Regular article

Enhancement of skin permeation of a hydrophilic drug from acryl-based pressure-sensitive adhesive tape

Takamasa Suzuki[a], Tomohiro Aoki[b], Masato Saito[b], Ichiro Hijikuro[c], Shoko Itakura[b], Hiroaki Todo[a,b], Kenji Sugibayashi[a,b]

[a]*Graduate School of Pharmaceutical Sciences and* [b]*Faculty of Pharmacy and Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan;*

[c] *Farnex Inc., Tokyo Institute of Technology Yokohama Venture Plaza, 4259-3 Nagatsuta, Midori-ku, Yokohama 226-8510, Japan*

[*]Corresponding author at: Faculty of Pharmacy and Pharmaceutical Sciences, Josai University, 1-1 Keyakidai, Sakado, Saitama 350-0295, Japan. Tel.: +81-49-271-7367; Fax: +81-49-271-8137.

E-mail address: sugib@josai.ac.jp

Abstract

Purpose Penetration enhancers are necessary to overcome a formidable barrier function of the stratum corneum in the development of topical formulations. Recently, non-lamella liquid crystal (NLLC)-forming lipids such as glycerol monooleate and phytantriol (PHY) are gaining increasing attention as a novel skin permeation enhancer. In the present study, fluorescein sodium (FL-Na) was used as a model hydrophilic drug, and acryl-base pressure-sensitive adhesive (PSA) tape containing NLLC forming lipids, mono-O-(5,9,13-trimethyl-4-tetradecenyl) glycerol ester (MGE) or PHY, was prepared to enhance drug permeation through the skin.

Methods A PSA patch containing FL-Na was prepared by mixing FL-Na entrapped in NLLC and acrylic polymer. FL permeation through excised hairless rat skin, and also human skin, was investigated. Changes in lipid structure, folding/unfolding state of keratin in the stratum corneum, and penetration of MGE into the stratum corneum were investigated using confocal Raman microscopy.

Results Enhanced FL permeation was observed by the application of a PSA patch containing MGE and PHY. Especially, dramatically enhancement effect was confirmed by 15% of MGE contained formulation. Penetration of MGE provided diminished orthorhombic crystal structure and a peak shift of the aliphatic CH_3 vibration of keratin chains toward lower wavenumbers.

Conclusion The present results suggested that the formulation development by adding MGE may be useful for improving the skin permeation of mal-permeable drugs such as hydrophilic drugs.

Keywords; drug-in-adhesive patch; self-assembling lipid; skin permeation enhancer; skin permeation; hydrophilic drug

Abbreviations
NLLC: non-lamella liquid crystal
FL: fluorescein sodium
PSA: pressure-sensitive adhesive
TDDS: transdermal drug delivery system
MGE: mono-O-(5,9,13-trimethyl-4-tetradecenyl) glycerol ester
PHY: phytantriol
MW: molecular weight
PET: polyethylene terephthalate
SAXS: small angle X-ray scattering
ER: enhancement ratio
Q_{8h}: cumulative amount of FL permeated over 8 h

1. Introduction

Transdermal drug delivery systems (TDDSs) can offer several advantages, such as the avoidance of first-pass metabolism in the liver, easy to use by patients and caregivers, prolonged therapeutic action, and the ease of stopping administrating at the onset of side effects (1). However, the stratum corneum, the outmost layer of the epidermis, acts as the primary barrier against the skin permeation of topically applied drugs. Thus, skin permeation enhancers such as sulphoxides, pyrrolidones, alcohols, alkanols, glycols, surfactants and terpenes have been widely used to overcome the skin barrier (2, 3). In addition, emulsions and liposomes composed of immiscible lipids have also been applied to increase the skin permeation of drugs. In recent years, lipids such as 2,3-dihydroxypropyl oleate (glycerol monooleate, GMO) and 3,7,11,15-tetramethyl-1,2,3-hexadecanetriol (phytantriol, PHY) have been the focus of attention as self-assembling amphiphilic molecules that form a non-lamella liquid crystal (NLLC) when exposed to water (4, 5). Constructed NLLCs structures, such as normal discontinuous cubic, normal hexagonal, normal bicontinuous cubic, inverted bicontinuous cubic, inverted hexagonal and inverted discontinuous cubic, exhibit extensive porosity and a remarkably high surface area (6). Because these structures provide a large, complex

internal surface area, a high encapsulation of drugs despite their lipophilicities may be possible compared with liposomes.

Yamada et al. (7) and Kadhum et al. (8, 9) reported that the skin permeation of hydrophilic drugs was dramatically improved by application entrapped in NLLC gels, suggesting that NLLC gels may be a promising formulation to improve the permeation of mal-absorbable drugs through the skin. In addition, many reports have been published on the skin permeation enhancement effects of NLLC gels and its suspensions (10, 11). Therefore, NLLC formulations may be useful to develop dermatological formulations with high skin permeation enhancement effects.

The amount of drug absorbed into the systemic circulation using TDDSs is associated with the concentration of the applied drug, application area, and formulation. Among the pharmaceutical dosage forms used in TDDSs, patches (i.e., poultices and tapes) are possible to apply quantitative doses. Hydrophilic polymers, such as polyacrylic acid polymer, and lipophilic polymers, such as acrylic polymer, are widely used for fabricating poultices and PSA patches, respectively. The physicochemical properties of most, but not all, of the marketed drugs used in patches, should normally be moderately lipophilic (log *K*o/w range from 1 to 5), have a low molecular weight (MW < 500 Da), and a low melting point (MP < 200°C) (12). Few studies have been reported to develop tape formulations

that provide enhanced skin permeation of hydrophilic drugs due to quite a low solubility of hydrophilic drugs in lipophilic polymers. Therefore, it is unfeasible to develop a patch containing hydrophilic drugs with a lipophilic polymer.

PSA patches are widely used in TDDSs due to their compatibility with drugs. PSA patches containing NLLC-forming lipids may be useful for the preparation of formulations with high skin permeation enhancement effects for hydrophilic drugs. For NLLC-forming lipids, PHY and a novel amphiphilic lipid, mono-O-(5,9,13-trimethyl-4-tetradecenyl) glycerol ester (MGE), were used. An isoprenoid type fatty chain in the MGE structure exhibited a highly self-organized NLLC structure with close packing at the interface over a wide range of temperatures (13, 14).

In the present study, acrylate-based PSA patches containing MGE or PHY were prepared with fluorescein sodium salt (FL-Na) as a model hydrophilic drug with mal-permeation, and skin permeation enhancement effects were investigated to clarify the usefulness of PSA patches containing NLLC-forming lipids to improve the skin permeation of hydrophilic drugs. FL-Na is mal-permeable

2. Materials and methods

2.1. Materials

FL-Na was purchased from Fujifilm Wako Pure Chemical Corporation (Osaka, Japan). MGE was kindly provided by Farnex Inc. (Yokohama, Japan). PHY was purchased from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Duro-tak® 87-5216 (Henkel Japan Ltd., Tokyo, Japan), polyethylene terephthalate (PET) film and silicone-coated PET film (Filmbyna®, each thickness 75 μm and width 200 mm, Fujimori Kogyo Co., Ltd., Tokyo, Japan) were kindly provided by Teikoku Seiyaku Co., ltd (Higashi-Kagawa, Kagawa, Japan). All other reagents and solvents were of reagent grade or HPLC grade and used without further purification. Figure 1 shows the structural formulas of MGE and PHY.

Figure 1

2.2 Skins

Eight-week-old male hairless rats (WBN/lla-Ht) weighting about 180 g were obtained from Ishikawa Laboratory Animals (Saitama, Japan). The rats were housed in a room at 25 ± 2°C and the light cycle 12 h on and 12 h off. The rats were allowed free access to water and diet (Oriental Yeast Co., ltd., Tokyo, Japan). All animal feeding and experiments were approved by the Institutional Animal Care and Use Committee of Josai

University (Sakado, Saitama, Japan). Excised abdominal human skin (donor No. 1; age: 51-year-old, ethnicity: Caucasian, gender: female, thickness: 460 μm, donor No. 2; age: 56-year-old, ethnicity: Caucasian, gender: female, thickness: 426 μm) were purchased from Biopredic International (Rennes, France) through KAC (Kyoto, Japan). The use of excised human skin was approved by the KAC Ethics Committee for human-derived products.

2.3. Preparation of formulations

PSA patch formulations were prepared by mixing NLLC gel formulation or FL-Na solution with Duro-tak (387-2516, acrylic PSA) at 500 rpm for 5 min. using a magnetic stirrer (SW-RS 077, Rika Nikonaga, Tokyo, Japan). Table 1 shows the mixing weight ratio of the prepared PSA patches. An adhesive layer was prepared by spreading the solution onto silicone-coated PET film using a Baker type film applicator (Yasuda Seiki Seisakusho Ltd., Tokyo, Japan) with 1.0 mil (25.4 μm) thickness. The spread adhesive layer (10 cm ×30 cm) was dried for 30 minutes at a temperature of 20 ± 2°C and a humidity of 20 ± 5%, and further in an incubator at a temperature of 32°C and a humidity of 20 ± 2% for 30 minutes. Then, it was laminated with PET film (backing layer) using a print roller (Taniguchi Shoyudo Co., Ltd., Kyoto, Japan).

NLLC gel formulations were prepared by mixing an NLLC-forming lipid (MGE or PHY) and FL-Na solution prepared in pH 7.4 phosphate buffer solution (PB) with two connected microsyringes (volume 0.25 mL, MS-GAN 025, Mitsubishi Gas Chemical Company, Inc., Tokyo, Japan). Mixing was repeated one-hundred times to prepare the NLLC gel formulations. NLLC gel composed of MGE was prepared without heating because it exists in a liquid state at room temperature. On the other hand, NLLC gel composed of PHY was prepared after heating to 100°C for 30 min due to its semi-solid state at room temperature. PSA patches without NLLC gel were also prepared by spreading DURO-tak as a control. All formulations were prepared with a final FL-Na concentration of 1.0 mM. The NLLC gels were abbreviated as $MGE_{3:1}$, $MGE_{1:1}$, $PHY_{3:1}$, and $PHY_{1:1}$; consisting of the NLLC lipid named and the mixing ratio of NLLC-forming lipid and FL solution. No decreased fluorescence intensity derived from FL was detected by applying heat to prepare the formulations containing PHY.

Table 1

2.4. Measurement of adhesive layer thickness

The thickness of the prepared PSA patch formulation was measured using a hand clipper (Synnex Gauge, Teclock Co., Ltd., Okaya, Nagano, Japan). The thickness of the adhesive layer was obtained by subtracting both the thickness of the backing layer and the liner layer from the thickness of the prepared PSA patch. The thickness was measured at the center of the PSA patch and at 7.5 cm intervals from the edges (total of three points for each tape). Triplicate measurements were done for each point.

2.5. Observation of FL-Na distribution in the adhesive layer

FL-Na distribution in the adhesive layer in the prepared PSA patch was observed using a fluorescence microscope (BZ-X700, Keyence Corporation, Osaka, Japan). The objective lens was a CFI Plan Apo λ 2×, the excitation and emission filters were for GFP (OP - 87763 BZ - X filter). The excitation/emission wavelengths and dichroic mirror wavelengths of the filters were 470/40 nm, 525/50 nm, and 495 nm, respectively. The exposure time was set to 1/175 s for PSA patches containing NLLC gel formulation and 1/5 s for PSA patches without NLLC gel.

2.6. *In vitro* release experiments

A PSA patch was attached directly into a horizontal diffusion cell (cell volume: 3 mL, effective release area: 0.95 cm^2). PB was used as the receiver solution. The temperature of the receiver was kept at 32°C, and the receiver solution was continuously stirred with a star-head-type magnetic stirrer at 500 rpm. Aliquots (0.5 mL) were withdrawn from the sampling port for 8 h to determine drug content. After each sampling, the same amount of fresh PB was added to maintain the volume in the receiver. When FL release from the NLLC gel was investigated, a semi-permeable membrane (molecular cut-off; 12,000–16,000, Viskase Companies Inc., Darien, IL, U.S.A.) was mounted between the donor and receiver compartments. The gel formulations (200 μL) were applied on the semi-permeable membrane. The cumulative percentage of FL release was calculated from the applied amount of FL in the formulation.

The drug release profile from the prepared formulation was analyzed by the following eq. (1).

$$Q = kt^n \quad (1)$$

where Q is the cumulative amount of drug released percentage from the skin, k is the release rate constant, t is time after injection, and n is the release exponent. When the release exponent, n, had a value of 0.5, the drug release profile showed Fickian release following the W.I. Higuchi equation (15). When the release exponent had a value greater

than 0.5 ($0.5 < n < 1$), the release behaved as non-Fickian diffusion (16). When the release exponent was $n = 1$, the profile showed zero-order release.

2.7. *In vitro* skin permeation experiment

Hairless rats were anesthetized using an intraperitoneal injection of three types of anesthesia (medetomidine hydrochloride; 0.15 mg/kg, midazolam; 2 mg/kg and butorphanol tartrate; 2.5 mg/kg) and sacrificed by cervical dislocation. Full-thickness skin was excised from the body. Then, 1.0 mL of PB was applied on the stratum corneum side for 1.0 h as hydration. After removal of the applied purified water from the stratum corneum side, the prepared PSA patch or 1.0 mL of 1.0 mM FL-Na solution prepared with PB was applied to the stratum corneum side. The skin was mounted in a Franz-type diffusion cell (cell volume: 6.0 mL, effective permeation area: 1.77 cm^2). PB was used as a receiver solution, and 6.0 mL was applied to the dermis side. The experimental conditions were the same as in release experiment. Sink conditions of FL in the receiver compartment were maintained throughout the experiment.

In the case of *in vitro* skin permeation experiments with human skin, Frozen human skin was thawed at room temperature and mounted in a Franz-type diffusion cell. Two donor skins were cut into 2–3 cm^2 pieces, and the skin was mounted in a Franz-type

diffusion cell at random. Skin impedance was measured using an impedance meter (Asahi Techno Lab. ltd., Yokohama, Japan, alternating current voltage: 5 V, frequency: 12 Hz, measurement range: 0.5–100 kΩ) after 1 h hydration with PB before application of the formulation. Skin with impedance over 10 kΩ·cm^2 was used for the experiment. The other method used the same procedure as for excised rat skin to conduct skin permeation experiments. The cumulative percentage of FL permeated was calculated with the applied amount of FL in the formulation.

2.8. FL determination

FL concentration in the sample solution was measured using a fluorescence spectrophotometer (RF-5300 PC, Shimadzu Corporation, Kyoto, Japan) (excitation wavelength: 485 nm, fluorescence wavelength: 535 nm). The obtained sample from the *in vitro* skin penetration experiments was centrifuged at 15,000 rpm for 5 min, and FL concentration in the supernatant was measured.

2.9. Observation of NLLC structure using small angle X-ray scattering

The NLLC structure in the PSA patch was observed using a Rigaku NANOViewer small angle X-ray scattering (SAXS) system equipped with an X-ray

generator (Akishima, Tokyo, Japan) (Cu Kα radiation, λ = 1.5418 Å) operated at 30 kV and 40 mA. The camera focal length was set to 700 mm. The scatter pattern was acquired on a blue imaging plate for 1 h. The obtained pattern was analyzed using a Rigaku NANO-Solver program. All processes related to the measurement operation were conducted by a qualified researcher at Kanazawa University (Kanazawa, Ishikawa, Japan). Crystalline interplanar spacing, d, was determined, in accordance with the Bragg equation.

2.10. Confocal Raman microscopy

Neat MGE, $MGE_{1:1}$, 1% l-menthol in 40% EtOH aq, and 40% EtOH aq were applied to excised hairless rat skin set in a Franz type diffusion cell for 12 h at 32°C. In the present study, l-menthol and EtOH are used as positive controls to affect the lipid structure and keratin in the stratum corneum. Penetration of MGE into the stratum corneum and change in ordered skin structure were investigated using a confocal Raman spectrometer (Model 3510, RiverD International B.V., Rotterdam, The Netherlands). Measurement with Raman spectroscopy was performed at the headquarters of Integral Co., Ltd. (Tokyo, Japan). The measured fingerprint area (400–1800 cm^{-1}) and high wavenumber area (2500–4000 cm^{-1}) were selected in the present study. A 785 nm laser (20 mW) and a 671 nm laser (17 mW) were used for analysis in the fingerprint region and high wavenumber

region, respectively. The exposure time for each measurement was 5 s in the fingerprint region and 1 s in the high wavenumber region. Raman spectra were recorded every 2 μm depth from the skin surface. The acquired data at a 4-μm depth from the skin surface were analyzed using Skintools (RiverD International B. V.). Fitting was performed according to Gaussian–Lorentzian functions after subtracting the baseline. The spatial depth resolution was ≤5 μm and the spectral resolution was 2 cm^{-1}, respectively. Three to four samples were analyzed to obtain profile for each experiment.

2.11. Statistical analysis

The statistical significance of differences in data for FL release, FL permeation, and Raman spectra were evaluated using one-way analysis of variance (ANOVA) followed by the Tukey–Kramer post-hoc test. The significance level was set at $p < 0.05$.

3. Results

3.1. Observation of FL distribution in the adhesive layer

Figures 2 shows visible light (a-c) and fluorescence photographs (i-iii) of prepared PSA patches, respectively. PSA patches without MGE or PHY displayed a non-uniform appearance; areas where a non-adhesive layer lying on the PET film was

confirmed (Fig. 2a). On the other hand, PSA tape containing MGE (Fig. 2b) and PHY (Fig. 2c) ($TD_{80}M_{10}$ and $TD_{80}P_{10}$, respectively) showed a homogeneous appearance, and yellow color derived from the FL distribution was uniformly observed. Homogeneous appearance was also observed in the other PSA tapes containing MGE and PHY (data not shown, $TD_{80}M_{15}$ and $TD_{80}P_{15}$). The thicknesses of the PSA patch with MGE and PHY are shown in Table 2.

PSA patches containing MGE and PHY were successfully prepared when the acrylic PSA composition was more than 80% (data not shown for the observation data of patches with less than 80% acrylic polymer). Thus, in the following experiments, $TD_{80}M_{10}$, $TD_{80}M_{15}$, $TD_{80}P_{10}$ and $TD_{80}P_{15}$ were used to observe skin permeation enhancement effects.

Figure 2

Table 2

3.2. FL release from PSA patches and gels

Figure 3a shows the FL release profile from PSA patches. Almost 100% of FL-Na was released from the patches by 8 h. The highest FL release rate was observed in

$TD_{80}M_{15}$ compared with the other patches. When the release rate was compared with patches containing the same NLLC-forming lipid, the FL release rate was decreased with a decrease in the NLLC lipid content in the patch.

In addition, FL release was also investigated with gel formulations that were used in the process of patch preparation (Figure 3b). Unlike FL release from the patch, the percentage of the cumulative amount of FL release did not reach close to 100% in the experimental period. FL release from gels was decreased with increasing amounts of MGE or PHY in the gel formulation independent of the kind of NLLC-forming lipid. Table 3 shows calculated *K* and *n* values for FL release from the gels and patches. The *n* value of prepared patches was almost n=0.5, whereas the *n* value obtained from $MGE_{3:1}$ and $PHY_{3:1}$ were larger than n=0.5.

Figure 3

Table 3

3.3. FL permeation from PSA patches and gels

Figure 4a shows FL permeation profiles through hairless rat skin after topical application of the patches. The cumulative amount of FL permeated over 8 h (Q_{8h}) from

PSA patches containing MGE or PHY was significantly higher than that from FL-Na solution. Higher FL permeation was observed in the following order; $TD_{80}M_{15} > TD_{80}P_{15} \approx TD_{80}P_{10} > TD_{80}M_{10} >$ FL-Na solution. When skin permeation was compared between the patches containing MGE or PHY, significantly higher FL permeation ($p<0.05$) was observed in $TD_{80}M_{15}$ compared with $TD_{80}M_{10}$. On the other hand, almost the same skin permeation profile was observed in PHY-containing patches ($TD_{80}P_{10}$ and $TD_{80}P_{15}$).

When the enhancement ratio (ER) calculated with Q_{8h}, ER values for $TD_{80}M_{15}$, $TD_{80}M_{10}$, $TD_{80}P_{15}$ and $TD_{80}P_{10}$ were about 65-fold, 15-fold, 23-fold, and 21-fold, respectively, compared with the FL-Na solution. In addition to skin permeation experiments with hairless rat skin, the ERs with $TD_{80}M_{15}$ and $TD_{80}P_{15}$ were evaluated with excised human skin (Figure 4b). About 50- and 7-fold ERs were observed with $TD_{80}M_{15}$ and $TD_{80}P_{15}$ compared with FL-Na solution, respectively.

Figure 5 shows the FL permeation from NLLC gels composed of MGE ($MGE_{1:1}$ and $MGE_{3:1}$) or PHY ($PHY_{1:1}$ and $PHY_{3:1}$). Only $MGE_{1:1}$ showed a higher Q_{8h} compared with the FL solution, whereas the other formulations ($PHY_{3:1}$, $PHY_{1:1}$, and $MGE_{3:1}$) displayed a lower Q_{8h}.

Figures 4 and 5

3.4. Confirmation of the construction of NLLC structures

Figure 6 shows SAXS analysis results for the NLLC gels and PSA patches, and the spacing lattices are summarized in Table 4. NLLC gels composed of MGE exhibited an H_2 structure independent of the mixing ratio of MGE and water. NLLC gels composed of PHY exhibited a *Pn3m* structure. When these NLLC gels were mixed with acrylic PSA, the H_2 and *Pn3m* structures were diminished according to the SAXS analysis.

Figure 6

Table 4

3.5. Skin structure analysis using confocal Raman microscopy

Figure 7a shows a Raman spectrum of skin in the fingerprint region at a depth of 6 μm from the skin surface. The peak around 1420 cm^{-1} indicating orthorhombic packing and the peaks around 1440 and 1460 cm^{-1} representing hexagonal packing were clearly observed at a depth of 6 μm from the skin surface in non-treated skin. When *l*-menthol in 40% EtOH solution was applied, peak shifts around 1440 and 1460 cm^{-1} were clearly observed. The peak around 1420 cm^{-1} disappeared after the application MGE, although

345 no peak shifts around 1440 and 1460 cm^{-1} were confirmed. A peak shift and disappearance at around 1420 cm^{-1} was also observed after the application of NLLC gel.

Figure 7b shows the Raman spectra of neat MGE, non-treated skin, $MGE_{1:1}$-treated skin and *l*-menthol-treated skin in the wavelength range from 1600 to 1700 cm^{-1}. The Raman spectra of neat MGE was compared with rat skin, and a distinctive peak
350 related to MGE was observed around 1675 cm^{-1}. The peak around 1675 cm^{-1} was also detected in MGE- -treated skins, whereas no peak was detected around this wavenumber after the application of the other formulations.

Figure 8 shows the three-dimensional keratin structure in the skin. The peak shift was detected after the application of 40% EtOH. The peak derived from three-
355 dimensional keratin shifted significantly after the application of MGE and $MGE_{1:1}$. A peak shift was also detected after the application of 40% EtOH.

Figures 7 and 8

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4. Discussion

Polymers widely used in PSA patches include polyisobutylenes, silicones, and polyacrylates. These have lipophilic domains in their structures to achieve good adhesive performance. It is necessary to develop hydrophilic PSAs that are capable of dissolving a broader range of drugs with good skin adhesion. Hydrocolloids are used to prepare hydrophilic PSAs that acquire adhesive properties through their water content. Modifying polymer chains to obtain hydrophilic properties may be another method to prepare PSA patches that are capable of containing hydrophilic drugs, but this may be challenging because polymer chains with hydrophilic properties have weaker adhesion properties due to an increase in their glass-transition temperature (17). In the present experiment, a commercially available acryl-based polymer containing a hydrophilic model drug, FL-Na, was successfully prepared by blending MGE or PHY in the formulation. Furthermore, the skin permeation enhancement effect from gel consisted of NLLC-forming lipid was also investigated to clarify the effect of the NLLC structure on the skin permeation of hydrophilic drugs.

In vitro drug release from topical formulations generally shows a very close relationship with its skin permeation profile (18), and analysis of the drug release profile is helpful in understanding the presence of a drug in the formulation. FL release from gel formulations composed of MGE was much slower than that from PSA patches. When the

FL release profiles were compared among patches, the FL release rate increased with an increase in the amount of NLLC-forming lipid in the PSA patch. Aqueous channel (interplanar spacing) size and tortuosity of the aqueous channels in the NLLC structure should be related to the drug release rate (19). In addition, constructing structure also affect the release rate of an entrapped drug. The release rate was faster in the order lamellar phases, cubic phases, hexagonal phases, and micellar cubic (20, 21).

Conductivity of electricity was measured through prepared PSA patches to confirm the existence of a continuous phase consisting NLLC-forming lipid in the adhesive layer. Because no electroconductivity was detected through the backing layer of the PET film, the measurement was performed after peeling off the PET films. To remove the PET film smoothly, PSA patches were prepared by spreading an acrylic polymer on the PET film, on which a dialysis membrane was laid. Electroconductivity was confirmed in PSA patches containing MGE and PHY despite of their concentrations, whereas no electroconductivity was detected through PSA patches without NLLC-forming lipids (data not shown). This result indicated that a continuous structure such as fibrous micelles might be present in the PSA layer, although the NLLC structure was not detected by SAXS analysis. The following results also supported the existence of a continuous structure in the PSA layer; FL release profiles from PSA patches containing MGE or PHY

were subjected to the Higuchi equation (n=0.5), and a homogeneous FL-Na distribution in the PSA patches was confirmed by microscopic observation.

GMO, known as a self-NLLC structure-organizing lipid, like MGE and PHY, has been used as a lipophilic solvent to enhance the skin permeation of drugs. Gupta et al. reported the enhancement mechanism of GMO (applied concentrations: 1%, 3%, and 5% w/w) and reveled that GMO can form small clusters in the lipid layer in the stratum corneum (22). As described above, no clear peaks derived from the NLLC structure were detected in the PSA patch according to SAXS analysis. Therefore, we assumed that a high skin permeation enhancement effect of FL with $TD_{80}M_{15}$ might be related to MGE penetration into the stratum corneum from the patch, although the existing state of MGE in the adhesive layer was unclear.

When MGE or PHY, FL-Na solution, and 1, 3-butyen glycol (BG) were mixed at a volume ratio of 35:35:30, respectively, the NLLC structure was not detected by SAXS analysis (data not shown). To confirm the skin permeation enhancement effect with or without constructing NLLC structure, an *in vitro* skin permeation experiment was performed with BG mixed formulations. Figure 9 shows the skin permeation profile of FL through hairless rat skin after the application of the BG mixed formulations. MGE_{BG} was BG-containing formulations (mixing ratio of MGE, FL-Na solution, and BG =

35:35:30). BG is known as a skin permeation enhancer, and FL-Na solution containing 30% BG was also applied as a comparison. No differences were observed in the FL permeation profiles after the application of FL-Na solution and its solution containing 30% BG. However, MGE_{BG} showed higher skin permeations compared with $MGE_{1:1}$. This result suggested that the penetration of MGE itself may be related to the skin permeation enhancement effect of FL.

Figure 9

Raman microscopic observation was performed to clarify the skin permeation enhancement effect by MGE itself at a depth of 6 μm from the skin surface because the peaks representing the orthorhombic crystal structure observed at a depth of 6 μm from the skin surface was diminished by the application of neat MGE. Furthermore, the Raman peak at around 2930 cm^{-1} originating from aliphatic CH_3 vibration in keratin chains was shifted toward lower wavenumbers (23). Its peak was associated with the exposure of CH_3-side chains to the surrounding water molecules, and a lower position of the peak corresponds to the keratin folding state (24). Keratin lies in the most folded state in the shallow layer in the stratum corneum and maximal unfolding of keratin is confirmed at 60–70% stratum corneum depth. Increased folding of keratin might be related to reducing

hydrogen bonding sites at shallow depth in the stratum corneum (25). This change might be a reason for the increase in FL skin permeation by decreasing FL-keratin interactions.

Changes in orthorhombic and hexagonal structures in the lipid structure have been investigated after the application of penetration enhancers. Many reports have been published that decrease the orthorhombic packing structure and a hexagonal packing structure by penetration enhancers to increase the skin permeation of drugs. In the present study, *l*-menthol and ethanol were selected as positive controls that affect the lipid structure and the three-dimensional structure of keratin in the stratum corneum (26, 27). The changes in the lipid structure and three-dimensional structure were also observed by the application of MGE suggested that these alternations may be reasons for the high skin permeation enhancement effect with PSA patches containing MGE. Although Raman microscopic observation was conducted with hairless rat skin, almost the same alternations can occur in human skin because a higher skin permeation enhancement was also confirmed even when human skin was used. Further experiments should be performed to reveal MGE states in the adhesive layer and the distribution of MGE into the stratum corneum.

An MGE-derived peak was clearly confirmed at a depth of 6 μm from the skin surface when neat MGE was applied, whereas the peak was slightly observed after the

application of NLLC gel composed of MGE. The diffusion of substances in the skin is theoretically expressed by Fick's second law of diffusion (28). The diffusion coefficient is proportional to the reciprocal of the cubic root of the molecular weight (molecular volume), which is given by the Stokes–Einstein equation (29). Because the molecular volume of the assembled structure composed of MGE was larger than that of MGE itself, these results suggested that the MGE state (e.g. mono-dispersion state or NLLC constructed state) in the formulation or the state during the penetration process through the stratum corneum can greatly affect the skin permeation enhancement of FL. A higher MGE concentration in the formulation resulted in higher skin distribution. Thus, the skin permeation enhancement effect with $TD_{80}M_{15}$ was much higher than that with $TD_{80}M_{10}$.

The MGE distribution and change in lipid structure at deeper depths (> 6 μm) were also investigated using confocal Raman microscopy. However, the obtained weak peaks at deeper sites (data not shown) and the spatial depth resolution (≤ 5 μm) made the analysis inconclusive. In addition, in the present study, no observations by confocal Raman microscopy were performed after PSA patch application. This is because that peak obtained from the applied PSA patches interfered with the MGE and structural change derivative peaks. In addition, shallow layers of the stratum corneum might be stripped by

the removal process of the patch from the site of application, and the residue of the patch component on the skin may affect the obtained results after the removal process.

Skin occlusive effect is well known to enhance drug permeation. In the present study, the occlusive effect by applying the patch was not evaluated. Further experiments should be done to evaluate the effect of skin occlusion on the FL permeation after application of patch formulation containing MGE or PHY.

Conclusion

A hydrophilic drug, FL-Na, entrapped in a PSA patch was successfully prepared by adding NLLC-forming lipids such as MGE and PHY in an acryl-based adhesive layer. In particular, PSA patches containing MGE showed significantly improved skin permeation of FL even in excised human skin. Although NLLC gel constructed using MGE in the PSA tape was not revealed, partitioning of MGE into the stratum corneum induced structural changes in the lipid layer of the orthorhombic packing and three-dimensional structure of keratin. Although further experiments should be performed to reveal more detail on the mechanism and to evaluate the adhesive properties of the patch (180° peel test, 90° peel test, self-adhesion test, and probe tack test), the present results

485 suggested that topical application of PSA patches with MGE may be useful to improve the skin permeation of mal-permeable drugs such as hydrophilic drugs.

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Figure captions

Figure 1. Chemical structures of MGE (a) and PHY (b).

Figure 2. Light and fluorescence microscopic images of the prepared patches. (a) $T_{cont.}$, (b) $TD_{70}M_{15}$ and $TD_{80}M_{10}$. i), ii) and iii) show the measurement area 7.5 cm from the left edge of the patch. The area enclosed with a white dotted line shows the observation area. Formulation codes are same as in Table 1.

Figure 3. Cumulative amount of FL released from PSA patches (a) and gels (b). Symbols: (a); □:$TD_{80}P_{15}$, ■: $TD_{80}M_{15}$,○: $TD_{80}P_{10}$, ●: $TD_{80}M_{10}$, (b); □:$PHY_{1:1}$, ■: $MGE_{1:1}$,○: PHY $_{3:1}$, ●: $MGE_{3:1}$. Formulation codes are same as in Table 1. Mean ± S.E (n = 3–4).

Figure 4. Cumulative amount of FL permeated through (a) hairless rat skin or (b) human skin from the PSA patches. Symbols: (a); □:$TD_{80}P_{15}$, ■: $TD_{80}M_{15}$,○: $TD_{80}P_{10}$, ●: $TD_{80}M_{10}$, △: FL-Na solution (b); □:$TD_{80}P_{15}$, ■: $TD_{80}M_{15}$, △: FL-Na solution. Formulation codes are same as in Table 1. Mean ± S.E (n = 4–7). *: $p < 0.05$ compared with Q_{8h} value of FL-Na solution, #: $p < 0.05$ between Q_{8h} values of $TD_{80}M_{15}$ and $TD_{80}M_{10}$.

Figure 5. Cumulative amount of FL permeated through hairless rat skin from the gels.

Symbols: □:$PHY_{1:1}$, ■: $MGE_{1:1}$,○: $PHY_{3:1}$, ●: $MGE_{3:1}$, ▲: FL-Na solution. Formulation codes are same as in Table 1. Mean ± S.E (n = 4–7). *: $p < 0.05$ compared with Q_{8h} value of FL-Na solution.

Figure 6. Small-angle X-ray analysis of PSA patches (a-d) and gels (e-h). Formulation codes are same as in Table 1. (a) $TD_{80}M_{10}$, (b) $TD_{80}M_{15}$, (c) $TP_{80}M_{10}$, (d) $TP_{80}M_{15}$, (e) $MGE_{1:1}$, (f) $MGE_{3:1}$, (g) $PHY_{1:1}$, (h) $PHY_{3:1}$.

Figure 7. Raman spectra in the stratum corneum derived from intercellular lipids (a) and derived from MGE (b) at a depth of 6 μm from the skin surface 12 h after application of the formulations. The peak at 1420 cm^{-1} (vertical solid line) indicates orthorhombic packing and the peaks around 1440 and 1460 cm^{-1} (vertical broken lines) represents hexagonal packing. Lines; solid line in black: Non-treated skin, solid line in gray: MGE-treated applied skin, dotted line in black: $MGE_{1:1}$-treated skin, dotted line in gray: 40% ethanol solution-treated skin, broken line in black: 1% l-menthol in 40% ethanol solution-treated skin, broken line in gray: Neat MGE. Arrow indicates the specific Raman spectra derived from neat MGE.

Figure 8 Change in Raman spectra peak derived from tertiary keratin structure in hairless rat skin at a depth of 6 μm from the skin surface. (a) Non-treated skin, (b) 40%

ethanol-treated skin, (c) MGE-treated skin, (d) $MGE_{1:1}$-treated skin. Each line shows mean ± S.E. (n = 3–4). *: $p < 0.05$ compared with non-treated skin.

Figure 9. Effect of pretreatment with neat MGE and $MGE_{1:1}$ on the skin permeation of FL through hairless rat skin. FL-Na solution was applied 2 h after the pretreatment process. The applied formulation was removed and blotted with a Kim wipe, then 1.0 mL of FL-Na solution (1.0 mM) was applied. Symbols: ○: pretreatment with $MGE_{1:1}$/BG, ●: pretreatment with $MGE_{1:1}$, □: pretreatment with BG, △: pretreatment with PBS. Mean ± S.E (n = 4–+7). The formulation code of $MGE_{1:1}$/BG was prepared as a physical mixture of $MGE_{1:1}$ and BG with a mixing ratio 2:1. *: $p < 0.05$ compared with Q_{8h} value of FL-Na solution.

Table 1 Composition of prepared formulations.

(%)	$T_{cont.}$	$TD_{70}M_{15}$	$TD_{80}M_{10}$	$TD_{80}M_{15}$	$TD_{80}P_{10}$	$TD_{80}P_{15}$
DURO-TAK	90	70	80	80	80	80
MGE	-	15	10	15	-	-
PHY	-	-	-	-	10	15
FL-Na sol.	10	15	10	5	10	5

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Table 2 Thickness of the adhesive layer in the prepared patches.

Area	$T_{cont.}$ (μm)	$TD_{70}M_{15}$ (μm)	$TD_{80}M_{10}$ (μm)	$TD_{80}M_{15}$ (μm)	$TD_{80}P_{10}$ (μm)	$TD_{80}P_{15}$ (μm)
Area 7.5 cm from the left edge	80	20	15.7 ± 2.8	18.0 ± 1.0	16.4 ± 2.0	15.0 ± 1.7
Middle	45	20	15.3 ± 2.2	18.1 ± 0.4	16.6 ± 2.1	16.0 ± 1.6
Area 7.5 cm from the right edge	<1	20	15.3 ± 2.5	16.6 ± 1.7	15.7 ± 1.5	15.3 ± 2.2

Triplicate measurements were conducted for each area. Mean ± S.D. (n=3)

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Table 3 Fickian release parameters for FL release profile from gels and patches

	Formulation	k (h^{-1})	n
Patch formulations	$TD_{80}M_{15}$	58	0.5
	$TD_{80}M_{10}$	33	0.5
	$TD_{80}P_{15}$	59	0.5
	$TD_{80}P_{10}$	43	0.5
Gel formulations	$MGE_{3:1}$	6.0	0.6
	$MGE_{1:1}$	12	0.5
	$PHY_{3:1}$	4.0	0.8
	$PHY_{1:1}$	14	0.5

Table 4 Constructed liquid crystal structure and interplanar spacing of the formulations

	Formulation	Liquid crystal structure	*d* (nm)
Patch formulations	$TD_{80}M_{15}$	---	---
	$TD_{80}M_{10}$	---	---
	$TD_{80}P_{15}$	---	---
	$TD_{80}P_{10}$	---	---
Gel formulations	$MGE_{3:1}$	*H2*	4.27
	$MGE_{1:1}$	*H2*	4.32
	$PHY_{3:1}$	*Pn3m*	4.60
	$PHY_{1:1}$	*Pn3m*	4.77

The relative positions of the Bragg peaks are in a ratio of $\sqrt{2}:\sqrt{3}:\sqrt{4}:\sqrt{6}:\sqrt{8}$ showing Pn3m structure. The spacing ratio of $1:\sqrt{3}:\sqrt{4}$ shows H_2 structure. The peak positions were calculated with SAXS data shown in Fig. 6.