Regular Article

Promoting Activity of Terpenes on Skin Permeation of Famotidine

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Famotidine (FMT) is a competitive histamine-2 (H2) receptor antagonist that inhibits gastric acid secretion for the treatment of Gastroesophageal reflux disease. To study the promoting effect and mechanism of terpenes, including *l*-menthol, borneol, and geraniol, as chemical enhancers, FMT was used as a model drug. Attenuated total reflectance-Fourier transform IR spectroscopy (ATR-FTIR) and differential scanning calorimetry (DSC) were used to explore the effects of terpenes on the skin. Hairless mouse skin was mounted on Franz-type diffusion cell, and skin permeation experiment of FMT hydrogel was carried out. The results suggested that the thermodynamic activity influenced the permeability of the drug, and the main mechanism of terpenes to enhance skin permeation of the drug was based on increasing the fluidity of the intercellular lipids. Moreover, it was revealed that *l*-menthol simultaneously relaxed the packing structure and lamellar structure, whereas geraniol had a great influence on the lamellar structure only. Collectively, all terpenes had a promoting effect on skin permeation of FMT, indicating their potential as chemical enhancers to change the microstructure of stratum corneum and improve the permeation of FMT through the skin, and it has great potential to be used in transdermal formulations of FMT.

Key words transdermal drug delivery, famotidine, chemical enhancer, terpene

Introduction

Gastroesophageal reflux disease (GERD) is a digestive condition characterized by partially digested foods, acidic stomach juices, and fluid coming up into the esophagus from the stomach. It may affect individuals of any age, including infants. Heartburn occurs when you get a burning sensation in your chest due to stomach acid going up into the throat. It develops into GERD if it goes on for some time.[1)] Some of the most common symptoms were trouble swallowing or painful swallowing, a feeling of food stuck in the throat, abdominal or chest pain, and blood in the vomit or black and tarry stools.[2)]

Histamine-2 (H2) receptor blockers are also frequently used to relieve the symptoms of GERD.[3)] The frequent exposure to stomach acid can irritate the esophagus and lead to uncomfortable symptoms as described.

Famotidine (FMT) is a competitive H2 receptor antagonist that inhibits gastric acid secretion for the treatment of GERD. It is used *via* orally route associated with gastric acid secretion for GERD patients.[4)] But, FMT is usually administrated as orally, considering symptoms of GERD as mentioned previously, it seems to be better for the GERD patients to take FMT thorough parenteral administration. Moreover, following oral administration, the absorption of FMT was dose-dependent and insufficient. The oral bioavailability ranges from 40–50%, and the C_{max} is reached in 1–4 h post administration. The elimination half-life is approximately 2–4 h, which is expected to increase nonlinearly in patients with decreased renal function.[5–7)] Due to the adverse effects of the central nervous system, longer dosing intervals or reduced doses may be used instead to adjust for the resulting longer elimination half-life of FMT.[8–10)] Intravenous formulations have local adverse effects such as irritation at the injection site; moreover, frequent administration results in low patient compliance. Thus, development of potential dosage form of FMT is required to promote drug absorption as well as improve GERD patient compliance.

Transdermal drug delivery system (TDDS) is an alternative to minimize the limitations associated with oral and parenteral administration of drugs.[11)] At present, TDDS for various drugs such as nicotine patches, estradiol, fentanyl, lidocaine, and rotigotine have been developed and approved by the U.S. Food and Drug Administration.[12,13)] However, the skin, which is the largest tissue in the human body, is a barrier against the extent and rate of drug absorption by TDDS. Stratum corneum is the superficial layer of the epidermis and the primary diffusion barrier of the skin.[14)] Stratum corneum consists of keratinocytes embedded in a lamellar lipid matrix, mainly comprising ceramides (41%), cholesterol (27%), cholesteryl esters (10%), and fatty acids (9%), with a small fraction of cholesterol sulfate (2%).[15,16)] Intercellular, intracellular, and follicular pathways are the three main permeation routes through the stratum corneum.[17)] FMT has a small partition coefficient (log *P*) value, indicating that it is a hydrophilic drug, which prefers to pass through the stratum corneum using the intracellular pathway through the corneocytes.[18)] However, according to its chemical structure and experimental data, water solubility of FMT is very low.[19)] These findings suggest difficult penetration of FMT through stratum corneum, thus indicating the assistance of other means to penetrate. To date, there have been few reports of transdermal studies of FMT. Mehra and Geevarghese[20)] had developed transferosomes as a transdermal delivery system for delivery of famotidine and had optimal permeation flux of 22.4 μg/cm^2/h. But transferosomes are expensive due to its lipid excipients and equipment

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needed, as well as its complex processes. It is therefore urgent to find more convenient and inexpensive ways to promote the transdermal efficiency of FMT.

Transdermal absorption enhancers are most commonly used to help drugs penetrate the skin. Terpenes are frequently used transdermal absorption enhancer; they act on the polar hydrophilic end of the lipid bilayer, destroy the hydrogen bonding network, increase lipid fluidity, and extract lipids to form new polar channels.[21] In this study, we evaluated the effect of three terpenes in promoting the skin penetration of FMT. At present, many studies have confirmed the efficacy and safety of *l*-menthol as a transdermal permeation enhancer. And in some report, novel terpenes borneol and geraniol has shown excellent transdermal promoting effect of drugs. Thus, borneol and geraniol were selected as transdermal absorption enhancers in the present study. Borneol is a traditional Chinese medicine obtained from castoreum and is used in the Chinese plaster. Yang *et al.*[22] investigated the safety and promoting effect of borneolum from three sources on transdermal permeation of compounds with different octanol-water partition coefficient values and molecular weights. Here, the mechanism needs to be investigated further. Geraniol is the primary component of rose oil and possesses a good smell. Its effect as a penetration enhancer for transdermal drug delivery has attracted the attention of many researchers. The percutaneous absorption of geraniol from an oil-in-water emulsion was studied by Doan *et al.*[23] However, there is not much research on their mechanism for promoting transdermal, especially for geraniol. Thus, being a sesquiterpene, in contrast to the cyclic terpenes *l*-menthol and borneol, geraniol has been selected in the present study to examine the penetration effect of terpenes having different structures on stratum corneum.

Collectively, in this study, we used novel compound borneol and geraniol as skin permeation enhancers for developing a novel FMT transdermal system, and to compare the results with commonly used terpene *l*-menthol. Furthermore, elucidated the mechanism of these terpenes by differential scanning calorimetry (DSC) and attenuated total reflectance-Fourier transform IR spectroscopy (ATR-FTIR) measurements.

Experimental

Materials FMT, hydroxypropyl cellulose, hydroxyethyl cellulose, isopropyl alcohol (IPA), *l*-menthol, borneol, and geraniol were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan). All other chemicals used in this study were of reagent grade.

Skin Permeation Study

Preparation of Hydrogel

Hydroxypropyl cellulose and hydroxyethyl cellulose (1% (w/w)) and FMT (3% (w/w)) were dispersed in purified water and allowed to swell overnight to obtain the hydrogel formulation base. Different terpenes (3% (w/w)) dissolved in 30% IPA were added dropwise to the hydrogel formulation base while stirring overnight to obtain a homogeneous FMT hydrogel. Table 1 shows different formulations of FMT hydrogel.

In Vitro Skin Permeation Study

Hairless mouse skin (Labskin® Hos: HR-1, 7-weeks-old male, Sankyo Labo Service Corp., Tokyo, Japan) was mounted on a Franz-type diffusion cell. The donor cell was fixed facing towards the stratum corneum side, while the receiver cell faced towards the dermis side. Phosphate-buffered saline (PBS; pH 7.4; 16 mL) containing 1% acetic acid was heated in a water bath to 32 °C and added to the receiver cell. The prepared hydrogel (1 mL) was applied to the donor cell, and the top of the cell was covered with parafilm. Aliquots containing 8 mL of the receiver solution were collected at predetermined time intervals (every 2 h for 24 h) and replaced immediately; FMT concentration in the samples was measured by HPLC.

Preparation of Stratum Corneum Sheet Stratum corneum was separated from the excised skin of the abdominal region of hairless mouse (Labskin® Hos: HR-1, 7-weeks-old male, Sankyo Labo Service Corp.) by digestion with 0.1% (w/w) trypsin in PBS (pH 7.4) at 37 °C for 24 h. The separated stratum corneum was rinsed with purified water and dried *in vacuo*. The stratum corneum was incubated in different chemical enhancer-IPA solutions for 1 h at 32 °C and dried at room temperature of about 25 °C until it reached an acceptable predetermined weight (125% of the pretreatment weight). Table 2 shows various formulations of the terpene-IPA solutions. Procedures involving animals and their care complied with the regulations of the Committee on Ethics in the Care and Use of Laboratory Animals of Hoshi University.

DSC Measurements Thermal analysis of stratum corneum was performed using DSC-60 Plus (Shimadzu Corp., Kyoto, Japan). Five milligrams of stratum corneum was placed on aluminum seal pans, and analysis was performed from 20 to 140 °C at a heating rate of 10 °C/min. All experiments were performed in triplicates. Thermograms were analyzed using the ORIGIN® 2016 software (LightStone Corp., Tokyo, Japan).

Attenuated Total Reflectance-Fourier Transform IR (ATR-FTIR) Measurements ATR-FTIR measurements were carried out using FTIR-4200 spectrometer fitted with ATR PRO (JASCO International Co., Ltd., Tokyo, Japan). Stratum corneum sheets were placed on a sample stage containing ZnSe crystal. The contact area with the sample was 1.5 mm^2. The incident angle of the IR radiation was 45°, and the radiation penetrated the sample to a depth of 1–2 μm. All spectra were obtained as an average of 256 scans recorded from 4000 to 600 cm^{-1} at a resolution of 2 cm^{-1}. The sample stage temperature was increased at a heating rate of 1.0 °C/min to the target temperature ±0.3 °C. Spectra were acquired from 20 to 100 °C at 5 °C increments. All experiments were performed in triplicates. Spectral analysis was performed

Table 1. Formulations of FMT Hydrogel (%)

	FMT	IPA	HPC	HEC	Chemical enhancers			Water
					l-Menthol	Borneol	Geraniol	
Control	3	30	1	1	—	—	—	65
l-Menthol	3	30	1	1	3	—	—	62
Borneol	3	30	1	1	—	3	—	62
Geraniol	3	30	1	1	—	—	3	62

Table 2. Formulations of Terpenes-IPA Solution (%)

	IPA	*l*-Menthol	Borneol	Geraniol	Water
Control	30	—	—	—	70
l-Menthol	30	3	—	—	67
Borneol	30	—	3	—	67
Geraniol	30	—	—	3	67

using the ORIGIN® 2016 software (LightStone Corp.). All parameters were determined by fitting a Gaussian function to the IR data.

HPLC Analysis FMT concentration in the samples was determined using HPLC (Shimadzu Corp.) equipped with an auto sampler, an UV detector, and a C18 column (YMC-Pack ODS-A; $150 \times 4.6\,mm^2$ I.D.). Methanol: 1% acetic acid (30 : 70, v/v) was used as the mobile phase at a flow rate of 1 mL/min. The injection volume was 20 μL, and scanning was performed at 267 nm at room temperature of about 25 °C. A calibration curve (peak *versus* drug concentration) was constructed by running standard FMT solutions in methanol for every series of chromatography samples. Calibration curves were observed to be linear in the range of 25–100 μg/mL.

Data Analysis and Statistical Evaluation The cumulative amount of drug permeating through the skin from FMT hydrogel was plotted as a function of time. The cumulative drug permeation (Q_n) was calculated using the following Eq. (1):

$$Q_n = \left(C_n \times V_0 + \sum_{i=1}^{n-1} ci \times Vi/S \right) \quad (1)$$

where C_n is FMT concentration of the extracted receiver medium, V_0 and V_i represent the volume of the receiver medium and sample, ci is FMT concentration in the sample solution, and S is the effective penetration area ($S = 2.01\,cm^2$).

The steady-state flux (J_{ss}) was calculated using the following Eq. (2) at a steady state:

$$J_{ss} = \frac{\Delta Q_t}{\Delta T \times S} \quad (2)$$

$\triangle Q_t$ is the change in the quantity of the drug (μg) passing through the skin into the receptor compartment. The flux was calculated from the slope of the linear portion of the curve. The lag time was determined by extrapolating the linear portion of the curve to the abscissa, where J_{ss} is the steady-state skin flux in $\mu g/cm^2/h$, S is the active diffusion area in cm^2, $\triangle t$ is the change in time, and T_{lag} is the lag time in h.

Statistical Analysis One-way ANOVA was used for statistical analysis of the results. Statistical significance was set at $p < 0.05$.

Results

Skin Permeation of FMT Figure 1 shows skin permeation of FMT. The cumulative permeation curve of FMT showed a similar trend in both 20 and 30% IPA solutions. FMT in 30% IPA solution showed slightly better permeability than that of FMT in 20% IPA solution. The solubility data of FMT in 20 and 30% IPA solutions is shown in Table 3, which indicates almost the same solubility of FMT, with 13.65 μg/mL in 20% IPA solution and 12.12 μg/mL in 30% IPA solution. Area under the curve $(AUC)_{0-12h}$ and flux were calculated using the trapezoidal rule from the cumulative permeation *versus* time curve and are shown in Table 4. Differences in the values of AUC_{0-12h} among different formulations were insignificant. Moreover, differences in the value of permeability coefficient of the saturated hydrogels based on the flux and solubility were observed to be insignificant.

Effects of Different Terpenes on FMT Skin Permeation The cumulative amount *versus* time curve of *in vitro* skin permeation experiments using *l*-menthol, borneol, and geraniol is shown in Fig. 2.

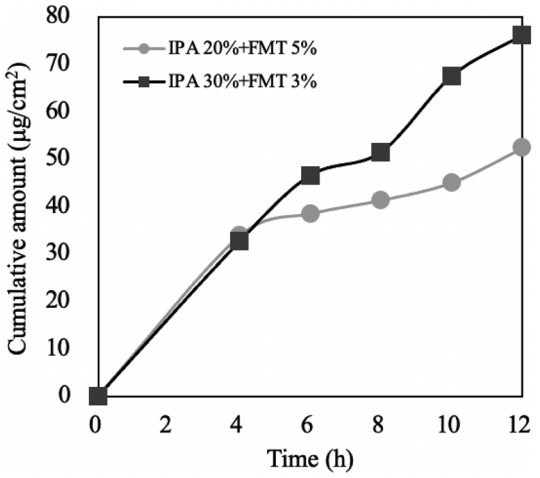

Fig. 1. FMT Permeation across the Mice Skin between Different Concentration of IPA and FMT

Each point represents the mean ± standard deviation (S.D.) ($n = 3$).

Table 3. FMT Solubility in 20% IPA Solution and 30% IPA Solution

	Solubility (μg/mL)
IPA 20%	13.65 ± 2.60
IPA 30%	12.12 ± 0.16

These results confirmed that all terpenes had a promoting effect on FMT skin permeation, and the flux of FMT with terpenes was significantly higher than that of the control group in the order of *l*-menthol > geraniol > borneol. The cumulative permeation of FMT reached a steady state after a certain lag time, and it was observed that FMT continuously permeated through the skin. The cumulative permeation of the formulation containing geraniol showed the highest value, which was 13-times higher than that of the formulation containing IPA only with the lowest permeation.

Furthermore, the steady-state flux and lag time, which used to evaluate skin permeability can be calculated according to the Fick's law of diffusion (Eq. (2)) from the cumulative permeation *versus* time curve shown in Fig. 3. Collectively, all terpenes increased FMT transdermal permeation rate compared to the control, and the extent of the increase was proportional to the concentration; however, *l*-menthol showed the highest steady-state flux. In addition, the lag time after using with borneol is shortest, this was followed by *l*-menthol and geraniol. The control group had the longest lag time.

Effects of Terpenes on DSC Profile of Stratum Corneum After applying different terpenes on stratum corneum, the peak position of intercellular lipids was observed, and the endothermic curve of 30% IPA solution is shown in Fig. 4. The endothermic peak was observed at approximately 40, 50, and 70 °C. The endothermic curve was then analyzed for second-order differentiation, and the phase transition temperature was determined. The changes in phase transition temperature due to the application of terpenes are shown in Fig. 5. The results indicated that the phase transition temperature of all chemical enhancers applied to stratum corneum was lower that of the control group. The phase transition temperature of *l*-menthol

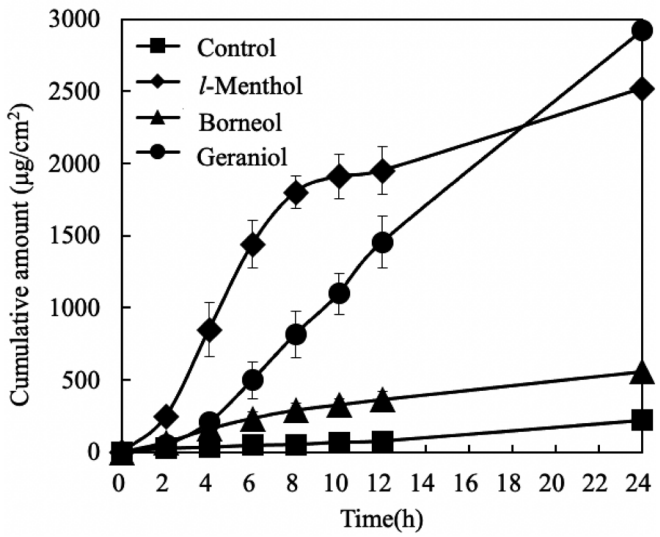

Fig. 2. FMT Permeation across the Mice Skin

Each point represents the mean ± S.D. ($n = 3$). ◆: *l*-Menthol, ▲: Borneol, ●: Geraniol, ■: Control.

and geraniol was observed at 40, 50, and 70 °C, and at 50 and 70 °C, respectively. Among the three terpenes, *l*-menthol significantly reduced the three typical phase transition temperatures in stratum corneum. Geraniol and borneol did not show a significantly low phase transition temperature at 40 °C, but the phase transition temperature of geraniol at approximately 50 °C was the largest change among the three chemical enhancers. For borneol, there were only slight changes at 50 °C, and it showed a typical temperature change at approximately 70 °C compared with the control group and the other two enhancers.

Effects of Terpenes on ATR-FTIR Profile of Stratum Corneum The mechanism of permeation enhancement of terpenes on transdermal delivery of FMT could be investigated through various spectral shifts. As shown in Fig. 6, main peaks appeared near 2800–3000 cm^{-1} IR absorption spectra of hairless mouse skin after treatment with different terpenes.

Figure 6 shows two obvious peaks of asymmetric CH_2 stretching vibrations and symmetric CH_2 stretching vibrations at approximately 2920 and 2850 cm^{-1}, respectively. It can be observed that all terpenes slightly shifted these two peaks to higher wavenumbers. In addition to the bands originating from the hydrophobic groups [$\nu_{as(CH2)}$ and $\nu_{s(CH2)}$], the bands of CH_3 asymmetric stretching vibrations also revealed a simultaneous shift to a lower wavenumber and broadening for borneol and to a higher wavenumber for geraniol.

Subsequently, the peak position, the wavenumber of the top of the peak, was calculated by subjecting the ATR-FTIR spectrum to the second derivative of the profiles, and then changes in the peak position of the CH_2 asymmetric stretching vibration were analyzed. As shown in Fig. 7, at 32 °C, which represents the skin surface temperature, the CH_2 symmetric and asymmetric stretching vibration absorption peaks were shifted to a higher wavenumber, compared with the control group. At this temperature, *l*-menthol and borneol revealed a similar trend of wavenumber shifts, while geraniol showed slight difference compared the control group in asymmetric CH_2 stretching vibrations and the largest change in symmetric CH_2 stretching vibration. Figure 8 shows a greater shift to a higher wavenumber by *l*-menthol and geraniol, whereas borneol exhibited a smaller

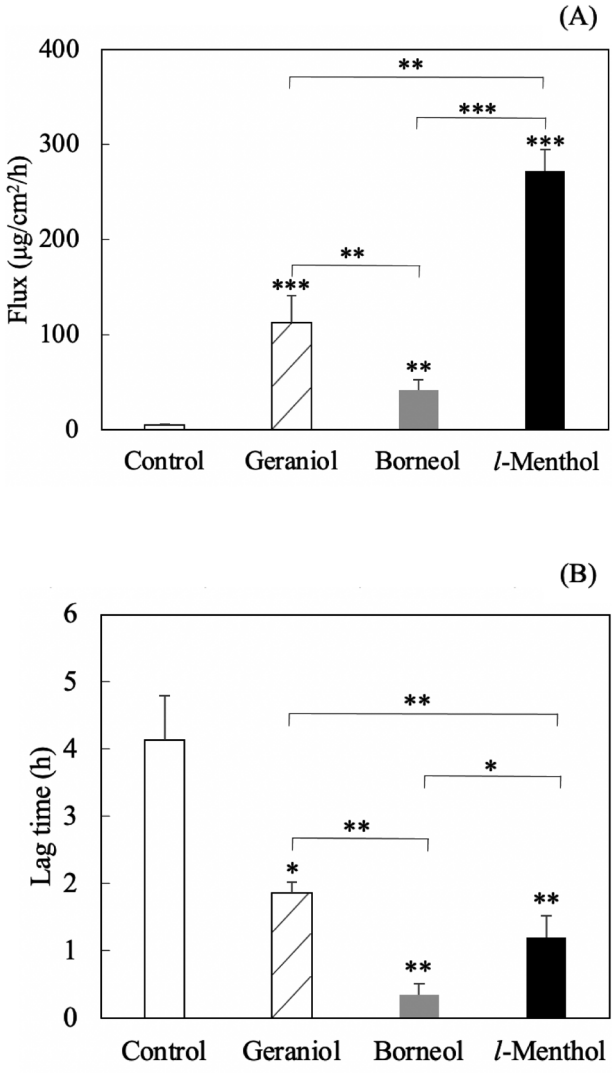

Fig. 3. (A) Steady-State Flux and (B) Lag Time of FMT through Hairless Mouse Skin

Each point represents the mean ± S.D. ($n = 3$). $^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* Control. Control was SC treated with only IPA 30% formulation.

shift to a higher wavenumber. Moreover, at a low temperature of 32 °C, geraniol did not show a large shift to a higher wavenumber of the asymmetric CH_2 stretching vibration, but magnitude of the red shift of geraniol was obviously higher at high temperatures than that of the other two terpenes.

Discussion

Skin Permeation of FMT from Different Formulation Figure 1 shows the skin permeation results of the two samples with different concentrations of IPA (20 and 30%) and FMT (5 and 3%). It was observed that increasing the IPA concentration and simultaneously decreasing the drug concentration resulted in the cumulative amount of FMT *versus* time curves of almost similar shape. As shown in Table 4, the difference between AUC_{0-12h} and flux of the two formulations was insignificant. FMT is very slightly soluble in water, slightly soluble in methanol, and freely soluble in glacial acetic acid.[23] The solubility of FMT in IPA solution was relatively low, indicating that both solutions (5% FMT in 20% IPA solution and 3% FMT in 30% IPA) were saturated. This result indicates that

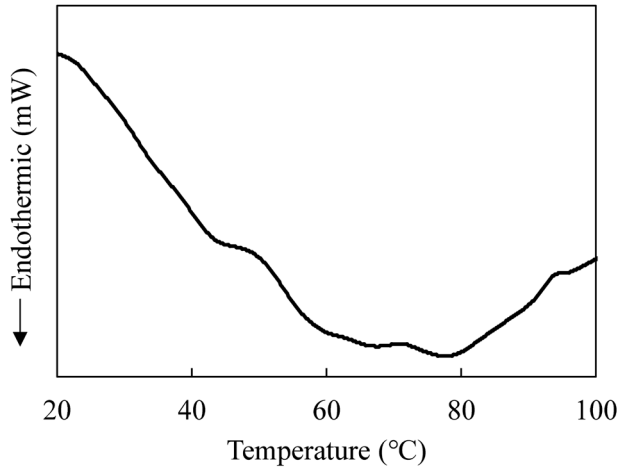

Fig. 4. The Endothermic DSC Curve of 30% IPA Solution

IPA had no significant effect on drug solubility. Based on the solubility of FMT in IPA solution (Table 3), it was considered that the thermodynamic activity of FMT in the hydrogel was maximized. Therefore, the two formulations had similar thermodynamic activity and escaping tendency of FMT from the hydrogel, and similar permeation rates (Fig. 1). Moreover, IPA was expected to promote skin permeation of the drug; however, in this study, the skin permeation promoting effect was not observed with increase in the concentration of IPA. Since IPA had only slight effect on drug solubility and the promotion of drug partition to the skin surface was considered to be the main mechanism for promoting permeation of IPA, different concentrations of IPA had slight effect on partition of FMT to the skin surface. In addition, no other direct action of IPA on the skin surface was observed.

Effects of Terpenes on FMT Skin Permeation The results of FMT skin permeation revealed that FMT in IPA solution could not efficiently penetrate the skin. Therefore, other methods are required to promote FMT skin penetration. There are several physical and chemical methods to improve drug permeation, including microneedles, iontophoresis, microemulsions, and penetration enhancers. Among these methods, the use of penetration enhancer is an established and widely used approach. Here, we compared the penetration promoting effect of three terpenes as penetration enhancers.

In order to investigate the effect of terpenes on skin permeation of drugs, an *in vitro* permeation experiment was performed using IPA as a common additive, which was incorporated at 30% into all aqueous hydrogel formulations. The hydrogel formulations, widely used in commercial preparations, were used as dosage forms in this study. The receiver cell was filled with PBS (pH 7.4) containing 1% acetic acid to ensure sink conditions during the permeation studies.

In the previous experiment, we compared the penetration ability of 5% FMT in 20% IPA and 3% FMT in 30% IPA and found that different concentrations of IPA solution had similar effect on FMT skin permeation. Then, we added terpenes to observe their effects on FMT penetration. Since the three terpenes are hydrophobic compounds, we selected 30% IPA solution as the control group, considering the better enhancement effect of the higher IPA content.

As shown in Fig. 2, the formulation containing geraniol had the highest cumulative amount. As shown in Fig. 3, the high-

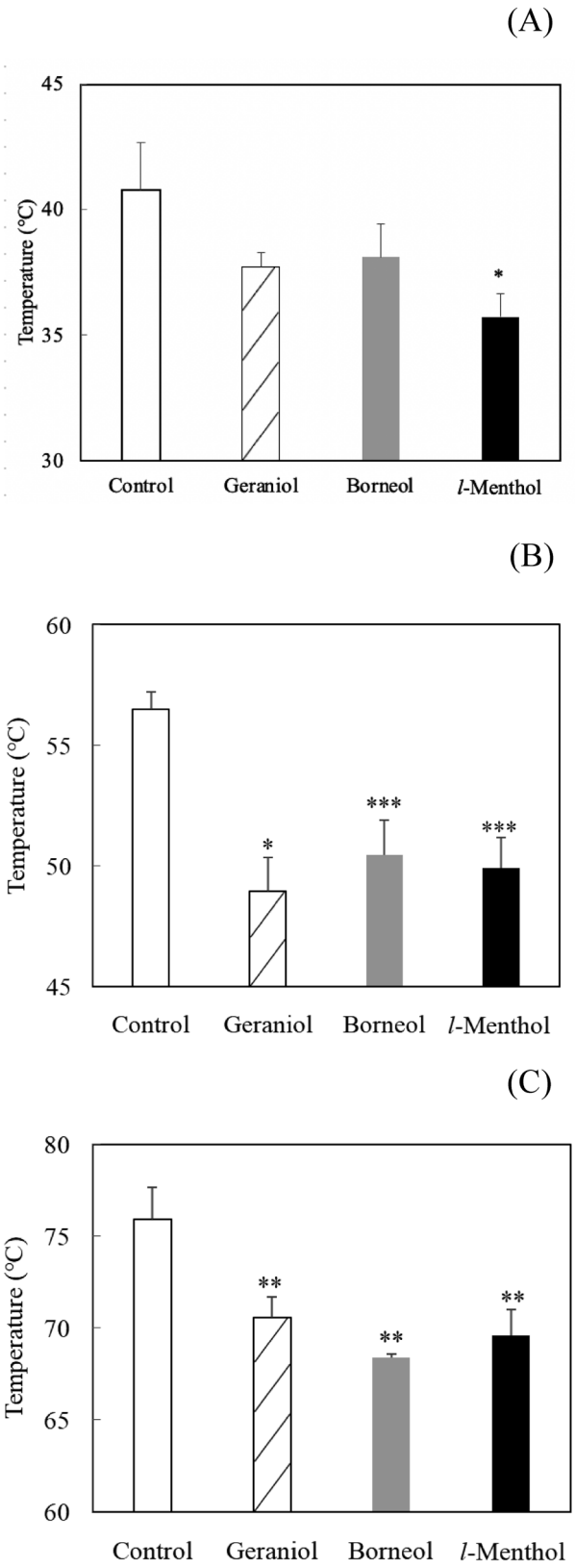

Fig. 5. Changes in the Phase Transition Temperature of the Hairless Mouse Stratum Corneum Treated with Various Terpenes

The phase transition temperatures were calculated from the DSC curves, around (A) 40 °C, (B) 50 °C, (C) 70 °C. Each point represents the mean ± S.D. ($n = 3$). $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ *vs.* Control. Control was SC treated with IPA 30% formulation.

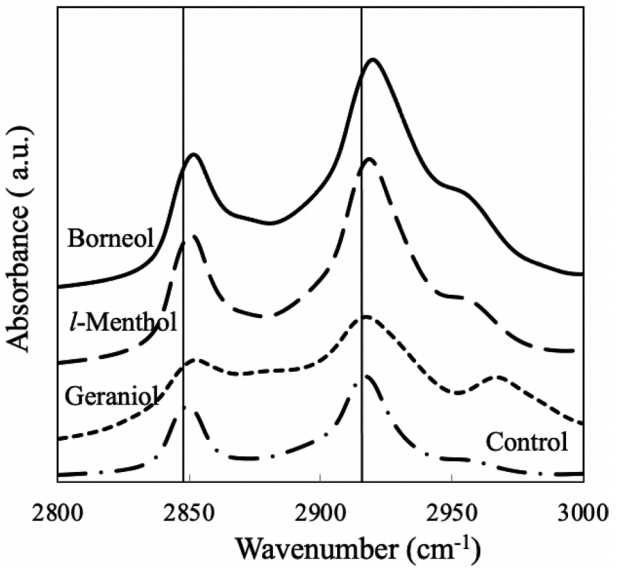

Fig. 6. ATR-FTIR Spectra of Hairless Mouse after Treatment with Different Terpenes

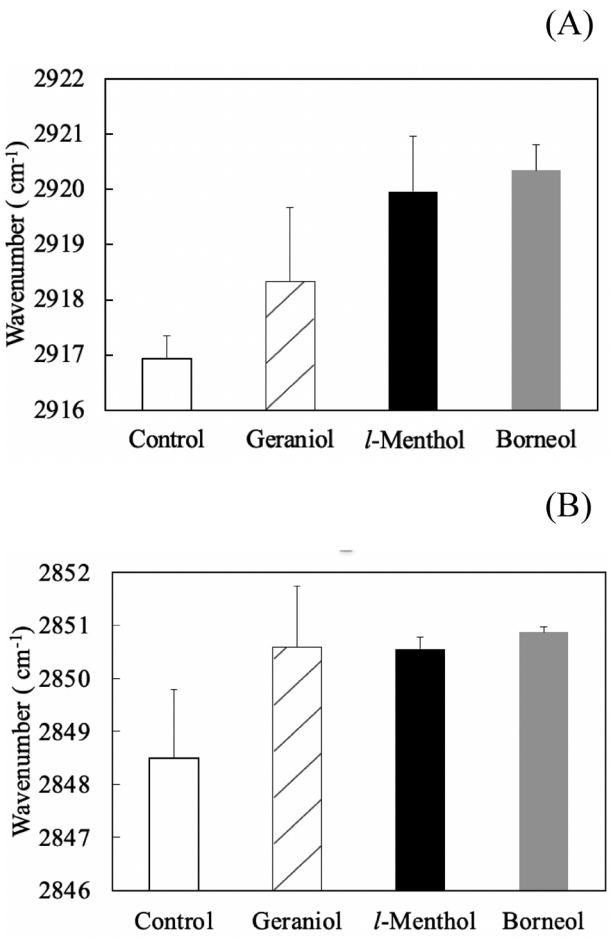

Fig. 7. The (A) Asymmetric and (B) Symmetric CH_2 Absorption Peak Position of Hairless Mouse SC after Treatment with Control, Geraniol, *l*-Menthol, and Borneol at 32 °C

Each column represents the mean ± S.D. ($n = 3$).

Table 4. AUC_{0-12h} Derived from Cumulative Amount and Time Curve of IPA 20% and IPA 30%

	AUC_{0-12h} ($\mu g \cdot h/cm^2$)	Flux ($\mu g/cm^2/h$)	Permeability coefficient
IPA 20%	370.13 ± 14.28	6.15 ± 2.62	0.45 ± 0.08
IPA 30%	472.44 ± 26.09	10.86 ± 1.69	0.89 ± 0.11

est flux for *l*-menthol and the relatively shorter lag time also explained this; the higher the flux, the shorter is the lag time. However, comparing with the 24 h cumulative curve of FMT, *l*-menthol and geraniol, particularly geraniol, showed a strong effect on FMT permeation. Despite the flux of *l*-menthol, the curve showed that the permeation influence of the FMT with *l*-menthol decreased after around 12 h. In contrast, geraniol had a longer effect on FMT transdermal penetration compared to *l*-menthol, resulting in a reverse drug cumulative amount beyond *l*-menthol at 24 h. In the case of borneol, the shortest lag time of FMT was shown, which mean that borneol would be a possible option to promote rapid onset of drug action that leads to faster therapeutic effects, but borneol had only slight effect compared with the control group. This means that sufficient plasma drug concentration would not be reached. Moreover, at the end of the skin permeation study using the borneol, we observed a few white particles of residual drug on the skin surface. This indicated that thermodynamic of FMT was maximum or not change, while borneol did not affect the solubility of FMT in the gel. Although it was not straightforward to draw conclusions, we assumed that the transdermal enhancement effect of borneol related to the drug solubility in donor compartment on FMT had been maximized, as will be discussed later in the text. This indicated that even though the lag time was short, the transdermal efficiency of FMT was affected by the small flux. The lag times for *l*-menthol and geraniol were also significantly shorter compared to the control group, suggesting that terpenes contributed significantly to the rapid onset of action of FMT. This may be attributed to the differences in their chemical structures and the mechanism underlying their penetration enhancement effects on stratum corneum. The log *P* values of *l*-menthol, geraniol, and borneol were 3.40, 3.56, and 3.24, respectively.[22,23] They are hydrophobic compounds with nearly same log*P* values. Therefore, it is speculated that differences in the mechanism underlying their penetration enhancement effects is mainly attributable to this result, which was further elucidated by DSC and FTIR measurements. For clinical use, the predictable permeation rate and time are required for an efficient formulation. The cumulative amount of FMT with *l*-menthol, borneol and control group gradually become stable at around 12 h, while the cumulative amount of geraniol still showing an increasing trend. This also contributed to the longer effect of geraniol on FMT transdermal penetration. These properties are beneficial to the patients, making it a favorable compound for FMT transdermal delivery.

Effects of Terpenes on DSC Profile of Stratum Corneum

DSC is a thermal analysis method for studying the relationship between sample properties and temperature. When stratum corneum was subjected to DSC scanning, several endothermic peaks appeared. The structure and mechanism of action of penetration enhancers on stratum corneum can be elucidated by studying the changes in these peaks. DSC is commonly used to study the lipid phase transition properties of stratum corneum.[24]

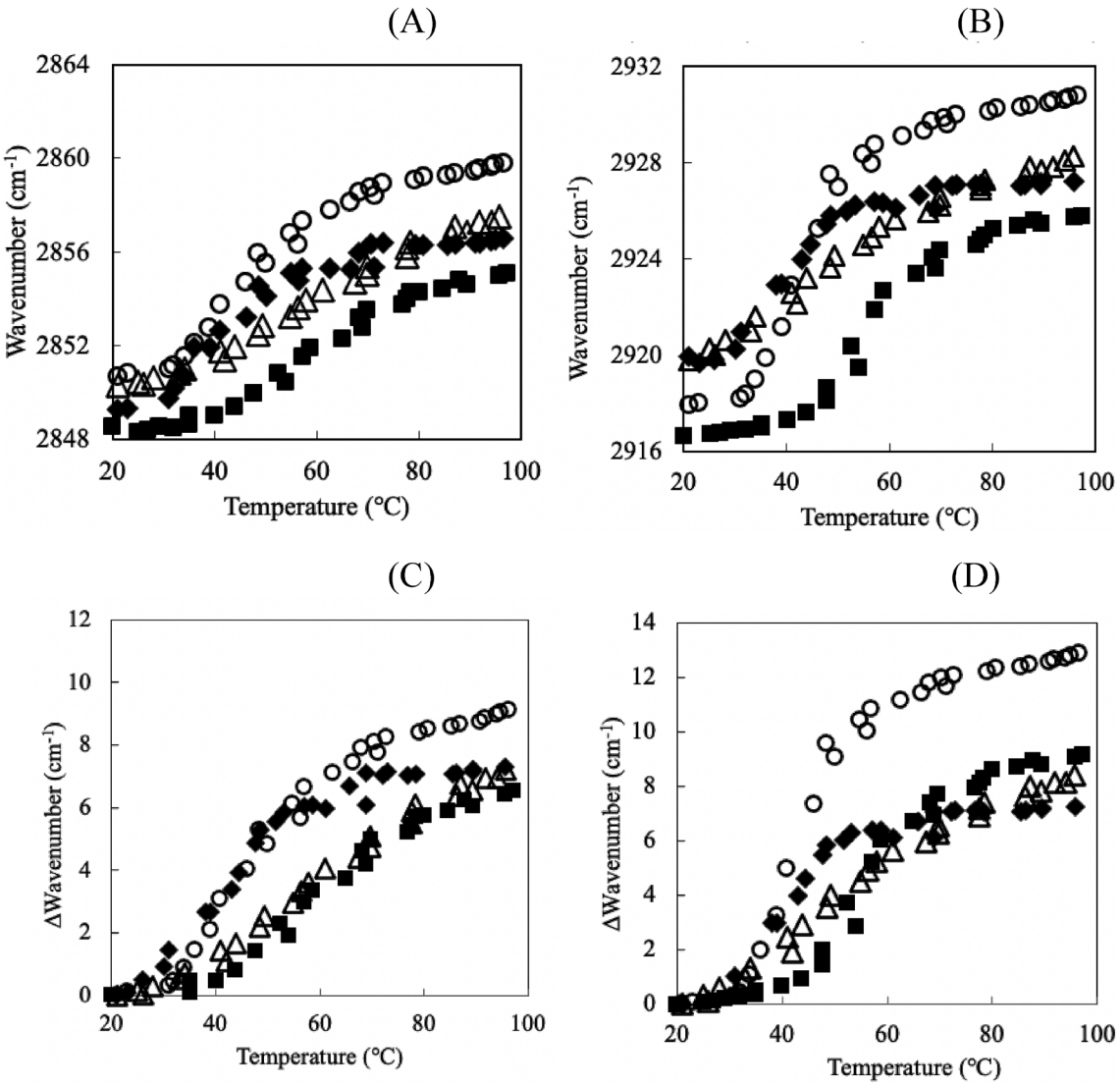

Fig. 8. Thermally Induced Changes in Peak Wavenumber of (A) Asymmetric and (B) Symmetric CH_2 Stretching Frequencies of Intercellular Lipids in the Stratum Corneum Treated with Various Terpenes

The difference in wavenumber with blue shift of (C) asymmetric and (D) symmetric CH_2 stretching frequencies of intercellular lipids treated with various terpenes. Control is the stratum corneum treated by 30% IPA-solution. ◆: *l*-Menthol, △: Borneol, ○: Geraniol, ■: Control.

The effects of chemical enhancers on thermotropic behavior of intercellular lipids in stratum corneum can be observed by DSC thermograms. The phase transition of stratum corneum is known to be liquid crystallization of orthorhombic packing at a temperature of 40 °C, dissolution of long-period lamella at 50 °C, melting of short-period lamella, and liquid crystallization of hexagonal packing at 70 °C.[25,26] In contrast to the short periodicity phase, the presence of long periodicity phase is unique for stratum corneum lipid organization, which suggests that this phase plays an important role in the cutaneous barrier function.[27]

As mentioned above, three typical phase transitions occur for stratum corneum with increase in temperature that represent structural changes in the intercellular lipids in stratum corneum. In the DSC spectrum, all terpenes showed changes in the phase transition temperature. This suggests that chemical enhancers may directly act on intercellular lipids, resulting in distortion of the lipid structure, thereby changing the phase transition temperature to lower values. Moreover, *l*-menthol exhibited significant changes in all three typical phase transition temperatures, which revealed that *l*-menthol simultaneously relaxed both the packing structure and lamellar structure of the intercellular lipids. In contrast, geraniol at 40 °C did not show significant change compared with the control group, but a significant temperature drop was observed at 50 °C, which indicated that geraniol had a greater influence on the lamellar structure than that of the other two terpenes. Borneol had less influence on temperature change compared with the other two terpenes; however, it showed a notable change at approximately 70 °C. These results indicate that borneol had only slight effect on the lamellar structure and no influence on the packing structure of stratum corneum. Collectively, *l*-menthol simultaneously relaxed the packing structure and lamellar structure of the intercellular lipids, whereas geraniol and borneol had only slight influence on the lamellar structure. Differences in the extent of the effects of terpenes on the packing structure and lamellar structure might be attributed to different chemical structures of the terpenes.

Effects of Terpenes on ATR-FTIR Profile of Stratum Corneum ATR-FTIR spectroscopy is considered a useful

tool for determining the molecular vibrations of compounds in stratum corneum. This technique has been used to evaluate functional group interactions between skin penetration enhancers and intercellular lipids of stratum corneum.[24–27] ATR-FTIR spectrum can be used to reveal the complex composition of stratum corneum, including lipids, proteins, minerals, and amino acids, through many IR absorption bands.[28] Previous studies have shown that the peak near 2800–3000 cm^{-1} disappeared when the intercellular lipids of stratum corneum were extracted by a mixture of chloroform and methanol, indicating that the absorption peaks of CH_2 symmetric and asymmetric stretching vibrations are derived from intercellular lipids.[29]

ATR-FTIR measurements can be used to observe changes in the fluidity of intercellular lipids after application of terpenes. ATR-FTIR spectra showed that all terpenes can increase the fluidity of intercellular lipids but had limited effect on keratinocytes. The result of Fig. 7 suggests that the fluidity of intercellular lipids may be increased at the skin surface temperature.

It is known that the absorption peaks of CH_2 symmetric and asymmetric stretching vibrations shift to a higher wavenumber with increase in temperature due to the increase in lipid fluidity. Because the shift of the C–H stretching frequency can be elucidated at the molecular level, the shift of the peak position to a higher wavenumber occurs when CH_2 groups along the alkyl chain of lipids change from the trans to the gauche conformation, indicating that the intercellular lipids in stratum corneum are disturbed. The extent of the shift in the C–H stretching vibrations is directly related to the ratio of trans to gauche conformers in the alkyl chain.[25] In this study, the wavenumber of the top of the peak (peak position) was calculated by subjecting the ATR-FTIR spectrum to the second derivative of the profiles, and then changes in the peak position of the CH_2 asymmetric stretching vibrations were analyzed. Figure 8 shows the shift in the peak position with increase in temperature in different terpene groups and the control group. These results indicated that the maximum wavenumber of the symmetric and asymmetric CH_2 stretching vibrations shifted to higher wavenumbers after the application of terpenes. In addition, shift of *l*-menthol and geraniol to higher wavenumber was observed; borneol also revealed a shift to a higher wavenumber here, but the magnitude of the shift was not large. Compared with the other groups, the magnitude of the red shift of geraniol was obviously higher at high temperatures, which might be due to the presence of unsaturated double bond in geraniol; however, further research is required.

For the typical symmetric CH_2 stretching vibration, geraniol exhibited the highest shift among the three terpenes, which might be attributed to the chemical structure of geraniol. In contrast to the other two terpenes, geraniol has a chain structure, which eases the lipid extraction compared with the ring structure. Furthermore, geraniol contains two unsaturated double bonds. This might make it perform better at high temperatures than the other two terpenes, but the specific mechanism requires further research.

In addition to the CH_2 symmetric and asymmetric peaks, a weak peak at 2960 cm^{-1} corresponding to the CH_3 asymmetric stretching vibration exists, as shown in Fig. 6, causing difference in the performance of the three terpenes. Borneol and *l*-menthol caused a slight shift to a lower wavenumber, whereas geraniol caused shift to a higher wavenumber. However, the bands of CH_3 vibrations originate not only from a specific component of stratum corneum, but from multiple components, including protein side chains, phospholipids, ceramides, and fatty acids.[29] In addition, the bands of CH_3 vibrations overlapped with the bands of CH_2 vibrations with lower intensity. Therefore, further analysis based on these bands was not performed in this study.

FMT Penetration Enhancement Effect of the Three Terpenes The results of ATR-FTIR and DSC suggested that the terpenes had different degrees of effects on intercellular lipids to promote the skin permeation of drugs. Among them, *l*-menthol caused simultaneous relaxation of the packing structure and lamellar structure and increased the fluidity of the intercellular lipids of stratum corneum, thereby promoting FMT permeation. Geraniol showed a strong promoting effect on drug permeation, but it had only a slight effect on the packing structure and strong effect on the lamellar structure of intercellular lipids. In addition, geraniol had a longer effect of stratum corneum than other terpenes. Borneol had only a slight effect on the lamellar structure of intercellular lipids, and its promoting effect was not obvious.

Finally, based on the results of the present study, we have considered the dosage of FMT required for the treatment of GERD. The blood concentration of FMT for the treatment of GERD is still unclear. Therefore, we calculated the dosage of FMT applied for 24 h based on the maximum flux value obtained from our results and compared dosage of FMT administrated transdermally with the oral FMT dosage to determine whether our results are effective in treating GERD. Based on an oral dose of 20 mg twice per day[30] and about 50% bioavailability for the clinical use of FMT in GERD, 20 mg needs to be absorbed transdermally to achieve the treatment of GERD. The highest flux of *l*-menthol and geraniol achieved in this study was 272.18 and 112.56 $\mu g/cm^2/h$, respectively, and the cumulative amount of FMT which permeated from the gel (2 cm^2) was 2518.30 and 2919.52 $\mu g/cm^2$ at the final determination at 24 h, respectively. Hence, transdermal FMT gel with an area of 3 cm^2 for *l*-menthol and 7 cm^2 for geraniol would be sufficient for the management of GERD patients. Although the transdermal promotion effect of borneol on FMT was less pronounced compared to *l*-menthol and geraniol, the maximum flux of FMT when using borneol as a transdermal enhancer was 42 $\mu g/cm^2/h$. Therefore, transdermal FMT gel with an area of 19 cm^2 is also sufficient to meet the daily dosing needs of GERD patients. It is possible to use these terpenes as chemical enhancers for FMT transdermal gel.

Generally, human skin shows more resistance for drug permeation when compared to hairless mouse skin.[31] However, the significance of this study is that it supports the feasibility of developing a matrix-type TDDS for FMT that would increase patient compliance.

Conclusion

All terpenes increase the fluidity of the intercellular lipids and improve the permeability of the substances through stratum corneum. However, terpenes may have different degrees of effect on the packing structure and lamellar structure of intercellular lipids in stratum corneum, which is dependent on the type of terpenes. The maximum flux of FMT was increased by the administration of terpenes. Terpenes are effective chemical enhancers to promote FMT penetrability

through the skin; however, among the administered terpenes, geraniol was the most effective. The penetration mechanism of terpenes may include interaction with the lipids in stratum corneum to increase the fluidity of the rigid barrier, thereby increasing skin permeation of the drug; however, the effects and mechanisms may vary depending on the type of terpenes. Geraniol had great influence on the lamella structure of the stratum corneum, which might be the main mechanism influencing permeation of FMT through the skin. Meanwhile, *l*-menthol induced a phase transition at approximately 30–40 °C and relaxation of the structure that influenced the orthorhombic packing. Although borneol had little effect on the lamella structure of stratum corneum, it would slightly promote transdermal efficiency of FMT comparing to the absence of terpenes. Thus, geraniol could also be a suitable candidate for promoting FMT transdermal delivery. The present findings indicate the potential of chemical enhancers in changing the inner structure of stratum corneum and improving the permeation of FMT through the skin, and it has great potential to be used in transdermal formulation of FMT.

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Conflict of Interest The authors declare no conflict of interest

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