Antimicrobial Activity of Curcumin-Loaded Myristic Acid Microemulsions against *Staphylococcus epidermidis*

Chi-Hsien Liu* and Hsin-Ying Huang

Graduate Institute of Biochemical and Biomedical Engineering, Chang Gung University; 259 Wen-Hwa 1st Road, Kwei-Shan Tao-Yuan, Taiwan 333, R.O.C. Received March 2, 2012; accepted June 12, 2012

The baccericial properties of myristic acid and curcumin were revealed in a number of studies. However, whether curcumin-loaded myristic acid microemulsions can be used to inhibit *Staphylococcus epidermidis*, which causes nosocomial infections, has not been reported. Our aim was to develop curcumin-loaded myristic acid microemulsions to inhibit *S. epidermidis* on the skin. The interfacial tension, size distribution, and viscosity data of the microemulsions were characterized to elucidate the physicochemical properties of the curcumin microemulsions. Curcumin distribution in neonate pig skin was visualized using confocal laser scanning microscopy. Dermal curcumin accumulation (326 µg/g skin) and transdermal curcumin penetration (87 µg/cm²/d) were obtained with the microemulsions developed herein. Curcumin at the concentration of 0.86 µg/mL in the myristic acid microemulsion could inhibit 50% of the bacterial growth, which was 12 times more effective than curcumin dissolved in dimethyl sulfoxide (DMSO). The cocktail combination of myristic acid and curcumin in the microemulsion carrier synergistically inhibited the growth of *S. epidermidis*. The results we obtained highlight the potential of using curcumin-loaded microemulsions as an alternative treatment for *S. epidermidis*-associated diseases and acne vulgaris.

Key words curcumin; myristic acid; microemulsion; *Staphylococcus epidermidis*; transdermal

The skin commensal and opportunistic pathogen *Staphylococcus epidermidis* is an important cause of many relentless and chronic bacterial infections in hospitals. This is directly related to its capability to establish multilayered, highly structured biofilms on skin surface. At present, conventional systemic therapies using antibiotics represent the main strategy to treat and prevent *S. epidermidis*-associated infections. Antibiotics such as oxacillin, cefotaxime, ciprofloxacin and vancomycin are often used to treat nosocomial infections caused by *S. epidermidis*. The spread of multiple drug resistance in *S. epidermidis* indicates a growing need for new anti-microbial agents. Essential oils and plant extracts can exhibit antibacterial activities against *S. epidermidis*. Curcumin extracted from the spice, turmeric, has multiple biological activities such as anti-inflammatory, antimicrobial and antitumor effects. The major disadvantage of curcumin as a therapeutic agent is its low aqueous solubility and poor bioavailability. Curcumin loaded alginate foams have antibacterial activity against *Escherichia coli* in photodynamic therapy of infected wounds. However, few papers reported curcumin’s antibacterial activity against *S. epidermidis*, which is also the possible pathogen for acne vulgaris. Nontraditional antimicrobial agents have garnered tremendous interest in overcoming drug-resistance of pathogenic microorganisms. The effectiveness of antimicrobial nanoparticles and nanosized carriers in treating infectious diseases was proven. For example, silver nanoparticles impede the biofilm formation by *Pseudomonas aeruginosa* and *S. epidermidis*. Nanoemulsion composed of soybean oil, Triton X-100, and tri-n-butyl phosphate disrupted membranes of enveloped viruses and G(−) bacilli. New nanocarriers such as nanoemulsions, microemulsions and liposomes are developed and investigated for topical drug delivery. Microemulsions are single-phase, optically isotropic nanostructured solutions composed of a surfactant, cosurfactant, oil, and water. Advantages of microemulsion as drug carriers include easy preparation, high drug solubilization, optical transparency and high skin accumulation. The skin provides a natural physical barrier against foreign penetration, but there are opportunities to deliver therapeutic nanoparticles, especially in diseased skin and to the openings of hair follicles. Microemulsions were applied to deliver different lipophilic drugs including genistein, daidzein, and granisetron. These in vitro and in vivo studies demonstrated that lipophilic drugs incorporated into microemulsions can efficiently be delivered to the skin. Although topical curcumin delivery for treating skin disorders is promising, little work has focused on combining curcumin and myristic acid for topical inhibition of *S. epidermidis*. Our main purposes were to investigate the in vitro antimicrobial effects of curcumin-loaded myristic acid microemulsions and curcumin accumulation in neonate pig skin since it has not been clearly studied yet. This study evaluated the antimicrobial properties of fatty acids and curcumin in microemulsions against *S. epidermidis*. The delivery efficacy of curcumin-loaded vehicles in porcine skin was evaluated using neonate pig skin mounted on Franz diffusion cells. The transdermal distribution of curcumin in the skin was visualized by using confocal laser scanning microscopy.

Experimental

**Materials** Tween 80, F127, isopropanol, and fatty acids were obtained from Sigma (St. Louis, MO, U.S.A.). Curcumin was procured from Mastersasia (Taipei, Taiwan). BacTiter-Glo™ kit for microbial assay was purchased from Promega (Madison, WI, U.S.A.). All reagents were used without further purification. Water was freshly purified by the Milli-Q Gradient A10 system (Millipore, Molsheim, France).

**Preparation of Microemulsions** Myristic acid was mixed with isopropanol in the weight ratio of 1:2 to increase its solubility. Microemulsion composed by myristic acid, isopropanol, and surfactants (Tween 80 and F127) were prepared by drop-wise adding the required amount of water into the pre-mixed solution under gentle magnetic stirring for 10 min.

The authors declare no conflict of interest.
After being mixed for 10 min, the systems could form single-phase and transparent microemulsions.

**Characterization of Microemulsions** Microemulsions were prepared by mixing the oil with the surfactant or surfactant/cosurfactant mixture before adding the required amount of water under magnetic stirring. Curcumin (4 g/L) was then added to the prepared microemulsion. No phase change was noted after addition of the drug or after equilibration in the water bath. The flow properties and viscosity of the formulations were determined at 32±1°C. Viscosity determinations employed a Brookfield viscometer (DV II+, Brookfield, Stoughton, MA, U.S.A.). Interfacial tension measurements were carried out at room temperature using a thin platinum plate attached to a transducer amplifier (Kyowa CBVP-A3, Saitama, Japan). The average particle size was characterized using a Zetasizer Nano ZS 90 (Malvern, Worcestershire, U.K.) at a fixed angle of 90° and a temperature of 32°C. Microemulsion samples were measured without dilution by water or solvent in order to avoid the dilution effects on the size distribution in the microemulsion.

**In Vitro Curcumin Release** Full-thickness skin from the pig ear is a generally accepted model of permeation for human dermatological research. In this study, the skin of the outside of the ears of corpses of new-born piglets was used to study the permeation of curcumin in a Franz diffusion assembly. The skin was peeled from the underlying cartilage after cutting along the tips of the ears. The ear skin was mounted between the donor and receptor compartments with the stratum corneum side facing the donor compartment. The donor medium consisted of 0.5 mL of vehicle containing curcumin. The receptor medium (5.5 mL) was obtained by mixing 1:1 ethanol and phosphate-buffered saline (PBS, pH 7.4) in order to maintain sink conditions. Similar solution was successfully applied to monitor the skin delivery of curcumin from microemulsions.

The available diffusion area between the cells was 0.785 cm². The stirring rate and temperature were respectively kept at 600 rpm and 32°C. At appropriate intervals, all receptor medium was withdrawn and immediately replaced with equal volumes of fresh medium. Cumulative amounts of curcumin permeated after 24 h diffusion were used to calculate the transdermal drug flux. The permeated amount of curcumin was determined by high-performance liquid chromatography (HPLC). The curcumin accumulated in the skin was measured after the permeation experiment (24 h). The skin was washed three times using a cotton cloth containing ethanol. A skin sample with the 0.785 cm² permeated area was cut, weighed, and then homogenized in 50% ethanol PBS solution for 5 min at 10000 rpm rate. The resulting solution was centrifuged, and the supernatant was analyzed by HPLC.

**Curcumin Analysis** Quantification of curcumin was achieved using an HPLC system (Jasco, Tokyo, Japan) consisting of a pump, a UV detector, and a Microsorb-C18 column (Varian, Lake Forest, CA, U.S.A.). The mobile phase was consisted of 1% (w/v) acetic acid, 73% (w/v) methanol, and 26% (w/v) water. The flow rate of the mobile phase was 1.0 mL/min. The column effluent was monitored at 430 nm, and the chromatographic data was analyzed by the Borwin Program (Version 1.5, Jasco).

**Fluorescence Examination of Porcine Skin** Skin was treated with PBS and microemulsions (ME6, ME6+5% F127) respectively for 24 h on a Franz diffusion cell. For confocal laser scanning microscopy observation, the skin was removed from the diffusion cell, rinsed with 50% ethanol and then the surface of the skin was wiped gently. The skin was directly sandwiched between a glass slide and a coverslip in a 1:1 solution of PBS—glycerol, and examined confocal microscopy without additional tissue processing. Confocal laser scanning microscope (Leica, SP2, Heerbrugg, Switzerland) is used to analyze the delivery path of curcumin in the skin. The fluorescence of curcumin was excited at a wavelength of 420 nm by means of an argon laser. To visualize the distribution of curcumin, confocal images were first obtained in the xy-plane. The top surface of skin (z=0 μm) was defined as the fluorescence plane with a morphology characteristic of the stratum corneum surface. The skin sample was scanned from the skin surface (0 μm) to a depth of 160 μm at a 5.3-μm interval.

**Determination of Antibacterial Activity** *S. epidermidis* BCRC-11030 (ATCC 12228) was obtained from Bioresource Collection and Research Center (Hsinchu, Taiwan) and was cultured on Nutrient agar (Oxoid, Hampshire, England). Single colonies were inoculated in Nutrient broth and cultured at 37°C until reaching around OD600 1.0 (logarithmic growth phase) under shaking conditions. Then the seed culture was diluted with fresh Nutrient broth to the value of 0.1 (optical density (OD)600) for further microbial growth experiments. Drugs and drug-contained microemulsions were diluted by dimethyl sulfoxide (DMSO) in a 2-fold serial manner and added at the 1% volume ratio to the 96-well microtiter plates. The bacteria were cultured in plates (volume=0.2 mL) for 24 h and the growth was quantified by the BacTiter-Glo™ assay (Promega). The assay uses bioluminescence produced by luciferase to detect the intracellular concentration of ATP, which correlates with viable bacterial cell numbers. In brief, the reaction involves adding a single reagent directly to bacterial cells cultured in medium and measuring luminescence generated by bacterial ATP and a thermostable luciferase. The bioluminescence was measured by Epoch Spectrophotometer (Biotek, Winooski, VT, U.S.A.). The control group was *S. epidermidis* incubated with 1% (v/v) DMSO.

**Results and Discussion**

**Fatty Acids and Curcumin Inhibit in Vitro Growth of *S. epidermidis*** Since fatty acids were reported to inhibit several microorganisms, the inhibitory effects of fatty acids with different chain lengths on *S. epidermidis* were investigated to evaluate their antimicrobial effects at 50 μg/mL concentration (Fig. 1). The fatty acids and their derivatives tested in this study included butyric (C4), caproic (C6), amino caproic (NH2-C6), heptanoic (C7), octanoic (C8), decanoic (C10), lauric (C12), myristic (C14), palmitic (C16), and stearic acids (C18). Middle-chain fatty acids such as myristic, palmitic and lauric acid significantly inhibited the growth of *S. epidermidis* (p<0.05) compared to short-chain fatty acids (carbon number <10). The inhibition efficacy was ranked in the following order: myristic>palmi tic>lauric acid (Fig. 1). Among the tested fatty acids, myristic acid most effectively inhibited the growth of *S. epidermidis* at the inhibitory concentration of 80.6%. Azelaic acid and curcumin were chosen as controls since azelaic acid is approved for treating mild-to-moderate acne vulgaris and curcumin has antibacterial activity against several G(+) and G(−) bacteria in therapy of infected wounds. The dose effects of curcumin, azelaic acid, and myristic acid on *S.
epidermidis were evaluated using DMSO as the dissolving vehicle (Fig. 2). Curcumin and myristic acid strongly inhibited microbial growth compared to azelaic acid. Their inhibition abilities were ranked in the order of curcumin > myristic acid > azelaic acid with respective IC50 concentrations of 10.5, 52.0, and 452.2 µg/mL. Azelaic acid, the topical drug for treatment of acne, showed the least antimicrobial activity against *S. epidermidis*.

In contrast, curcumin and myristic acid inhibited 79.9% and 71.2% of the growth of *S. epidermidis* at concentrations of 31.2 and 62.5 µg/mL, respectively. Curcumin showed greater inhibitory ability against *S. epidermidis* compared to myristic acid. Notably, there was a 43-fold decrease in the IC50 concentration for curcumin compared to azelaic acid. Myristic acid also had an 8.7-fold increase in inhibition compared to azelaic acid. The results indicated the potency of curcumin and myristic acid as candidates for treating acne vulgaris. Recently curcumin and its derivatives exhibited antibacterial activity against *Staphylococcus aureus*, *E. coli*, *Bacillus cereus*, and *Yersinia enterocolitica*.\(^\text{19}\) The antibacterial activity of curcumin is due to inhibition of the cytokinetic Z-ring assembly from FtsZ protofilaments, which playing critical roles in bacterial cytokinesis.\(^\text{20}\) Fatty acids inhibit gram-positive cocci by disrupting the electron transport chain and oxidative phosphorylation of the bacteria.\(^\text{17}\) However curcumin and myristic acid both have poor water solubility which limits their therapeutic applications. Microemulsions were applied to deliver hydrophobic drugs with the advantages of high drug-solubility, high stability, and easy dermal penetration.\(^\text{21}\) Microemulsions are composed of four major components including oil, water, a surfactant, and/or a cosurfactant. All four ingredients are essential for the formation and stability of microemulsions. Isopropanol can act as a cosurfactant to increase the solubility of myristic acid and enhance the stability of the oil–water interface of the microemulsions. Myristic acid was the only oil in the microemulsions that used to accommodate curcumin. The dermal properties of curcumin-loaded myristic acid microemulsions and the antimicrobial activity against *S. epidermidis* were systematically evaluated in the following sections.

Transdermal Curcumin Accumulation and Antimicrobial Activity of Microemulsions Microemulsions are composed of an aqueous phase, oil phase, and surfactant/cosurfactant from which transparent and mono-phase emulsions are formed. Since myristic acid is a saturated fatty acid with limited water solubility, isopropanol can act as a cosurfactant to increase the solubility of myristic acid and enhance the stability of the oil–water interface. The formulations of 18 microemulsion tested were shown in Table 1. Curcumin accumulation and delivery in porcine ear skin of microemulsions with various surfactant/water ratios and a fixed myristic acid/isopropanol concentration were evaluated. The results of curcumin penetration and dermal curcumin accumulation are shown in Figs. 3 and 4. The curcumin accumulation in the skin increased from 1.0 to 35.2 µg/g skin after 24h diffusion experiments as the water content increased from 10 to 50%. Curcumin penetration after 24h of diffusion increased from 0.2 to 88.6 µg/cm² as the water/Tween 80 ratio increased from 0/5 to 5/1. Completely removal of Tween 80 in the formulation caused the decrease of curcumin penetration and the increase of curcumin accumulation as comparing ME5 with ME6 (Figs. 3 and 4). The stratum corneum is the main barrier of the skin to transdermal curcumin delivery. The fact that ME5
could overcome the barrier to deliver curcumin might be related to the following reasons. First, 10% Tween 80 maintained the droplet size at around 36 nm as indicated in Table 1. The small size of the ME5 formulations could increase curcumin’s penetration. Second, the viscosity of ME5 was very low since only 10% Tween 80 was added. A high viscosity hampers the efficacy of transdermal delivery as it decreases the diffusion rate. Finally, the combined effects of all ingredients in the microemulsions may have played a role in improving curcumin penetration through the stratum corneum by modulating its microstructure. However, these hypotheses require further confirmation. The objective of this study was to develop microemulsions for curcumin accumulation on the topical skin, not for curcumin penetration into the circulatory system. Therefore, the ME5 formulation was not further developed or investigated in this study.

When the water content in microemulsions increased, the curcumin penetration increased (Fig. 3). Microemulsions would change from water-in-oil to oil-in-water types when the water content increased. In this study oil-in-water microemulsion had better curcumin accumulation and penetration in the porcine skin. Similar increasing of transdermal domperidone delivery was observed when decreasing Tween 80 concentration. Microemulsion types (water-in-oil or oil-in-water) would affect the delivery efficacy of curcumin. F127 was incorporated into microemulsions to stabilize the microemulsions. The good emulsifying capacity, low toxicity, and sustained release characteristics of F127 render it an attractive candidate as a pharmaceutical vehicle for drugs. Effects of the surfactant/water ratio and F127 content on the size distribution of emulsions were indicated in Table 1 by using a dynamic light-scattering method. Colloid size directly impacts the stability, bio-distribution, and release kinetics. Nanosized particles with a large surface area could closely contact tissues and have better chances to penetrate bio-barriers and transport drugs in a more-controlled fashion. Surfactant/water ratios greatly impacted on the size distributions as shown in Table 1. The size distribution of these 18 emulsions was in the range 1.45–448 nm. The addition of 5% F127 reduced the colloidal sizes of ME6 from 236 (0% F127) to 4.12 nm. Further increase in F127 concentration (10%) will increase the size of particles to 46.6 nm in ME6 microemulsion. Particle sizes of the microemulsions decreased as the water content in the formulation increased except in ME6. The change in droplet size during storage is a good indicator of the stability of a microemulsion. According to Table 1, the droplet size of ME6 (without F127) was around 235 nm, and phase separation appeared after 1 week of storage. After the addition of 5% F127 to the ME6 formulation, the droplet size decreased to 4.12 nm, and no phase separation or aggregation was observed after 3 months of storage. These results indicate the stability of ME6+5% F127. Since Tween 80 and F127 are pharmaceutical-grade surfactants, they are considered to be safe excipients for drug formulations. By comparing the dermal curcumin accumulation levels, 5% F127 was found to be better than Tween 80 (Fig. 4). Simplifying the complex formulation is important for academic studies and clinical applications. However, microemulsions composed of three components (no cosurfactant or surfactant) were not stable as indicated by their large droplet sizes in Table 1. The dermal accumulation of curcumin with the surfactant-free solution (ME6) was also less than that of the surfactant-containing microemulsion (ME6+5% F127) as indicated in Figs. 3 and 4. Surfactants can reduce the colloidal size by reducing the surface tension and fluidizing the interfacial droplet film. Here, 5% F127 combined with myristic acid microemulsions (ME1–ME6) elevated curcumin accumulation as indicated in Fig. 4. However, a higher F127 content (10%) did not enhance curcumin accumulation in the skin compared to the controls (0% and 5% F127). The rate of curcumin transdermal penetration was ranked ME6+5% F127>ME6>ME6+10% F127 at a high water content (50%) as shown in Fig. 3. It was noted that hydration by water in

---

Table 1. Formula and Size Distribution for Microemulsions (ME)\(^a\)

<table>
<thead>
<tr>
<th>Microemulsion</th>
<th>Tween 80 (%)</th>
<th>Water (%)</th>
<th>Myristic acid/isopropanol (%)</th>
<th>Particle size (nm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ME1</td>
<td>50</td>
<td>0</td>
<td>16.7/33.3</td>
<td>2.55±0.17</td>
</tr>
<tr>
<td>ME2</td>
<td>40</td>
<td>10</td>
<td>16.7/33.3</td>
<td>1.45±0.51</td>
</tr>
<tr>
<td>ME3</td>
<td>30</td>
<td>20</td>
<td>16.7/33.3</td>
<td>2.55±0.38</td>
</tr>
<tr>
<td>ME4</td>
<td>20</td>
<td>30</td>
<td>16.7/33.3</td>
<td>22.64±2.19</td>
</tr>
<tr>
<td>ME5</td>
<td>10</td>
<td>40</td>
<td>16.7/33.3</td>
<td>36.60±2.62</td>
</tr>
<tr>
<td>ME6</td>
<td>0</td>
<td>50</td>
<td>16.7/33.3</td>
<td>235.83±107.59</td>
</tr>
</tbody>
</table>

\(^a\) All microemulsions contain 0.4% (w/v) curcumin (mean±S.D., n=3).
the stratum corneum can enhance drug delivery as previously described.28) F127 is an U.S. Food and Drug Administration (FDA)-approved biocompatible surfactant for sustained drug delivery. For example, F127 was used to enhance the delivery of 5-aminolevulinic acid for treating actinic keratosis in photodynamic therapy.22) F127 combined with lecithin enhanced the stability and absorption of sumatriptan across the skin.29) Percutaneous absorption of nonvamide from F127 vehicles was reported by using Wistar rats as an animal model.30) In this study, we found that the addition of 5% F127 to myristic acid microemulsions had the maximal curcumin’s accumulation in porcine skin.

The antimicrobial activities of three curcumin-loaded vehicles were shown in Fig. 5. Curcumin-loaded microemulsions (ME6 and ME6+5% F127) and the mixture of curcumin–myristic acid (1:1) were diluted in DMSO and incubated with S. epidermidis in the growth medium for 24h to determine their inhibitory activities. The results indicated that 0.86 µg/mL curcumin in ME6+5% F127 (also containing 35.7 µg/mL myristic acid) inhibited 50% bacterial growth. The mixture of 10.7 µg/mL curcumin and 10.7 µg/mL myristic acid in the DMSO vehicle inhibited 50% growth of S. epidermidis. In contrast, 52 µg/mL of myristic acid in DMSO solvent could only inhibit 50% growth of S. epidermidis as indicated in Fig. 2. The IC50 values of curcumin in different vehicles of DMSO, ME6 and ME6+F127 were 10.5, 1.38 and 0.86 µg/mL, respectively. The addition of 5% F127 to ME6 slightly decreased the curcumin’s IC50 value. Significantly the antimicrobial activity of curcumin was enhanced by loading it in myristic acid microemulsions (Figs. 2, 5). The spread of multiple drug resistance in S. epidermidis indicates a growing need for new antimicrobial agents. To address this microbial threat, a combination of myristic acid and curcumin in microemulsions against S. epidermidis was developed herein. The possible mechanisms for the enhanced effectiveness of myristic acid microemulsions might be due to the following reasons. Nanosized microemulsions can help drugs (myristic acid and curcumin) contact with bacteria and efficiently penetrate membranes. Combination of two agents possessing distinct mechanisms may exert a more-potent inhibitory effect than a single agent. For example, the combination of 5% azelaic acid and 2% clindamycin is used to clinically treat acne vulgaris.17) The cellular target of myristic acid is the electron transport chain and oxidative phosphorylation.17) The antibacterial mechanism of curcumin is an alteration of the assembly and stability of FtsZ protofilaments in bacterial cytokinesis.25) Only 0.86 µg/mL curcumin loaded in ME6+5% F127 achieved 50% microbial inhibition, which required 10.5 µg/mL of curcumin by itself as indicated in Figs. 2 and 5. Our in vitro results indicate that the cocktail combination of myristic acid and curcumin in the microemulsion carrier may provide a new strategy for anti-S. epidermidis therapy. The physicochemical features of microemulsions were characterized in the next section in order to investigate the accumulation-enhancing effects of curcumin-loaded myristic acid microemulsions.

**Physicochemical Properties of Microemulsions**

The characteristics of surface tension and viscosity are used to describe the colloidal properties of microemulsions.31) In this study, the physical properties such as viscosity and surface tension of curcumin-loaded microemulsions were analyzed. The information may provide some clues to explain why the surfactant/water content affected the efficacy of curcumin dermal delivery. The interface tension and viscosity of the 18 formulations are summarized in Fig. 6. One of the useful properties of microemulsions is the typically low interfacial tension at liquid–liquid interfaces that could stabilize colloidal systems. The interfacial tension of myristic acid microemulsions slightly changed from 26.1 to 16.6 mN/m when the water contents increased. The increase of F127 in ME6 also increased the interfacial tension from 16.6 to 21.8 mN/m. The low interfacial tension may facilitate formation of nanosized colloids in suspension which prevents the oil separation from the microemulsion. Additionally, low interfacial tension may help the contact of microemulsions with the skin and enhance the curcumin accumulation in the skin.

The viscosity of curcumin-loaded myristic acid microemulsions was also analyzed. The viscosity of microemulsions increased from 16.2 to 246.5 cP when F127 increased from 0 to 10% in ME6 (Fig. 6). The amount of Tween 80 (0–50%) could enhance the viscous property of the microemulsions from 60.2 to 246.5 cP in ME6. The viscosity of drug-loaded microemulsions was reported to influence drug partitioning into the skin.32) High viscosity hampers the efficacy of transdermal delivery as it decreases the diffusion rate.20) The most viscous microemulsion with 10% F127 had the lowest transdermal flux compared to 0% and 5% F127 as indicated in Fig. 3. The oil-in-water microemulsion containing 5% F127 had the best curcumin accumulation in the skin. The microemulsion structure was changed from water in oil, bicontinuous phase to oil in water as the water content increased. Additionally, the water content in microemulsions has been reported to contribute the indomethacin accumulation in the skin.32) The skin distribution of curcumin was investigated for the understanding of the possible mechanisms for the developed microemulsions.

**Curcumin Distribution in the Skin**

Confocal laser scanning microscopy was used to monitor the gradient of...
curcumin in the porcine skin. Confocal microscopy is extensively used as a tool to visualize fluorescent model compounds in skin. The 540 nm fluorescence of curcumin excited by HeNe laser was used to trace its transdermal route. This method does not require fixation of skin samples and thereby reduces the redistribution of the stain and tissue damage. Curcumin resided in the skin for three formulations was visualized by confocal microscopy. Figure 7 presents fluorescent images of curcumin distribution in the skin with three different vehicles of PBS, ME6, and ME6+5% F127. The fluorescence intensities of curcumin for three vehicles were ranked ME6+5% F127 > ME6 > PBS. These results are consistent with curcumin accumulation in the diffusion experiments (Fig. 4). The fluorescence intensity of curcumin at different skin depths is summarized in Fig. 8. The formulation (ME6+5% F127) exhibited the steepest penetration of curcumin, which provided a driving force for the curcumin accumulation. Among the tested formulations, the ME6+5% F127 formulation had the highest curcumin accumulated in the skin after 24h diffusion experiment (Fig. 7C). The maximal fluorescence intensity of curcumin appeared at the same depth of 55 µm in the skin when ME6 and ME6+5% F127 were applied (Fig. 8). Interestingly, the depth of maximal fluorescence intensity for curcumin/PBS was 23 µm, which was shallower than with the two microemulsions. The results indicated that a curcumin reservoir in the skin formed with the three vehicles.

The confocal results of the present study also showed distinct profiles of curcumin localization within the skin that were dependent on vehicle compositions. The fluorescent tubules in Figs. 7B and C might be hair follicles on the skin which could accumulate a large amount of curcumin and form the reservoir for curcumin. Hair follicles and sebaceous glands could participate in drug penetration through the skin for a wide range of compounds. The fluorescence images of curcumin confirmed the conclusion of the diffusion experiment that ME6+5% F127 could enhance the curcumin accumulated in the porcine skin. Finally, various mechanisms could be proposed to explain the enhancing effects of ME6+5% F127 on dermal curcumin accumulation. First, F127 can reduce the droplet size as indicated in Table 1. The high surface/volume ratio of oil droplet increases curcumin’s contact area with the skin and raises the amount of curcumin deposited in hair follicles of the skin. Second, F127 adsorbed in the skin may modulate the stratum corneum and increase the partitioning of curcumin into the skin. This curcumin reservoir also created a high drug concentration within the upper layers of the skin and resulted in a high curcumin gradient as a driving force for dermal curcumin delivery. This possibility was supported by our confocal results that efficient delivery of curcumin was accompanied by high curcumin retention in the skin (Figs. 7, 8). The third possibility is that myristic acid is a well-known...
skin penetration enhancer which disturbs the double layers of the epidermis. Finally, the combined effects of all ingredients in the microemulsions may also have played a role in improving curcumin permeation and accumulation. However, these hypotheses need further confirmation.

Conclusion
Curcumin-loaded myristic acid microemulsion was developed to study the \textit{in vitro} inhibition of \textit{S. epidermidis} and cumulative accumulation in the skin. Our results demonstrated that myristic acid microemulsion was a good vehicle for delivering curcumin and inhibiting \textit{S. epidermidis}. Combined effects of curcumin and myristic acid were confirmed in inhibiting \textit{S. epidermidis}. Maximal curcumin accumulation in the skin was observed with myristic acid microemulsions composed of 5% F127, 31.6% isopropanol, 15.9% myristic acid, and 47.5% water. The important findings revealed in this study are that the inhibitory activity against \textit{S. epidermidis} was enhanced, and stratum corneum could be penetrated using the curcumin-loaded myristic acid microemulsion. Our \textit{in vitro} results indicate that the cocktail combination of myristic acid and curcumin in the microemulsion carrier may provide a new strategy for anti-\textit{S. epidermidis} therapy. Further animal tests of the pharmacological activities on the model disease would confirm the efficacy and synergism of the microemulsion formulations. In summary, curcumin-loaded myristic acid microemulsions are a promising tool for the topical delivery of curcumin to inhibit the growth of \textit{S. epidermidis} on the skin.

Acknowledgments The project was supported by Grants from the National Science Council (NSC 100-2628-E-182-002) and Chang Gung Memorial Hospital (CMRPD 2A0101), Taiwan.

References