Regular Article

Potential of Stratum Corneum Lipid Liposomes for Screening of Chemical Skin Penetration Enhancers

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Received March 28, 2017; accepted May 26, 2017

The evaluation of effective skin chemical penetration enhancers (CPEs) is a crucial process in the development of transdermal and dermal formulations with the capacity to overcome the stratum corneum barrier. In the present study, we aimed to investigate the potential of stratum corneum lipid liposomes (SCLLs) as an alternative tool for the screening of various types and concentrations of CPEs. SCLLs were prepared using a thin-film hydration technique, and two types of fluorescent probes (sodium fluorescein [FL] or 1,6-diphenyl-1,3,5-hexatriene [DPH] were entrapped separately into SCLLs (FL-SCLL and DPH-SCLL, respectively). FL leakage from SCLLs as well as the fluidity of DPH-SCLLs were determined after incubating with various types of CPEs as a function of their concentrations. The obtained results showed a concentration-dependent relationship for most CPEs both for FL leakage and the fluidity of SCLLs. When observing these data in detail, however, the concentration profiles could be classified into five main categories depending on the mode of action of the CPEs. These results strongly suggest the usefulness of SCLLs for high-throughput screening of effective CPEs as well as the understanding of their possible mode of action, especially in the early stage of skin formulation development.

Key words stratum corneum lipid liposome; chemical skin penetration enhancer; liposome; high-throughput screening

Skin has been recognized as an application site of therapeutic drugs for several decades. However, only small numbers of drugs are available nowadays intended for systemic absorption through the skin. The stratum corneum (SC), the outermost layer of the skin, provides a strong barrier to protect against extraneous molecules from entering the body, and thus limits the skin penetration of drugs. The structure of the SC consists of keratin-rich corneocytes embedded in an intercellular lipid matrix.[1,2] This lipid-rich region plays a key role in the penetration of drugs, because it is a part of both intercellular and transcellular penetration pathways. According to several studies, the skin-penetration-enhancing effects of various chemicals have been reported to act by disrupting these lipids in the SC.[3–5]

Various chemical penetration enhancers (CPEs) have been investigated to enhance the skin penetration of drugs. Such investigations are conventionally performed by skin permeation experiments using a Franz diffusion cell.[6] In addition, assessments to understand the mechanism of action of CPEs are carried out by differential scanning calorimetry (DSC), Fourier transform (FT)-IR, ESR, or X-ray diffractometry (XRD).[7–10] Although these techniques can observe changes in the SC structure, they require specialist manipulation techniques and are labor intensive, time consuming, and costly. High-throughput screening (HTS) techniques have been introduced using electrical resistance-based methods.[11,12] However, this technique requires animal skin, which still has ethical considerations.

Stratum corneum lipid liposomes (SCLLs) are a type of liposome prepared from a lipid mixture of SC lipids.[13] Generally, SC lipids are composed of ceramides, cholesterols, cholesterol esters, and fatty acids. Because of the similar bilayer morphology, as well as the similar composition of intercellular lipids, SCLLs have been investigated as a drug delivery carrier.[14–16]

Several researchers have already utilized SCLLs to investigate the mechanism of action of CPEs, by monitoring the release of fluorescent markers or chemical molecules from SCLLs, or change in the transition temperature of SCLLs.[15,17–19] Nevertheless, these studies focused on small numbers of CPEs, and thus there is still a lack systematic data that could determine the feasibility of this SCLL-based approach for HTS of various types of CPEs.

In the present study, the effects of various types of well-known CPEs with different concentrations were determined from the leakage of sodium fluorescein (FL) and the fluidity of SCLLs as an HTS method. Then, the effects of CPEs were categorized according to their concentration profiles on these penetration enhancing effects.

Experimental

Materials FL, lignoceric acid, palmitic acid, boric acid, potassium chloride, sodium hydroxide, chloroform, methanol, ethanol, *n*-propanol, Transcutol® (diethylene glycol monoethyl ether), dimethyl sulfoxide (DMSO), oleic acid, ethyl oleate, sodium dodecyl sulfate, Brij® 58, and 1-methyl-2-pyrrolidone were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Cholesterol, cholesteryl sulfate, octacosanoic acid, 1,6-diphenyl-1,3,5-hexatriene (DPH), and Pluronic® F-127, were obtained from Sigma-Aldrich (St. Louis, MO, U.S.A.). CER[NP] (*N*-(octadecanoyl)-phytosphingosine) and CER[AP] (*N*-(α-hydroxyoctadecanoyl)-phytosphingosine) were obtained from Evonik Industries AG (Essen, Germany). Sodium decanoate, 1,8-cineol, lauryl alcohol, cetylpyridinium chloride, polyoxyethylene sorbitan fatty acid esters (Tween 20, 40, 40, 80, 85), and sorbitan fatty acid esters (Span 20, 40, 60,

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80) were obtained from Tokyo Chemical Industry Co., Ltd. (Tokyo, Japan). Propylene glycol, benzyl alcohol, and polyethylene glycol (PEG) 400 were obtained from Kanto Chemical Co., Inc. (Tokyo, Japan). Sefsol 218 (propylene glycol monocaprylate) was a kind gift from Nikko Chemicals Co., Ltd. (Tokyo, Japan). Pluronic® P-84 was supplied by Adeka Corporation (Tokyo, Japan), and *l*-menthol was obtained from Fisher Scientific Japan (Tokyo, Japan). These reagents were used without further purification.

Preparation of Stratum Corneum Lipid Liposomes SCLLs were prepared using the thin film hydration method reported by Hatfield and Fung[20] with a slight modification. Total lipids (5.5 mg/mL), which includes 33% CER[NP], 22% CER[AP], 25% cholesterol, 5% cholesteryl sulfate, 7.5% lignoceric acid, 3.75% palmitic acid, and 3.75% octacosanoic acid were first dissolved in chloroform–methanol (2 : 1) in a round-bottomed flask. The solvent was then evaporated at 60°C under reduced pressure using a rotary evaporator until a thin film was obtained on the wall of the flask which was then purged with N_2 gas and allowed to stand overnight to remove any traces of organic solvents. Next, the lipid film was annealed in a water bath at 90°C for 30 min. FL in 0.1 M borate buffer pH 9.0 (2.5 mg/mL) for FL-SCLLs or plain buffer for blank-SCLLs was used to hydrate the lipid film. The resulting SCLL suspension was then sonicated using a probe sonicator (VCX-750, Sonics & Materials Inc., Newtown, CT, U.S.A.) at an amplitude of 20% for 30 s. A freeze-thaw process was performed by immersing the flask in liquid N_2 and in a water bath at 90°C, each for 3 min in 4 cycles. The obtained liposomes were further extruded using an extruder (Lipex™, Northern Lipids Inc., Burnaby, BC, Canada) with a membrane filter with pore sizes of 400, 200, and 100 nm (Nucleopore® track-etched membranes, GE Healthcare Japan, Tokyo, Japan), twice for each size of filter.

After preparation of FL-SCLLs, excess FL was removed by ultra-centrifugation (Hitachi CS100GXL, Hitachi Koki Co., Ltd., Tokyo, Japan) at 289000×***g***, 4°C twice for 15 min, twice for 10 min and 5 times for 5 min. At each centrifugation process, the supernatant was removed and the same volume of 0.1 M borate buffer was added and mixed.

Determination of FL Leakage from SCLLs FL-SCLLs were mixed 5 times by pipetting with different types and concentrations of CPEs solution in borate buffer, as shown in Table 1, at a ratio of FL-SCLLs–CPEs solution 1 : 9 v/v. However, 3% ethanol in borate buffer was used as solubilizing medium for oleic acid, ethyl oleate, sefsol 218, lauryl alcohol, *l*-menthol and 1,8-cineol. Each sample was allowed to stand for 30 min at room temperature and immediately ultracentrifuged (Hitachi CS100GXL, Hitachi Koki Co., Ltd., Tokyo, Japan) at 289000×***g***, 4°C for 5 min according to our previous protocol.[21] The supernatant was collected to determine the amount of FL that leaked from FL-SCLLs after mixing with CPEs solution (FL leakage), using a microplate reader (Spectra Max® M2^e, Molecular Devices, LLC., Sunnyvale, CA, U.S.A.) at excitation and emission wavelengths of 485 and 535 nm, respectively. This study was also performed using 75% ethanol and 0.1 M borate buffer to represent total and background FL leakage, respectively. Ethanol at 75% was found to totally disrupt the FL-SCLLs as no evidence of SCLL pellet precipitation was observed after the ultracentrifugation process. The calculation of FL leakage (%) is shown as follows:

$$\text{FL leakage (\%)} = \left(\frac{\text{FL}_{\text{sample}} - \text{FL}_{\text{background}}}{\text{FL}_{\text{total}} - \text{FL}_{\text{background}}} \right) \times 100 \quad (1)$$

Determination of SCLL Fluidity The fluidity of the SCLL membranes was determined according to the method of Tan *et al.* with some modification.[22] The blank SCLLs and 2×10^{-5} M DPH solution in phosphate buffered saline (PBS) (25 : 75 μL) was firstly mixed in a 96-well plate and then subjected to shaking in an orbital shaker (IKA® MS1 Minishaker, Sigma-Aldrich, Willmington, NC, U.S.A.) at 500 rpm for 30 min in the absence of light. Next, different concentrations of CPEs in 0.1 M borate buffer (100 μL) were added and shaken in the same conditions. The fluorescence polarization (P_f) was measured at 25°C using a microplate reader (Spectra

Table 1. List of CPEs and Their Concentrations Tested in This Study

Group of enhancers	Enhancers	Concentration (%)
Anionic surfactant	Sodium dodecyl sulfate	0.01, 0.05, 0.1, 1.0, 2.5, 5.0
Cationic surfactant	Cetylpyridinium chloride	0.01, 0.05, 0.1, 1.0, 2.5, 5.0
Nonionic surfactant		
Polysorbates	Tween 20, 40, 60, 80, 85	0.01, 0.05, 0.1, 1.0, 2.5, 5.0
Sorbitans	Span 20, 40, 60, 80	0.01, 0.05, 0.1, 1.0, 2.5, 5.0
Brij	Brij 58	0.01, 0.05, 0.1, 1.0, 2.5, 5.0
Pluronic	Pluronic P-84, F-127	0.01, 0.05, 0.1, 1.0, 2.5, 5.0
Urea and its derivatives	Urea	5, 10, 20, 30, 40, 50
Glycols	PEG 400, propylene glycol	5, 10, 20, 30, 40, 50, 75
	Transcutol	5, 10, 20, 30, 40, 50, 75
Short chain alcohols	Ethanol, *N*-propanol	5, 10, 20, 30, 40, 50, 75
Aromatic ring alcohols	Benzyl alcohol	2.5, 5, 10
Fatty acids	Sodium decanoate	1, 5, 10, 20, 30
	Oleic acid, ethyl oleate	0.01, 0.05, 0.1
Polyol fatty acid esters	Sefsol 218	0.01, 0.05, 0.1, 1, 2.5, 5
Fatty alcohols	Lauryl alcohol	0.01, 0.05, 0.1
Sulfoxides	DMSO	5, 10, 20, 30, 40, 50, 75
Pyrrolidones	1-Methyl-2-pyrrolidone	1, 5, 10, 20
Terpenes	*l*-Menthol, 1,8-cineol	0.01, 0.05, 0.1, 1.0

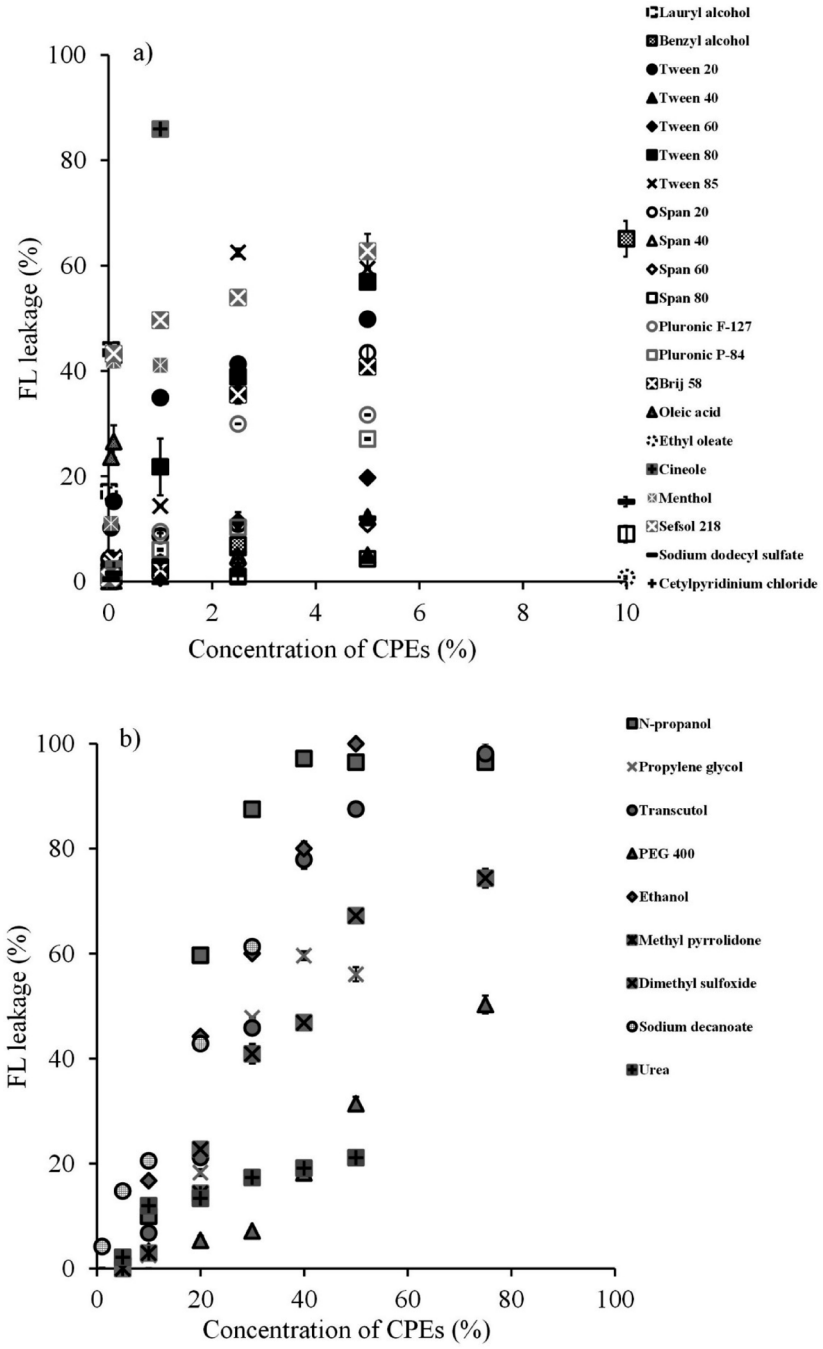

Fig. 1. Relationship between FL Leakage from SCLLs and CPE Concentration

a) Lipophilic and amphiphilic CPEs. b) Hydrophilic CPEs.

Max® M5e, Molecular Devices, LLC., Sunnyvale, CA, U.S.A.) at excitation and emission wavelengths of 358 and 425 nm, respectively. The P_f value was calculated according to the following equation:

$$P_f = \left(\frac{I_{\parallel} - GI_{\perp}}{I_{\parallel} + GI_{\perp}} \right) \quad (2)$$

where $I_{\parallel}$ and $I_{\perp}$ are the fluorescence intensities of the emitted light polarized parallel and perpendicular to the excited vertical light, respectively. G is an instrumental correction factor grating correction coefficient.[23,24]

In addition, the increase in SCLL membrane fluidity was calculated as follows:

$$\text{Increase in SCLL fluidity} = \left(\frac{P_{f,\,\text{control}} - P_{f,\,\text{sample}}}{P_{f,\,\text{control}}} \right) \times 100 \quad (3)$$

where $P_{f,\,\text{control}}$ was obtained from DPH-SCLL incubated with plain 0.1 M borate buffer.

Calculation of Effective Concentration for Promoting 10% FL Leakage ($EC_{10,\,\text{leakage}}$) and 10% Increase in SCLL Fluidity ($EC_{10,\,\text{fluidity}}$) of Each CPE $EC_{10,\,\text{leakage}}$ and $EC_{10,\,\text{fluidity}}$ of each CPEs was calculated by interpolation from

the FL-leakage or SCLL fluidity–concentration profile to estimate the effectiveness of each CPEs to act on SCLLs. In this study, 10% response was selected because most CPEs, within the tested concentrations range, could promote the FL leakage as well as SCLL fluidity higher than 10%. The relationship between $EC_{10,\ leakage}$ or $EC_{10,\ fluidity}$ value and $\log K_{o/w}$, obtained from ChemBioDraw Ultra 12.0 software, of each CPEs as well as the hydrophilic lipophilic balance (HLB) of surfactants were also investigated.

Results

FL Leakage from SCLLs FL leakage from SCLLs with the addition of different types of CPEs is shown as a function of their concentrations in Fig. 1. For lipophilic and amphiphilic CPEs, lower concentrations were used for evaluation compared with hydrophilic CPEs, as shown in Figs. 1a, b, respectively, because of their limited solubility in 0.1 M borate buffer, which was used as a dispersion medium for SCLLs. Most CPEs promoted FL leakage from SCLLs in a concentration-dependent manner except for ethyl oleate and cetylpyridinium chloride, which had no promoting effect on FL leakage. The concentration profiles of FL leakage from different CPEs could be classified into two types, as shown in Fig. 2. Type I (Fig. 2a) showed a linear relationship between FL leakage and CPE concentration, whereas type II (Fig. 2b) showed a concave downward profile, which indicated the limit level for the disruption of SCLLs. In both types, we sometimes observed an intercept of the profile, in which the FL leakage was not promoted at lower concentrations than the *x*-intercept.

The $EC_{10,\ leakage}$ of each CPE is shown in Table 2, we could observe that lipophilic CPEs tended to provide lower $EC_{10,\ leakage}$ than hydrophilic CPEs, as shown in the plot between $\log K_{o/w}$ and $EC_{10,\ leakage}$ (Fig. 3a). In addition, when comparing among amphiphilic CPEs, the more hydrophilic surfactants showing higher HLB tended to provide lower $EC_{10,\ leakage}$ (Fig. 3b).

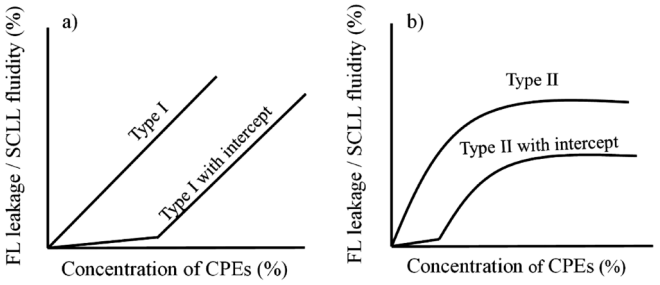

Fig. 2. Schematic Diagram of the Relationship between FL Leakage and CPE Concentration

a) Linear relationship (with intercept). b) Concave downward relationship (with intercept).

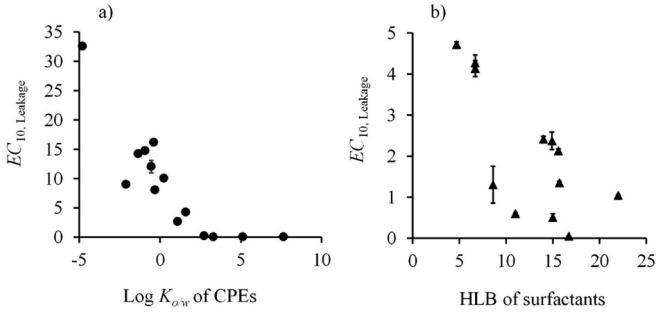

Fig. 3. Relationship between $EC_{10,\ leakage}$ and $\log K_{o/w}$ (a) and HLB (b) of CPEs

Each plot represents a single CPE.

The Effect of CPEs on SCLL Fluidity The relationship between fluidity of SCLLs and different concentrations of CPEs is shown in Figs. 4a, b for lipophilic and amphiphilic CPEs, and hydrophilic CPEs, respectively. A concentration-dependent pattern similar to the FL leakage profile was observed, unless type II with an intercept could not be observed. Most CPEs promoted the fluidity of SCLLs similar to the type II profile. The $EC_{10,\ fluidity}$ of each CPE is shown in Table 2). However, several CPEs promoted relatively low SCLL fluidity (less than 10%) within the concentrations used in this study; *e.g.* cetylpyridinium chloride, propylene glycol, Transcutol, urea, DMSO, PEG 400, lauryl alcohol, and 1,8-cineol. Among the CPEs, lipophilic CPEs provided lower $EC_{10,\ fluidity}$ in comparison with hydrophilic CPEs. For amphiphilic CPEs, no clear relationship was found between the HLB and $EC_{10,\ fluidity}$ value (data not shown).

The Relationship between Fluidity and FL Leakage from SCLLs Figure 5a shows the relationships between fluidity and FL leakage from SCLLs for various types of CPEs. According to the similarity in the profile patterns, we catego-

Table 2. EC_{10} Value Calculated from FL Leakage and SCLL Fluidity–Concentration Profile

CPEs	EC_{10} (mean±S.E.)	
	FL leakage	SCLL fluidity
Lauryl alcohol	0.006±0.000	>0.1%
Oleic acid	0.024±0.001	0.047±0.001
l-Menthol	0.047±0.003	0.304±0.048
Tween 20	0.048±0.001	0.189±0.019
Sefsol 218	0.061±0.000	0.236±0.010
1,8-Cineol	0.179±0.027	>1%
Tween 80	0.507±0.087	0.066±0.004
Tween 85	0.599±0.018	0.065±0.002
Pluronic F-127	1.040±0.019	1.331±0.027
Span 20	1.304±0.451	0.081±0.005
Brij 58	1.352±0.032	0.166±0.012
Tween 40	2.127±0.052	0.096±0.004
Tween 60	2.372±0.214	0.083±0.004
Pluronic P-84	2.415±0.069	2.372±0.172
Benzyl alcohol	2.650±0.026	7.310±0.012
Sodium decanoate	3.190±0.117	2.950±0.046
Sodium dodecyl sulfate	4.259±0.037	0.283±0.014
Span 40	4.266±0.197	0.324±0.048
Span 60	4.718±0.065	0.274±0.042
Ethanol	8.011±0.075	67.160±2.318
Urea	8.990±0.039	>50%
1-Propanol	10.001±0.038	44.533±1.023
Transcutol	12.021±1.056	>75%
DMSO	14.189±0.183	>75%
Propylene glycol	14.725±0.211	>75%
1-Methyl-2-pyrrolidone	16.130±0.396	>20%
PEG 400	32.548±0.297	>75%
Span 80	> 5%	0.048±0.004
Ethyl oleate	> 0.1%	0.051±0.002
Cetylpyridinium chloride	>5%	>5%

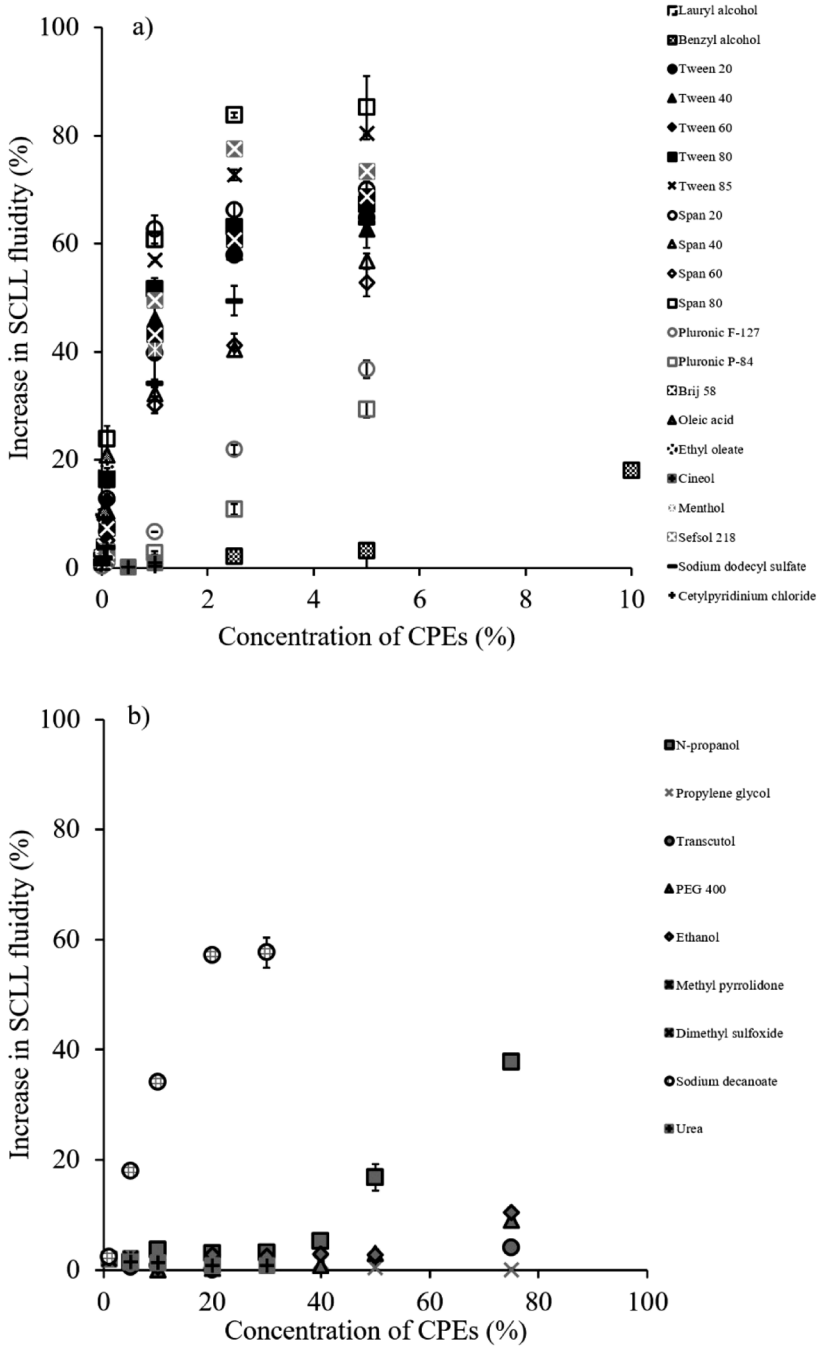

Fig. 4. Relationship between Fluidity of SCLLs and CPE Concentration
a) Lipophilic and amphiphilic CPEs. b) Hydrophilic CPEs.

rized the tested CPEs into 5 categories (A–E) as shown in Fig. 5b. Category A contains CPEs that promoted similar degree of FL leakage and the fluidity of SCLLs. The profile of this category showed a linear correlation between FL leakage and fluidity of SCLLs. Category B includes CPEs that promoted high fluidity but low FL leakage, the plots parallel to the *Y*-axis was observed. Similarly, CPEs in category C promoted high fluidity of SCLLs, however, at higher concentrations, higher FL leakage was then observed. The plots of this category were first parallel to the *Y*-axis, after a marked increase in fluidity, the curve bends toward the *X*-axis, therefore two slopes could be seen in this category's profile. Category D includes CPEs that promoted high FL leakage but low fluidity. This category CPEs' plot showed the trend parallel to *X*-axis. Finally, Category E containes CPEs that could not be classified into any of the previously defined categories. We then classified the CPEs that exhibited scattering profile in this category.

Discussion

CPEs represent an easy and well-established approach to enhance the skin penetration of drugs. One of the suggested mechanisms of the effects of CPEs is the lipid–protein-partitioning (LPP) theory,[25] in which CPEs act by disrupting the highly ordered lamellar structure of SC lipids, interacting with intracellular proteins to promote drug permeation through the corneocyte layer, and increasing the partitioning of drugs into

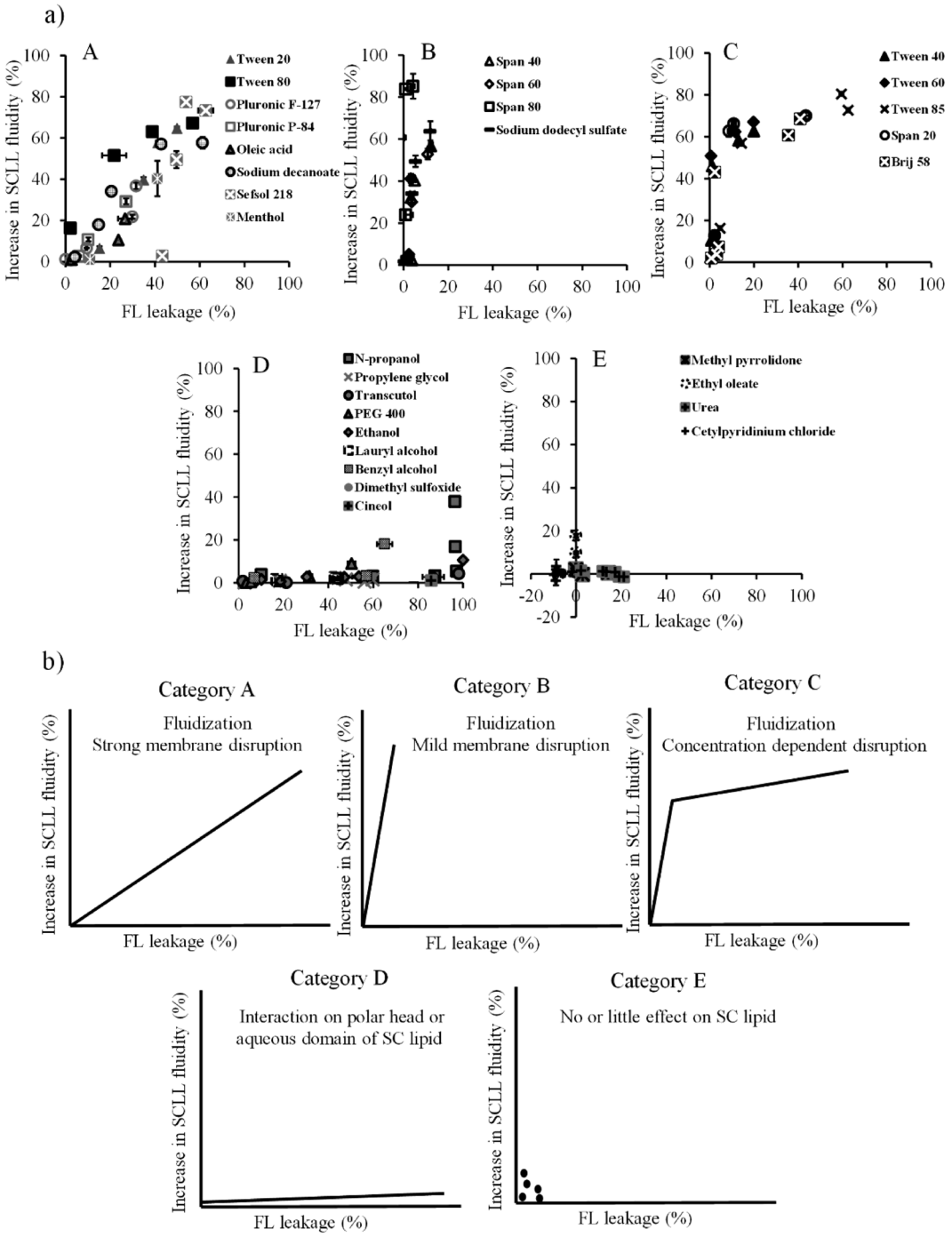

Fig. 5. Relationship between SCLL Fluidity and FL Leakage by Addition of Various Types of CPEs and Their Categorization
a) Observed data. b) Categorization.

the SC. SCLLs exhibit similar characteristics to SC intercellular lipids without the proteins associated with the SC, thus they were used in the present study as a model membrane to assess the interaction of various CPEs specifically on the SC lipid region.

The effect of CPEs on SC lipids is differentiated into three different locations; the lipid polar head group, the aqueous region between the lipid head groups, and the hydrophobic tail within the bilayers.[25] In this study, prepared SCLLs were entrapped with a hydrophilic fluorescent probe, FL, inside the SCLLs core due to its hydrophilic nature. The entrapment of FL was determined to be 8.09±0.18 μg/mg of total lipid or 1.78±0.04% of total FL loaded. The SCLLs prepared in this study were unilamellar vesicle as obtained from extrusion method. This SCLLs morphology can be more quantitatively characterized with regards to lipid concentration, surface area and volume as well as more stable than multilamellar vesicles which present similar multilamellar structure to SC lipids. Borate buffer pH 9.0 was used to prepare SCLLs as well as dissolve CPEs, regardless skin pH of 4–7, to maintain the stability of SCLLs' vesicle derived from ionized moieties of cholesterol sulfate and free fatty acids in this pH.[20] The degree of FL leakage from SCLLs refers to the degree of the disruption effect brought about by CPEs, which finally provide diffusion-

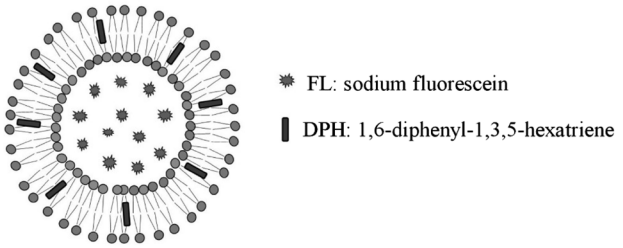

Fig. 6. Schematic Diagram of the Location of FL and DPH Fluorescence Probes in SCLLs

al paths or breaking of the enclosed structure of SCLLs and promote leakage of FL. Separately, a DPH probe was inserted between the hydrophobic tails of lipids in SCLLs.[26] The disruption of SCLLs by CPEs could be observed from the change in P_f of DPH molecules caused by the change in molecular orientation and mobility. Because DPH serves as a marker for molecular movement in the hydrophobic core, SCLL fluidity investigation using DPH represents the effect of CPEs only on the hydrophobic tails of SC lipids, not on the polar head group of lipids. A schematic diagram of the location of each fluorescent probe in SCLLs is shown in Fig. 6.

Fair correlations were observed between FL leakage, SCLLs fluidity and effect of ethanol on the enhanced skin permeation in our previous work.[21] Similarly, Yoneto *et al.*[17] found a good correlation between the enhancement of sugar molecule release from SCLLs and the enhancement factor on the lipoidal pathway of hairless rat SC in the presence of pyrrolidone derivatives. Furthermore, it was found in our present work that the effect of CPEs on FL leakage and SCLL fluidity were dependent on the CPE concentration.[21] Similarly, in the study by Suhonen *et al.*,[18] calcein leakage from SCLLs was concentration dependent for 1-dodecanol and concave downwards for Azone and dodecyl-2-(*N*,*N*-dimethylamino)propionate (DDAIP). Kim *et al.*[15] also observed that the lipid phase transition temperature of SCLLs shifted progressively towards lower temperature with the increased concentration of pyrrolidone derivatives. However, no relationship has been reported between change in SCLL fluidity and FL leakage to evaluate skin permeation enhancing effect of chemicals.

In the present study, we successfully categorized the CPEs into 5 categories by the different in characteristics of their profile between SCLL fluidity and FL leakage. We calculated the $EC_{10,\ \mathrm{leakage}}$ and $EC_{10,\ \mathrm{fluidity}}$ of various CPEs to compare the effectiveness of each CPE for promoting the same degree of SCLL leakage and fluidity. More obviously lower $EC_{10,\ \mathrm{leakage}}$ and $EC_{10,\ \mathrm{fluidity}}$ were observed from lipophilic CPEs than hydrophilic CPEs. It has been discussed previously that more lipophilic compounds penetrate more easily into the SCLL membrane and more effectively reduce the barrier function of the membrane.[15] However, some CPEs promoted very low leakage and fluidity, thus calculation of the exact EC_{10} value is difficult.

Category A contained several non-ionic surfactants *e.g.* Tween 20 and 80, Pluronic F-127 and P-84, fatty acids and its derivatives such as oleic acid, sodium decanoate, Sefsol 218, as well as menthol. Fatty acids such as oleic acid was reported to disturb the packing of lipids and thereby increase the fluidity of lipids as well as form a permeable defect within the SC lipids.[27,28]

Category B contained Span 40, 60, and 80, and sodium dodecyl sulfate. This group comprised mainly hydrophobic non-ionic surfactants (HLB 4–7) and anionic surfactants (HLB 40). These CPEs may insert into the hydrophobic region of SCLLs promoting higher membrane fluidity but not strong enough to promote the leakage from the SCLLs.

Most non-ionic surfactants such as Tween 40, 60, 85, Span 20, and Brij 58 were classified into Category C. These CPEs contained similar action to the CPEs in category B, by fluidization of SCLL. In addition, high leakage was also observed at higher concentration. This phenomenon might be due to the insertion of these non-ionic surfactants molecule in the hydrophobic area of SCLL, at higher concentration, the degree of fluidization is strong enough to disturb the SCLLs structure consequently allowing FL to leak outside.

We found that non-ionic surfactants could be categorized into either Categories A, B, or C. These findings are in good agreement with the penetration enhancing mechanisms reported that surfactants may intercalate into lipid bilayers of SC, resulting to increase fluidity of intercellular SC lipids which finally solubilize and extract the lipid components.[29] The effect of surfactants on SCLLs could vary from mild to strong effect as could be seen from each category's profile. However, the surfactants' effect on the keratin filament could not be observed using this model membrane.

The following CPEs, *n*-propanol, propylene glycol, Transcutol, PEG 400, ethanol, lauryl alcohol, benzyl alcohol, DMSO, and 1,8-cineol were classified in category D. Alcohols and glycols as classified under this category were reported to interact in the aqueous domain of the lipid bilayers which increase the solubility of drugs in the skin.[30] In addition, ethanol has been reported to extract and fluidize SC lipids.[31–33] Dimethyl sulfoxide has been shown to change keratin conformation and also interact with SC lipids.[34,35] However, the interactions of these CPEs with SC lipids was mainly on the polar head region.[34,36,37] Therefore, low fluidity in the hydrophobic chain of SCLL lipids was observed in our study.

1-Methyl-2-pyrrolidone, ethyl oleate, urea, and cetylpyridinium chloride were classified under Category E, in which the profile could not be categorized in any previous categories. The skin penetration-enhancing effect of urea and its derivatives are from keratolytic and moisturizing effects; cationic surfactants act on skin proteins; whereas pyrrolidone derivatives act on drug partitioning into SC and alter the solvent nature of SCs.[38,39] In addition, ethyl oleate is highly lipophilic compound ($\log K_{o/w}$ 6.89). Its low solubility in SCLLs dispersion medium might be a reason for low disruption effect on SCLLs, since it could not expose and penetrate into the SCLLs membrane. Thus, these reasons might support category E of exhibiting no trend in the SCLL fluidity and FL leakage plot.

The modes of action for each category CPEs could be proposed as follows: Category A, B and C CPEs might act mainly by fluidization of SC lipids. However, the degree of fluidization is different among each category. Category A CPEs the degree of fluidization is strong so that it could disrupt the SC lipids and promote the diffusion of drugs through the SC lipids which is evident from high FL leakage profile. Category B CPEs provides mild degree of fluidization, therefore they could not promote the diffusion of drugs through SC lipids. Category C CPEs is similar to category B, but at higher con-

centration, the degree of fluidization is strong enough to disrupt SC structure. On the other hand, since low fluidity was observed in category D, it might act at the lipid polar head group, or at the aqueous region between the lipid head groups. Finally, category E CPEs did not manifest any trend thus its mechanism of action cannot be identified using this test on SCLL model.

In the present study, different types and concentrations of CPEs was screened by the high-throughput approach using SCLLs. This approach provided the benefits over other previous SCLLs study in which small quantity of sample is required, rapid, simultaneous and simple determination can be performed using fluorescent microplate reader. This method was also faster and less complex compared to *in vitro* skin permeation experiment using animal skin which took about 4–48 h depending on drug permeability through skin. Moreover, the relationship between the SCLL fluidity and FL-leakage can roughly describe the mode of action of CPEs. This approach exhibits the good feasibility to test new candidate CPEs in the skin formulation development.

Conclusion

SCLLs could be a promising intercellular SC lipid model membrane for screening effective CPEs based on the investigation of FL leakage and SCLL fluidity using a DPH probe. The possible modes of action of CPEs could also be suggested from both tests. The present approach may be useful in the screening of large numbers of CPEs in the early phase of skin formulation development.

Conflict of Interest The authors declare no conflict of interest.

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