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INTRODUCTION

Transdermal route offers several potential advantages over conventional routes such as avoidance of first pass metabolism, predictable and extended duration of activity, minimizing under able side effects, utility of short half-life drugs, improving physiological and pharmacological response, avoiding the fluctuation in drug level, inter and intra patient valuations and most importantly. However one of the major problems in transdermal drug delivery is the low penetration rate through the outer most layer of the skin, stratum corneum. To date many skin penetration enhancement techniques have been developed to improve bioavailability and increase the range of drug for topical and transdermal delivery[1]. Some of skin penetration enhancement techniques include: heat, iontophoresis, electroporation, sonophoresis, microneedle or magnetophoresis[2, 3].

Microneedles (MNs) is one of the interesting physical enhancement method. This method can disrupt stratum corneum barrier by creating large aqueous microchannels enough for molecules to pass through without skin damage. Nevertheless, the length of aqueous microchannel does not reach the dermis layer, which is filled with nerves and blood vessels. Consequently, the patient does not experience pain or discomfort [4]. Ultrasound (US), also known as phonophoresis or sonophoresis, is a technique which involves the use of ultrasound energy to enhance skin penetration of drug. The mechanism of transdermal skin permeation involves the disruption of the stratum corneum lipids by the formation of gaseous cavities and heat generation, thus allowing the drug to pass through the skin. Transdermal enhancement is particularly significant higher at low frequency regimes (20 kHz - 100 kHz) than when induced by high frequency ultrasound (1 MHz – 16 MHz) [1, 5]. Therefore nowadays research fields of skin permeation using the synergistic effect of ultrasound with other methods such as chemical enhancer, microneedles are still important to be investigated. The objective of this study is to investigate the skin permeation of hydrophilic macromolecules by using MNs, low frequency US and both of MNs and low frequency US. Fluorescein isothiocyanate-dextrans (FITC-dextran) were used as model hydrophilic macromolecule.

MATERIALS AND METHODS

Material Fluorescein isothiocyanate–dextran(FD4) (MW 3,000- 5,000) was purchased from Sigma Aldrich (St. Louis, MO, U.S.A.) All other chemicals used in this study were analytical grade.

Skin preparation The pig skin is used in this experiment due to their similar physiological properties with the human skin[6]. The pig skin is obtained from slaughterhouse at Nakhon Pathom province. The skin was excised from pig immediately after the pig was sacrificed. The excised skin was fixed on top of a paraffin sheet with the epidermis layer facing up. The adhering fat and other visceral debris in the skin were then carefully removed. The underlying subcutaneous fat was gently scraped off until the skin was about 2.0-2.5 mm thick. The preparation skin was washed, wrapped in aluminum foil, and stored at -18°C.

Preparation of microneedles array The microneedles arrays were prepared by using 9 acupuncture needles (0.25 x 30 mm, DongBang acupuncture Inc., Boryeong, Korea). First, the silicone sheet with thickness 2 mm, and size 15 x 15 mm was cut. Each microneedle was cut into 4 mm in length and punctured through a silicone sheet allowing the needle tips out of a silicone sheet 1,000 μm in length. The other end of acupuncture needle was bended to obtain a perpendicular shape in order to anchor the needle. Then the microneedles array was fixed with an adhesive tape to ensure that the needles remained stationary.

Low frequency ultrasound Low frequency ultrasound at 20 kHz was delivered from a commercially available sonicator (Vibra-cell™, VCX130 PB, Sonics and Materials, Inc., Newtown, CT, USA). The amplitude of ultrasound was set at 25%. The radiating diameter of transducer was 6.0 mm. The ultrasound transducer was located approximately 3 mm. from surface of the skin. Continuous mode of ultrasound application was used in this study.

Microneedles array treatment of skin The skin was punctured by using MNs array with pressure of 10 N for 2 min. Then MNs array was removed from skin, and the skin was immediately placed on the Franz diffusion cell.

Ultrasound treatment of skin The skin was placed on the Franz diffusion cell. Before applied ultrasound, the donor compartment was filled with the FD4 solution. Then the pig skin was treated with with low frequency ultrasound for 2 minutes by using continuous mode.

Microneedles array and ultrasound treatment of skin The skin was punctured by using MNs array with pressure of 10 N for 2 min. Then MNs array was removed from skin and the skin was immediately placed on the Franz diffusion cell. After that the low frequency ultrasound was applied on the skin for 2 minutes by using continuous mode.

In vitro permeation studies Franz diffusion cell apparatus was used in vitro permeation studies. The diffusion cell has an average 2.022 cm^2 of diffusional area and the receptor compartment has a volume of 6 ml approximately. Before starting the experiments, the receptor compartment was filled with phosphate buffer saline (pH 7.4) and maintained at 32 °C using a water bath. The solution in the receptor compartment was continuously stirred at 400 rpm using magnetic stirrer in order to maintain the sink condition during the experiments. The donor and receptor compartment were then fixed by clamper. The top of the donor compartment was covered with parafilm® in order to prevent FD4 solution loss. To investigate the cumulative permeation profiles, 500 µl of the model drug solution in the receptor compartment were sampled at 5, 15 and 30 minutes, 1, 2, 4, 6, 8, 12, 16, 20 and 24 h. After sampling, the solution was immediately replaced with fresh PBS at the same volume. The samples were analyzed after finishing the experiments. All the tests were carried out in four experiments.

Preparation of FD4 Solution FD4 solution was prepared by dissolving FD4 in PBS pH 7.4 to obtain concentration 2.5 µg/ml of solution. After that, the solutions were then mixed to ensure that the FD4 was completely dissolved.

Quantitative Analysis The amount of FD4 in the solution was determined using a fluorescence spectrophotometer (RF 5300PC; Shimadzu, Kyoto, Japan) at excitation and emission wavelengths of 495 and 515 nm, respectively[7]. All experiments were performed 4-6 times.

RESULTS AND DISCUSSION

The skin permeation of FD4 by using MNs, low frequency US and both of MNs and low frequency US was investigated. Untreated skin was also studied in this experiment for representative of passive diffusion. After treatment skin with MNs, low frequency US and both of MNs and low frequency US at 24 hrs, the cumulative amount of FD4 permeated from skin treated with both of MNs and low frequency US, MNs and low frequency US increased up to 8-fold (100.120 ± 24.83 µg/cm^2), 6-fold (74.523 ± 11.47 µg/cm^2) and 2.37-fold (29.373 ± 4.35 µg/cm^2), respectively when compared with untreated skin (passive diffusion) (12.395 ± 4.84 µg/cm^2) (Table1). The permeation profiles of FD4 were shown in Figure 1. The combination of skin penetration enhancement techniques (microneedles and low frequency ultrasound) was significantly effective compared with microneedles alone or ultrasound alone. These results might be caused from a combination result of disruption of stratum corneum barrier by creating large aqueous microchannels by microneedle and disruption of the stratum corneum lipids by the formation of gaseous cavities and heat generation by low frequency ultrasound.

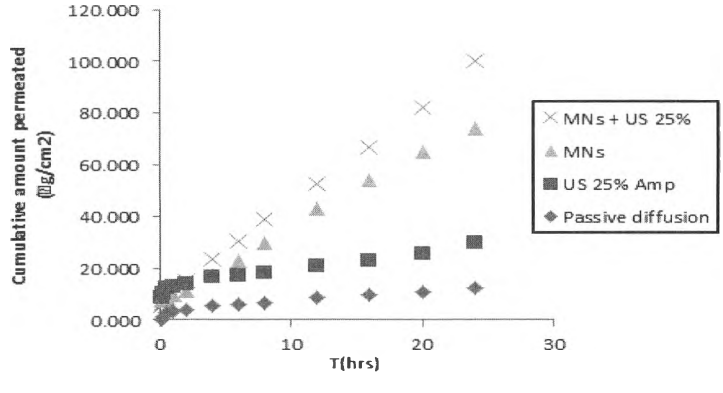

Figure 1 In vitro permeation of FD4 following treatment with MNs and low frequency US (x), with MNs (▲), with low frequency US (■) and passive diffusion (◆). Each point represents the mean S.E. of four experiments.

Table 1 Cumulative amount of FD4 permeated from skin after treatment with microneedles and low frequency ultrasound over 24 hrs.

Treatment	Cumulative amount of FD4 permeated from skin after treatment											
	5 mins	15 mins	30 mins	1 hr	2 hrs	4 hrs	6 hrs	8 hrs	12 hrs	16 hrs	20 hrs	24 hrs
Passive diffusion	0	1.262 ±2.525	3.064 ±3.538	3.463 ±4.024	3.868 ±4.497	5.598 ±4.139	6.281 ±4.492	6.694 ±4.694	8.962 ±3.048	9.969 ±3.546	10.879 ±4.063	12.395 ±4.841
Low frequency US, 25% amplitude	8.611 ±0.386	10.446 ±0.648	12.514 ±1.069	12.907 ±0.893	14.124 ±0.778	16.837 ±2.952	17.148 ±1.563	18.430 ±1.076	20.766 ±1.099	23.261 ±1.097	25.563 ±2.543	29.873 ±4.348
MNs	7.467 ±0.561	8.066 ±0.502	8.893 ±0.740	9.633 ±0.562	11.410 ±0.906	17.174 ±2.005	23.068 ±3.145	29.917 ±4.668	43.126 ±7.405	54.395 ±10.342	65.290 ±10.182	74.523 ±11.467
MNs + Low frequency US	5.748 ±0.878	6.451 ±1.168	7.360 ±1.310	9.504 ±1.326	15.149 ±3.372	23.687 ±8.951	30.635 ±5.145	38.876 ±7.289	52.801 ±13.629	66.975 ±17.271	82.444 ±21.974	100.120 ±24.830

Each value represents mean ± S.D., N=4 for all groups

CONCLUSION

In the present study, the synergistic effect with microneedles and low frequency ultrasound to transport hydrophilic macromolecules was obtained. The skin permeation of hydrophilic macromolecules in the combination of microneedles and low frequency ultrasound was significantly higher than microneedles alone or ultrasound alone because of different mechanisms in skin transport enhancement. In addition to increasing transdermal transport, the combination of skin penetration enhancement techniques also increased the safety by reducing the strength of individual skin penetration enhancement technique [8].

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DEVELOPMENT OF *GARCINIA MANGOSTANA* EXTRACT IN LIQUID CRYSTAL CREAM FOR TOPICAL DELIVERY SYSTEM

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KEYWORDS: *Garcinia mangostana*, Liquid crystal, α-mangostin, HPTLC, Cream

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INTRODUCTION

Garcinia mangostana L. is a medicinal plant that has long history in medicinal use in Southeast Asia for treatment of diarrhea, skin infection and chronic wounds[1]. The extract of its pericarp has demonstrated antibacterial activity against many types of microorganism[2, 3]. The ethanol extract of mangosteen fruit rinds was also active against *Propionibacterium acnes* and *Staphylococcus epidermis* with MIC of 7.81 and 15.63 µg/mL, respectively[4]. Currently, liquid crystal systems are used to modify drug delivery system for the delivery of topical drugs into skin[5]. Liquid crystals are highly anisotropic fluids that exist as a result of long-range orientation ordering among constituent molecules. They are also three-dimensional association structures that stabilize emulsions[6]. Our objective is to develop a liquid crystal cream containing *G. mangostana* fruit rind extract to be used as a topical anti-acne product.

MATERIALS AND METHODS

Instrument and reagents 1, 3-Butylene glycol was purchased from Kyowa Hakko (Tokyo, Japan). Carbopol ultrez polymer was purchased from Lubrizol (Ohio, USA). L-Arginine was purchased from CellMark (Balmoral Plaza, Singapore). NIKKOMULESE LC was purchased from Nikkol (Tokyo, Japan). Cetostearyl alcohol and caprylic/capric triglyceride were purchased from Parchem (New York, USA). The polarized light microscopy was performed on a Nikon Eclipse 50i POL (Tokyo, Japan). Viscosity measurement was performed using a Fungilab VISCOSTAR plus viscometer (Barcelona, Spain). All other reagents and solvents were reagent grade and used without further purification. TLC was performed on silica gel GF_{254} plates (Merck). For column chromatography, silica gel (Merck 230-400 mesh) was used. NMR spectra were recorded with a Bruker Avance (300 MHz) spectrometer. Chemical shifts are reported in ppm, and coupling constants are reported in Hz. All NMR spectra were obtained in deuterated chloroform ($CDCl_3$) and referenced to the residual solvent peak. Mass spectra were obtained from an Agilent GC/MS 5975C.

Plant material The fruit rinds of *G. mangostana* were bought from local drugstore in Nonthaburi province, Thailand. The material was identified by comparison with the specimen at the Forest Herbarium, Department of National Park, Wildlife and Plant Conservation, Ministry of Natural Resources and Environment, Bangkok. The voucher specimen of *G. mangostana* (SRU 026) was deposited at the Faculty of Oriental Medicine, Rangsit University, Pathumthani, Thailand.

Preparation of crude and partially-purified extracts The dried, powdered fruit rinds of *G. mangostana* (100 g) were extracted with 95% ethanol (400 mL) at room temperature for 7 days. The extract was filtered with Whatman No.1 filter paper and then evaporated under reduced pressure to obtain 9 g of crude dark brown extract. The crude extract (2 g) was dissolved in CH_2Cl_2-MeOH (8:2) (8 ml). The mixture was then subjected to silica gel column chromatography, eluted with CH_2Cl_2-MeOH (8:2), to obtain partially purified dark brown extract (1.2 g).

Isolation of α-mangostin The crude extract (1.2 g) was dissolved in 5 ml of CH_2Cl_2-MeOH (7:3), then subjected to silica gel column chromatography eluted with CH_2Cl_2-MeOH (7:3) as the mobile phase. After that, fractions 12-17 were collected and evaporated to obtain a yellow crystalline solid (212 mg) with melting point of 180-182 °C. UV [7] λ_{max} 244, 343 nm; IR [8] (KBr disc): 3256, 2925, 1639, 1460 cm^{-1}; ^1H NMR [9] (300 MHz, $CDCl_3$) δ [ppm]: 1.69 (s, 4H), 1.76 (s, 3H), 1.83 (s, 6H), 3.45 (d, J = 7.15 Hz, 2H), 3.81 (s, 3H), 4.09 (d, J = 6.26 Hz, 2H), 5.26 (m, 2H), 6.29 (s, 1H), 6.82 (s, 1H), 13.77 (s, 1H). ^{13}C NMR [9] (75 MHz, $CDCl_3$) δ [ppm]: 182.0, 161.6, 160.6, 155.8, 155.0, 154.5, 142.5, 137.0, 135.7, 132.2, 123.1, 121.4, 112.2, 108.5, 103.6, 101.6, 93.3, 62.0, 26.5, 25.8, 25.8, 21.4, 18.2, 17.9 and MS [9] (GC/MS): M^+ = 410.

Preparation of G. mangostana liquid crystal cream The *G. mangostana* liquid crystal cream was prepared as o/w emulsion cream. The process started with using NIKKOMULESSE LC, cetostearyl alcohol, caprylic/capric triglyceride and *G. mangostana* partially purified extract (0.1% w/w) as oil phase adding into 1,3-butylene glycol, Carbopol ultrez, L-arginine and water at 80 °C. The mixture was