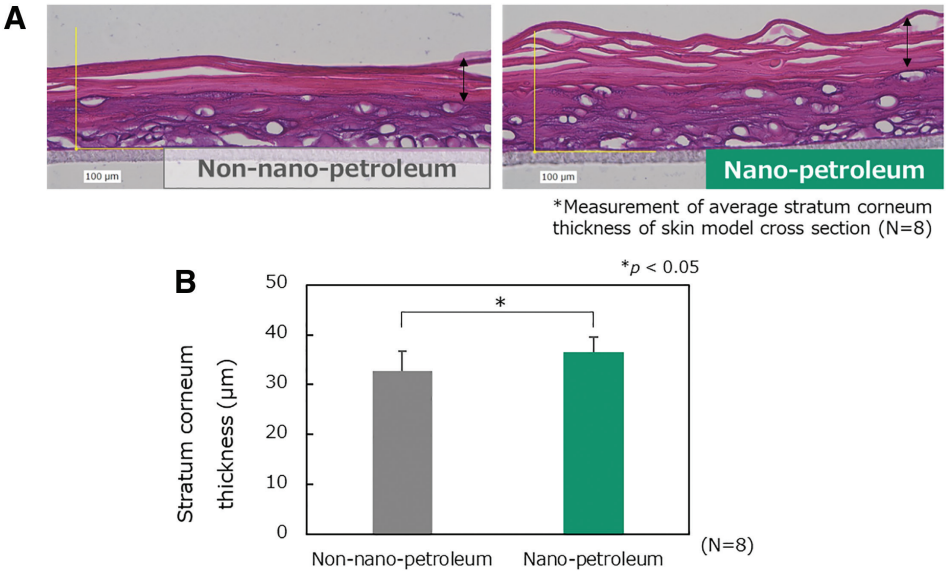


Fig. 4 SC thickness of skin model after topical nano-petroleum. Using a reconstituted human skin model, the thickness of SC is assessed when each formulation is applied. SC thickness was significantly increased after applying nano-petroleum compared with non-nano-petroleum. (A) H&E-stained image and (B) measured thickness of the SC from the acquired images.

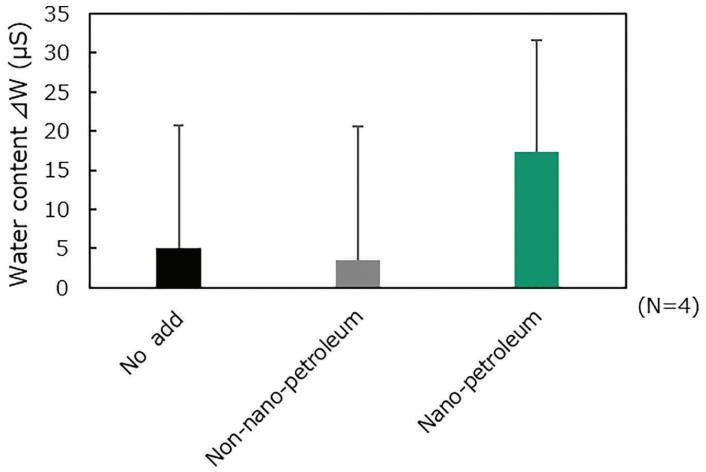


Fig. 5 Effect of nano-petroleum on SC water content. A reconstituted human skin model was used to assess SC water content when each formulation was applied. There is a trend toward increased water content after applying nano-petroleum compared with non-nano-petroleum (N = 4).

### 3.6. Swelling effect of nano-petroleum in SC

To determine the effect of petroleum on the actual SC, this study used SC obtained from excised human skin tissue. The thickness of the SC after 3 h of nano-/non-nano-petroleum application was measured using frozen cryostat sections. The results demonstrated that the thickness of the SC significantly increased after nano-petroleum applied compared with non-nano-petroleum (Fig. 8A). Optical microscopy images demonstrated that the SC swelled significantly only when nano-petroleum was applied (Fig. 8B). This is probably a result of enhanced penetration into the SC owing to nanosizing.

## 4. Discussion

SC penetration routes can be broadly divided into the intercellular lipid route and the intracellular route, with the exception of hair follicles. In general, molecules that are of medium lipophilicity (e.g., log P 1–3; Octanol-water partition

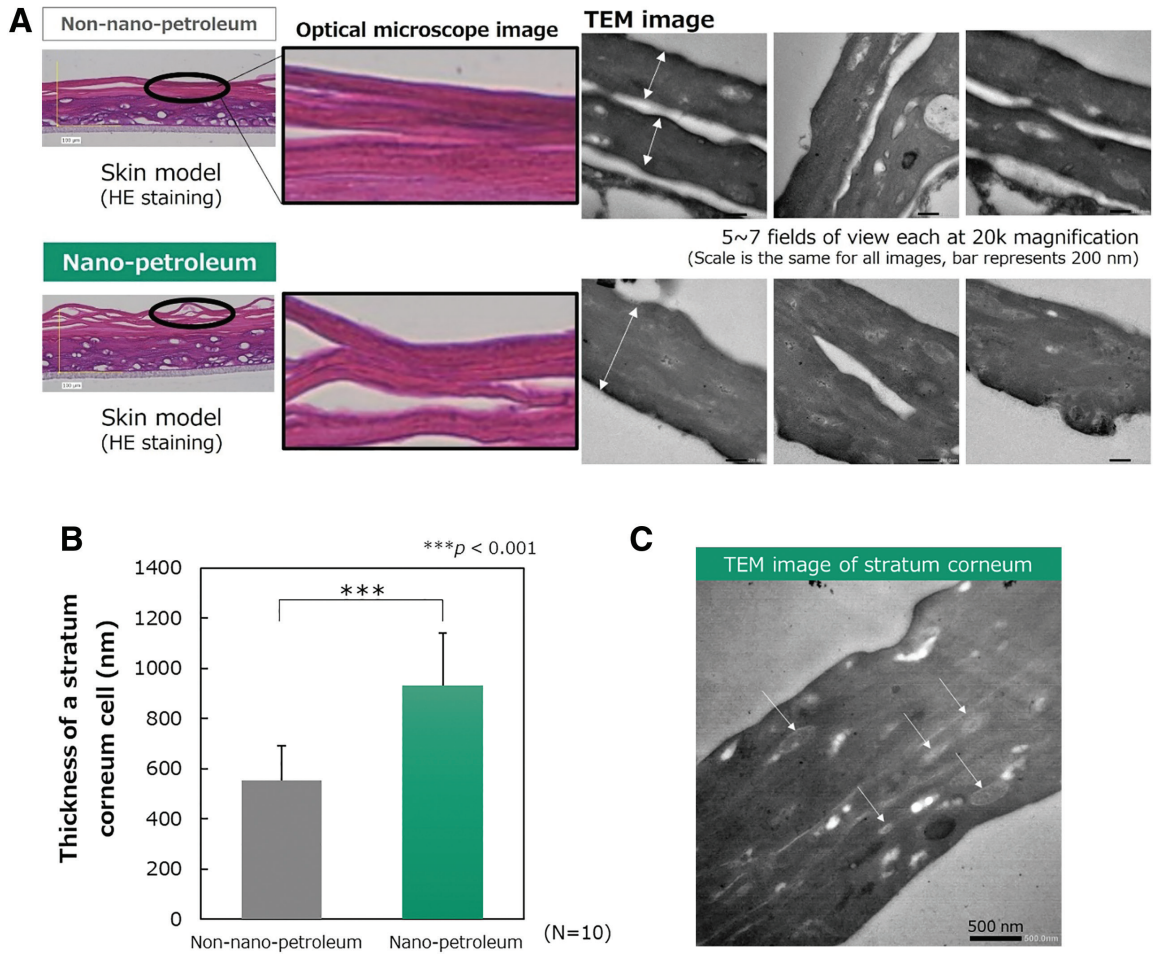


Fig. 6 Effect of nano-petroleum on SC swelling (increase in thickness). (A, B) Using a reconstituted human skin model, the thickness of SC upon the application of each formulation was assessed using TEM images. The thickness of a single SC cell measured from the TEM images was significantly increased after nano-petroleum application compared with that of non-nano-petroleum (N = 10). (C) Further magnification of the TEM image revealed multiple nano-sized particles that appeared to have been incorporated into SC cells.

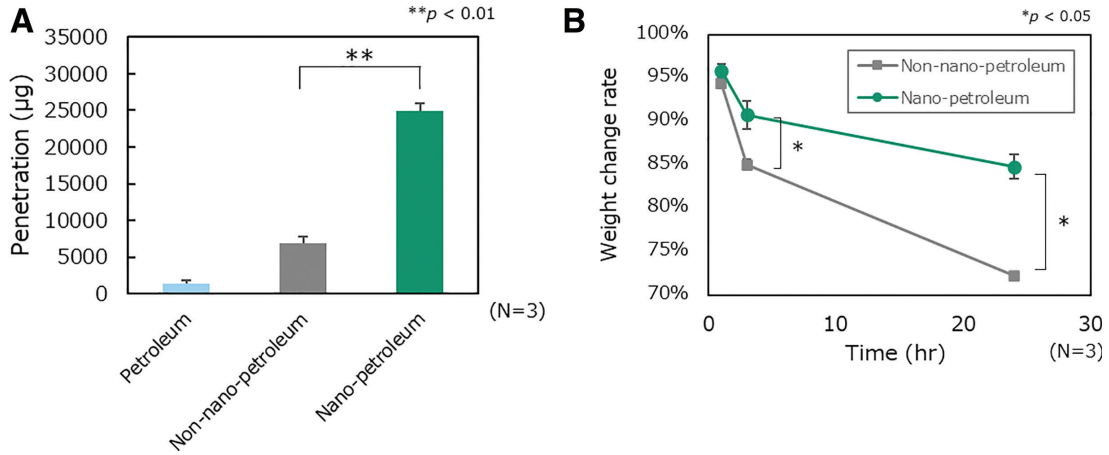


Fig. 7 Moisture retention effect of nano-petroleum. (A) The moisture retention capacity of each formulation was assessed by dissolving keratin protein in water and re-solidifying it to create pseudo-SC. High-purity petroleum and non-nano-petroleum used as raw materials have difficulty penetrating the SC (N = 3). (B) Water evaporation is higher for non-nano-petroleum than for nano-petroleum (N = 3)

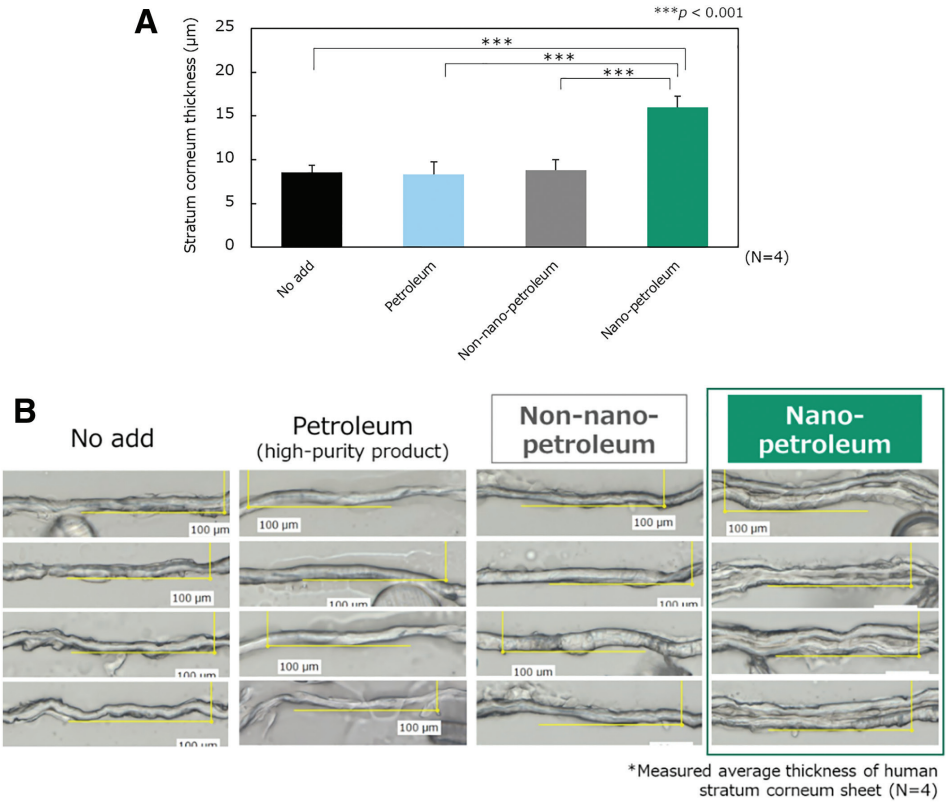


Fig. 8 Effect of nano-petroleum on SC swelling. (A) SC thickness after applying each formulation as measured by microscopy image using SC sheets prepared from excised human skin. The thickness of the SC did not change after the application of non-nano-petroleum. Only the SC treated with nano-petroleum showed increased thickness (N = 4). (B) Bright-field micrographs of the SC after the different treatments (N = 4).

coefficients) are considered to pass through intercellular lipids, while hydrophilic molecules pass across the intracellular pathway.[10] Long-chain hydrocarbons such as petroleum have very large LogP values and are considered to have very little penetration on their own.

However, several reports indicate that petroleum may penetrate directly into the skin. A report on hydrophilic and lipophilic moisturizers (including petroleum) applied to excisional skin and observations of its internal structure indicate that the application of petroleum swelled the SC more than the other hydrophilic moisturizers investigated.[11] In this report, the authors consider the potential increase in water content in the SC due to the occlusion effect. However, some studies have applied petroleum ointment to acetone-treated skin with a disrupted barrier and found that the barrier was restored.[12] The results showed that petroleum was localized in the intercellular lipids of the SC, as observed via electron microscopy. However, this localization of petroleum in the skin was observed after barrier disruption, and it remains unclear whether petroleum molecules fuse with intercellular lipids in the SC of healthy skin. If the skin barrier is compromised, petroleum may only slightly penetrate the SC. Based on the above results, petroleum may slightly penetrate SC under limited conditions.

Furthermore, recent studies have confirmed the enhanced skin penetration of large-molecular-weight compounds, such as hyaluronic acid, ceramide, and liposomes that contain various compounds.[13–16] These formulations are based on skin penetration mechanisms that are sufficiently designed for passage through SC cells. We believe that this is the first study investigating the enhanced skin penetration and moisturizing properties of petroleum due to nanoemulsification.

Next, we attempted to explain the mechanism underlying the enhanced water retention properties of nano-petroleum. We hypothesize two mechanisms. First, nanoemulsification increases petroleum penetration into the skin interior by increasing its fusion with intercellular lipids and coating the area around SC cells. This has also been reported for squalane, which has the same long-chain hydrocarbon molecular structure and is thought to coat the periphery of SC cells, near where the cornified envelope is present.[17] As a result, it was considered that, compared with the moisturizing effect when only the skin surface is covered (occlusion effect), the increased penetration of the nano-petroleum product, that is, its three-dimensional distribution inside the SC, contributes to its improved moisturizing effect. The increased

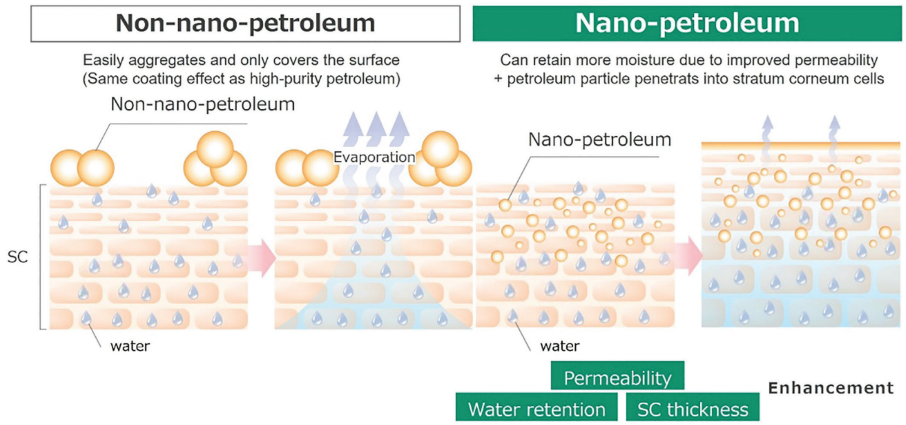


Fig. 9 Schematic representation of the skin penetration mechanism of nano-petroleum. The illustration shows the hypothesized effects of nano-petroleum on the skin based on previous results.

penetration of petroleum is shown in Fig. 3. Another study suggests the presence of a small fluid water layer (free water) within the intercellular lipids. The entire SC is easily coated by petroleum, which may reduce water evaporation. The penetration route within the stratum corneum may be revealed via detailed analyses such as X-ray scattering.[18)]

The second potential mechanism is via direct uptake into SC cells. The nano-petroleum formulation used in this study contains lecithin and sucrose stearate, which may result in a lipid particle structure with their hydrophilic heads facing outwards. Nano-petroleum exhibits moderate solubility, dispersibility, and surfactant properties, unlike viscous petroleum, which does not exhibit permeability. Based on the observed thickening of the individual SC layers and the results observed in SC cells via electron microscopy, we consider the possibility that nanoparticles are more easily fused into the SC cell membrane. A new finding of this report also suggests that some petroleum particles may be incorporated into SC cells (Fig. 6C). The mechanism by which the incorporated petroleum interacts with the side chains of keratin fibers and inhibits water evaporation from within SC cells, contributing to its moisturizing effect, has also been reported.[19)]

Considering these reports and our results, we speculate that nano-petroleum may act via both the intercellular lipid and intracellular routes, which may be involved in its moisturizing function. However, we believe that the route of entry of petroleum into the skin still needs careful discussion.

This study indicates that nano-petroleum particles may exhibit good moisture retention properties because of their enhanced penetration into the SC. The results indicate that the penetration of nano-sized petroleum into the SC reduces the rate of water evaporation, resulting in a long-term moisture retention effect. Additionally, our experimental results using human SC indicate that the enhanced water retention observed was due to the increased thickness of the SC (swelling effect, Fig. 9).

The results of this study demonstrated the enhanced penetration effect of nano-petroleum into the SC; however, its effect on skin physiological function remains unclear. Recent skin research has targeted various diseases, such as atopic dermatitis and the accumulation of inflammatory conditions because of aging, the relationship with skin immune function, and involvement with sensitive skin.[20,21)] To assess the effects of cosmetics and other topical formulations on the skin, verifying them based on dermatological evidence is crucial.

In this study, we focused on developing formulations that use petroleum for moisturization. If petroleum can be easily blended into various formulations, it may enable the development of products that enhance skin homeostasis. We believe that the development of cosmetics based on novel mechanisms is expected. The results of this study indicate that nano-petroleum exerts its moisture retention effect through a mechanism different from that of humectants (glycerin), which permeate the skin and retain water. We believe that the moisture retention effect of petroleum, which is elicited through full three-dimensional penetration into the interior of the SC rather than mere surface blocking, can be applied to develop novel cosmetics that eliminate stickiness.

## 5. Conclusion

In this study, we prepared a nano-formulation of petroleum and assessed its effect on the skin. The results demonstrated a moisture retention effect because of enhanced penetration into the SC, indicating that a nanosizing approach

may be useful to enhance the skin penetration of poorly soluble molecules. This approach shows promise in the development of cosmetics with both textural and moisturizing effects in a user-friendly water-based formulation.

**Conflicts of interest:** The authors declare no conflicts of interest associated with this manuscript.

**Data analysis and statistics:** Data are represented as mean ± SD. Significance: $*p < 0.5$, $**p < 0.01$, $***p < 0.001$ by Student's *t*-test (Fig. 3–7). $***p < 0.001$ compared with the control by Dunnett's Multiple Comparison Test (Fig. 8).

**Abbreviations:** SC, stratum corneum; TEM, Transmission electron microscopy

## References


1) S. Imayama, Y. Miyachi, K. Matsunaga, R. Utsugi, Scientific Study of Skin Care, Nankodo, 2008, p. 198
2) T. Hirao, Journal of Japanese Cosmetic Science Society, 2013, p. 95–100
3) E. Fuchs, Stem Cell Reports, 10, 1432–1438 (2018)
4) A. Kubo, I. Ishizaki, A. Kubo, H. Kawasaki, K. Nagao, Y. Ohashi, M. Amagai, Sci. Rep., 3, 1731 (2013)
5) K. Fukuda, Y. Ito, Y. Furuichi, T. Matsui, H. Horikawa, T. Miyano, T. Okada, M. Logtestijn, R. J. Tanaka, A. Miyawaki, M. Amagai, Nat. Commun., 15, 4062 (2024)
6) T. Bieber, Nat. Rev. Drug Discov., 21, 21–40 (2022)
7) S. Varothai, S. Nitayavardhana, K. Kulthanan, Asian Pac. J. Allergy Immunol., 31, 91–98 (2013)
8) P.M. Elias, J.S. Wakefield, M.-Q. Man, Skin Pharmacol. Physiol., 32, 1–7 (2018)
9) M. Hong, Y. Yating, W. Wenhai, L. Yuhong, L. Li, D. Yinmao, F. Yi, L. Yue, H. Yifan, Technol. Health Care, 29, 327–334 (2021)
10) R.H.H. Neubert, Eur. J. Pharm. Biopharm., 202, 114394 (2024)
11) J. Caussin, H.W.W. Groenink, A.M. de Graaff, G.S. Gooris, J.W. Wiechers, A.C.V. Aelst, J.A. Bouwstra, Exp. Dermatol., 16, 891–898 (2007)
12) R. Ghadially, L.H. Sorensen, P.M. Elias, J. Am. Acad. Dermatol., 26, 387–396 (1992)
13) M. Shigefuji, Y. Tokudome, Materialia (Oxf.), 14, 100879 (2020)
14) Y. Sugahara, M. Komorisono, M. Kuwajima, S. Yoshikawa, S. Yoshida, K. Maeda, Biosci. Biotechnol. Biochem., 86, 1240–1246 (2022)
15) C. Ni, Z. Zhang, Y. Wang, Z. Zhang, X. Guo, H. Lv, J. Control. Release, 357, 432–443 (2023)
16) P. Ghasemiyeh, S. Mohammadi-Samani, Drug Des. Devel. Ther., 14, 3271–3289 (2020)
17) T. Koike, N. Nakashima, H. Okumura, H. Okumira, J. Soc. Cosmet. Chem. Jpn., 45, 14–21 (2011)
18) I. Hatta, N. Ohta, H. Nakazawa, Pharmaceutics, 9, 26 (2017)
19) J.R. Bow, Y. Sonoki, M. Uchiyama, R.H. Dauskardt, Biochem. Biophys. Rep., 28, 101134 (2021)
20) V.V. Vanessa, W.S.L.W.A. Kammal, Z.W. Lai, K.N. How, Cosmetics, 5, 75 (2022)
21) T. Czarnowicki, D. Malajian, S. Khattri, J.C.D. Rosa, R. Dutt, R. Finney, N. Dhingra, P. Xiangyu, H. Xu, Y.D. Estrada, X. Zheng, P. Gilleaudeau, M.S. Whalen, M.S. Farina, A. Shemer, J.G. Krueger, E.G. Yassky, J. Allergy Clin. Immunol., 137, 1091–1102 (2016)