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*Research Article*

# Transcutol® P/Cremophor® EL/Ethyl Oleate–Formulated Microemulsion Loaded into Hyaluronic Acid–Based Hydrogel for Improved Transdermal Delivery and Biosafety of Ibuprofen

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***Abstract.*** In the present study, a novel transdermal delivery system was developed and its advantages were demonstrated. Ibuprofen is a commonly used anti-inflammatory, antipyretic, and analgesic drug; however, because of its short biological half-life, it must be frequently administered orally and is highly irritating to the digestive tract. To prepare a novel transdermal delivery system for ibuprofen, a microemulsion was used as a drug carrier and dispersed in a hyaluronic acid-based hydrogel (ME/Gel) to increase percutaneous drug absorption while avoiding gastrointestinal tract irritation. The prepared microemulsion had a droplet size of ~ 90 nm, and the microemulsion had good stability in the hydrogel. Rheological tests revealed that the ME/Gel is a pseudoplastic fluid with decreased viscosity and increased shear rate. It displayed a certain viscoelasticity, and the microemulsion distribution displayed minor effects on the rheological characteristics of the hydrogel system. There was no significant difference in the rheology of the ME/Gel at 25°C and 32°C (normal skin surface temperature), which is beneficial for clinical application. Drug transdermal flux was significantly higher than that of the hydrogel and commercial cream groups ($p < 0.01$). The 24-h cumulative drug permeation amount was 1.42-fold and 2.52-fold higher than that of the hydrogel and cream groups, respectively. By loading into the ME/Gel, the cytotoxicity of the drug to HaCaT cells was reduced. These results indicate that the prepared ME/Gel can effectively improve transdermal ibuprofen delivery and the biosafety of the drug and could therefore have applicability as a drug delivery system.

**KEY WORDS:** nanocarrier; percutaneous; permeability; stratum corneum.

## INTRODUCTION

Ibuprofen (Fig. 1a; molecular weight, 206.28 Da; logP (octanol/water), 3.68; pKa, 4.5–4.67) is a non-steroidal anti-inflammatory drug with anti-inflammatory, antipyretic, and analgesic effects (1,2). Ibuprofen is irritating to the gastrointestinal tract (3). Because of its short biological half-life, it must be frequently administered to maintain the therapeutic concentration (1). Long-term medication can cause adverse reactions in the digestive tract, including indigestion, stomach burning, stomach pain, nausea, and vomiting. Transdermal administration can avoid the irritating effect on the gastrointestinal tract owing to oral administration by reducing the first-pass effect of the drug, eliciting a sustained-release long-acting effect, and improving bioavailability.

The main limitation in the development of transdermal drug delivery preparations is the physiological barrier of the skin, especially the stratum corneum barrier. The stratum corneum is composed of tightly packed lipids that reduce or even impede drug penetration. Most drugs pass through the barrier at a very low flux; hence, it is difficult to achieve an effective therapeutic concentration (4). Many physical technologies have been applied to enhance transdermal drug delivery, such as iontophoresis, ultrasound, electroporation, and microneedles; however, these methods require special equipment and their operation is highly specialized. Nanocarriers and the chemical permeation enhancers can effectively promote penetration with convenient application. In recent years, microemulsion has been widely used in transdermal drug administration due to its prominent penetration-promoting effect (5–10).

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**Abbreviations:** *IBU*, Ibuprofen; *HA*, Hyaluronic acid; *ME*, Ibuprofen-loaded microemulsion; *ME/Gel*, Ibuprofen-loaded microemulsion-based hyaluronic acid hydrogel; *Gel*, Ibuprofen-loaded hyaluronic acid hydrogel; *NS*, Normal saline.

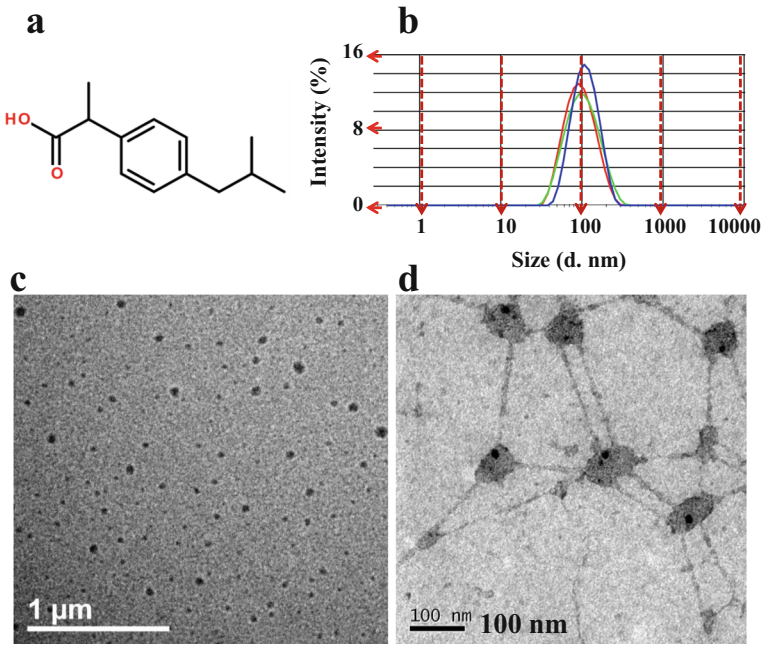


**Fig. 1.** Characterization of the ibuprofen-loaded microemulsion (ME) and ibuprofen-loaded microemulsion-based hyaluronic acid hydrogel (ME/Gel). **a** Ibuprofen chemical structure. **b** Size distribution of the blank microemulsion (Red), ME (green), and ME/Gel (blue). **c** and **d** The transmission electron micrographs of ME and ME in ME/Gel

Microemulsion refers to a well-defined, thermodynamically stable system formed by the self-assembly of an oil phase, an aqueous phase, a surfactant, and a co-surfactant. The microemulsion has a droplet size between 10 and 100 nm and is evenly distributed (11). Many surfactants and co-surfactants in the microemulsion formulation can form a stable interfacial film between oil and water. They can reduce the interfacial tension between the phases, ultimately reducing droplet aggregation and adhesion to achieve excellent formulation stability (12). The microemulsion is easy to prepare at room temperature, and the oil-in-water type microemulsion exerts a strong solubilizing effect on poor water-soluble drugs (9). The microemulsion also has high moisturizing property and high permeability, and after transdermal administration, a drug depot can be formed in the skin to achieve efficient transdermal delivery of the drug (13).

Due to the poor water solubility of ibuprofen, microemulsion is used as a carrier for transdermal administration, which can simultaneously promote solubilization and skin permeability. The highly purified diethylene glycol monoethyl ether under the trade name Transcutol® P, is widely used as a solubilizer and penetration enhancer in topical, transdermal, and oral drug delivery systems (14–16). The microemulsion containing imiquimod was prepared by using Transcutol® P as a co-surfactant with the amount of drug contained being 1/4 of that of the commercial cream; however, its percutaneous drug penetration was nearly twice as high as that of the latter (17). Ethyl oleate and polyoxyethylene castor oil (Cremophor® EL) are commonly used as excipients for the creation of microemulsions. The former has good stability and the latter has strong emulsifying properties; hence, the microemulsion that they form generally has high drug loading and small droplet size distribution, which are beneficial for the enhancement of drug permeation. Therefore, in the current work, the microemulsion was prepared with Transcutol® P/Cremophor® EL/ethyl oleate systems.

To facilitate transdermal administration, drug-loaded microemulsion can be dispersed in a hydrogel. Hyaluronic acid (HA) is an acid mucopolysaccharide (18). Due to the hydrogen bond between the monosaccharides on the linear axis, the HA molecules are spatially rigid spiral columnar structures, and the inside of the column is strongly hydrophilic due to the presence of many hydroxyl groups, thereby locking the water molecules. In its double-helix columnar structure, moisture is not easily lost; thus, it has a special water retention effect and is known as an excellent natural moisturizing factor (19). HA enhances skin hydration during external moisturizing, which further loosens the stratum corneum and improves skin permeability, ultimately contributing to its role as a good permeation enhancer (20,21). Herein, we evaluated the stability of microemulsion in HA-based hydrogels, as well as the transdermal penetration and skin safety of microemulsion gel systems, providing a foundation for further development and application of this new transdermal drug delivery system.

## MATERIALS AND METHODS

### Materials

Ibuprofen (API, purity > 99%) was purchased from Simeiquan Biotechnology Co., Ltd. (Shenzhen, China); ethyl oleate was a gift from the Tianzheng Pharmaceutical

Excipient Co., Ltd. (Xi'an, China); polyoxyethylene castor oil (Cremophor® EL) was purchased from BASF SE (Ludwigshafen, Germany); highly purified diethylene glycol monoethyl ether (Transcutol® P) was obtained from GATTEFOSSé (France); hyaluronic acid was provided by FREDA Pharmaceutical Group Ltd. (Jinan, China); ibuprofen cream was purchased from SK&F (Tianjin, China); and Cell Counting Kit-8 (CCK-8) was purchased from Dalian Meilun Biotechnology Co., LTD (Dalian, China). Other chemicals were procured from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China).

## Animals and cell lines

Healthy adult Sprague-Dawley rats (male, weight 120–140 g, clean grade) and BALB/c nude mice (male, 20 ± 3 g) were provided by the Laboratory Animal Center of Shanghai University of Traditional Chinese Medicine. The study protocol was approved by the Experimental Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine (License No. SYXK (HU) 2014-0008).

HaCaT cells were provided by the Cell Resource Center of the Institute of Basic Medicine, Chinese Academy of Medical Sciences.

## Ibuprofen Detection Method

Ibuprofen in the *in vitro* samples was measured by high-performance liquid chromatography (HPLC) (LC-2010A HT, Shimadzu, Japan) with a Platisil ODS column (250 × 4.6 mm, 5 μm) (DIKMA, Beijing, China); the mobile phase was methanol–0.01 mol/L potassium dihydrogen phosphate–phosphoric acid (700:300:0.1, v/v/v) and flow rate was 1.0 mL/min. Detection wavelength was 263 nm. The retention time of ibuprofen was approximately 9.6 min, and the solvents and impurities did not interfere with the peak for ibuprofen. The limit of quantitation was 0.04 μg/mL.

In the drug concentration range, 0.625to 80 μg/mL, the linear relationship between drug concentration (C) and chromatographic peak area (A) was good and the linear regression equation was $C = 1584.0 \times A + 646.7$ ($r = 0.999$).

## Preparation and Characterization of the Microemulsion and Hydrogel

### *Ibuprofen-Loaded Microemulsion-Based HA Hydrogel*

According to the formulations listed in Table I, the surfactant, co-surfactant, oil phase, and ibuprofen were mixed, and then magnetically stirred at 300 rpm at room temperature until the drug was completely dissolved. Following the slow addition of water to form a uniform microemulsion, Azone, a commonly used permeation enhancer in transdermal delivery studies, and HA were added under continued magnetic stirring overnight until a clear semi-solid hydrogel was formed.

### *Ibuprofen-Loaded HA Hydrogel*

The compositions listed in Table I in the ibuprofen-loaded HA hydrogel (Gel) formulation were mixed and magnetically stirred overnight at 300 rpm at room temperature until a semi-solid hydrogel with uniform drug dispersion was formed.

Measurement of microemulsion size distribution: The microemulsion and microemulsion gel were diluted 20-fold (v/v) with purified water. Subsequently, droplet size was measured by dynamic light scattering (Zetasizer Nano S90, Malvern Panalytical, UK). Each measurement was performed in triplicate.

The appearance of the microemulsions was examined using transmission electron microscopy (TEM) (JEM-1400; JEOL Ltd., Japan). Samples were prepared for negative staining as follows: copper nets carrying a formvar-supporting film (Xinxing Braim Technology Co., Beijing, China) were placed onto a stencil plate. MEs were dropped gently onto the film and then allowed to dry for ~ 20 min. A drop of 2% phosphotungstic acid was then added to the film and allowed to dry for 10 min. The film was observed under a transmission electron microscope.

## *In vitro* Transdermal Test

### *Preparation of Excised Skin*

Rats and nude mice were used to evaluate the transdermal delivery of the tested formulations (22,23). Animals were anesthetized with 10% chloral hydrate and humanely killed. The hair on the abdomen of rats was removed with an electric razor, and the skin was washed with saline. The skin of the abdomen was excised, the subcutaneous tissue and fat were removed, and the integrity of the skin was verified before washing with normal saline. The retrieved samples were subsequently stored at − 20°C and used within 1 week.

### *In vitro Transdermal Test*

*In vitro* transdermal drug delivery was investigated using a Franz diffusion cell. The rat skin was covered on a diffusion cell with the stratum corneum facing the donor cell and the dermis layer facing the receiving cell. One gram of the preparation was added to the donor cell before an even application onto the skin. The effective diffusion area was 1.6 $cm^2$. The receiving cell was filled with 12.5 mL of 20% (v/v) polyethylene glycol saline solution as the receiving medium to meet the sinking conditions. The receiving medium was kept at a constant temperature of 32°C ± 0.5°C and magnetically stirred at 300 rpm. Thereafter, 1 mL of the receiving medium was sampled at the predetermined time points (0, 1, 2, 3, 5, 7, 12, 24 h), with continued replenishing of the same volume of fresh receiving medium preheated to 32°C. The resulting samples were detected by HPLC. Every experiment was conducted in triplicate.

### *Fluorescence Distribution in the Skin*

The preparation was formulated by substituting Coumarin 6 with ibuprofen. Final concentration of Coumarin 6 in the preparation was 10 μg/g. The *in vitro* transdermal experiment was carried out by the method described above, and the entire process was protected from light. After 6 h of the experiment, the skin was removed, the remaining preparation

**Table I.** Composition of the Ibuprofen-Loaded Formulations

| Preparation | Ibuprofen (w/w, %) | Ethyl oleate (w/w, %) | Cremophor®EL (w/w, %) | Transcutol® P (w/w, %) | Azone (w/w, %) | HA (w/w, %) | Water (w/w, %) |
|---|---|---|---|---|---|---|---|
| ME/Gel | 5 | 4 | 18 | 18 | 1 | 1 | 53 |
| Gel | 5 | – | – | – | 1 | 1 | 93 |
| Cream[a] | 5 | – | – | – | – | – | – |

*ME, ibuprofen-loaded microemulsion; Gel, ibuprofen-loaded hydrogel; ME/Gel, ibuprofen-loaded ME-based hyaluronic acid hydrogel; HA, hyaluronic acid*

[a] *Commercially available preparation (Fenbid®)*

was wiped off, and the skin was washed with saline. Thereafter, the skin was cut into 0.5 × 0.5-$cm^2$ squares and placed onto the microscope slides. Full skin thickness was optically scanned through the *z*-axis of a CLS microscope (Confocal Laser Scanning Microscope, Carl Zeiss 710, Zeiss, Germany) with Ex/Em wavelength of 425/515 nm.

### Biocompatibility

#### *Cytotoxicity*

WST-8(4-[3-(2-methoxy-4-nitrophenyl)-2-(4-nitrophenyl)-2H-5-tetrazolio]-1,3-benzene disulfonate sodium salt) was used for cytotoxicity detection (24). HaCaT cells were seeded in 96-well plates at 5 × $10^3$/well and cultured for 24 h. The medium was subsequently discarded. Cells were divided into three groups and respectively added to 100 μL of the drug-containing medium (ibuprofen preparation group), 1% sodium dodecyl sulfate in fresh medium (positive control group), and fresh drug-free medium (negative control group), prior to a 24-h culture. Medium was then removed and 100 μL of 5% CCK-8 color developer was added per well prior to a 2-h incubation. The absorbance (ibuprofen preparation group, $A_{IBU}$; positive control group, $A_{Positive}$; negative control group, $A_{Negative}$) was detected at 450 nm with a microplate reader (Speotra Max 190, Gene Company Ltd., Hongkong). Cell viability was calculated using Eq. 1 (25):

$$\text{Cell viability}\ (\%) = \frac{A_{IBU} - A_{Negative}}{A_{Positive} - A_{Negative}} \times 100\% \quad (1)$$

#### *Primary Skin Irritation*

The hair on the back of the rat was shaved with an electric razor, and 0.3 g of ibuprofen-loaded microemulsion-based HA hydrogel (ME/Gel) was administered to their depilated skin (2 × 2 cm) every day for 3 days. To observe the pathological changes of the deep skin tissue and evaluate the safety of the tested formulation on the skin, treated skin was excised and fixed with 4% paraformaldehyde, embedded in paraffin, sectioned and stained with hematoxylin/eosin, and imaged with an optical microscope.

### Statistical Analysis

Data were analyzed by one-way ANOVA using software SPSS 18.0 (IBM, USA). A $p$ value < 0.05 indicated significant differences between groups.

## RESULTS AND DISCUSSION

The prepared microemulsion had a droplet size distribution of ~ 90 nm (Fig. 1b). Transcutol® P was used as an emulsifier to dissolve the oil phase containing the long-chain and penetrate the molecular branching structure of the surfactant; hence, the curvature of the interface film of the formed droplets increased, resulting in a relatively large microemulsion size compared with that formed using the medium and short chain oil phase (as used herein, ethyl oleate) (26). The smaller the size of the nanocarriers, the stronger the permeability; however, the particle size was too small and was not conducive to drug loading. The droplet size distribution of the microemulsion prepared by the formulation used in this study was moderate, which maintained the high solubility of ibuprofen, with a suitable size distribution below 100 nm. This also facilitated transdermal permeation (27). The size of the microemulsion was unchanged after drug loading ($p > 0.05$), indicating that ibuprofen did not affect size distribution; the drug was less distributed on the interface film of the oil-in-water microemulsion and was mainly distributed in the co-surfactant or oil phase. Transcutol® P acts as a co-surfactant for strong dissolution of the drug; hence, the drug was not embedded in the interface film formed by the surfactant, thereby avoiding the increase in droplet size, which is beneficial to the high penetration for microemulsion percutaneous administration (28). After the microemulsion was dispersed in the hydrogel, the droplet size showed no significant change ($p > 0.05$), indicating that the microemulsion retained the original form in the hydrogel. Under the transmission electron microscope, the microemulsion droplets were spherical-like, with no adhesion and uniform distribution (Fig. 1c). The morphology of the microemulsion dispersed in the hydrogel was similar to that of the liquid microemulsion, maintaining a complete morphology in the gel network (Fig. 1d). Such finding suggests that the microemulsion is stable in the HA-formed hydrogel, which is critical for stabilizing and achieving the penetration enhancement of microemulsions.

Viscosity is important for transdermal preparations. Appropriate viscosity can ensure good adhesion of the hydrogel. However, excessive viscosity can slow the diffusion

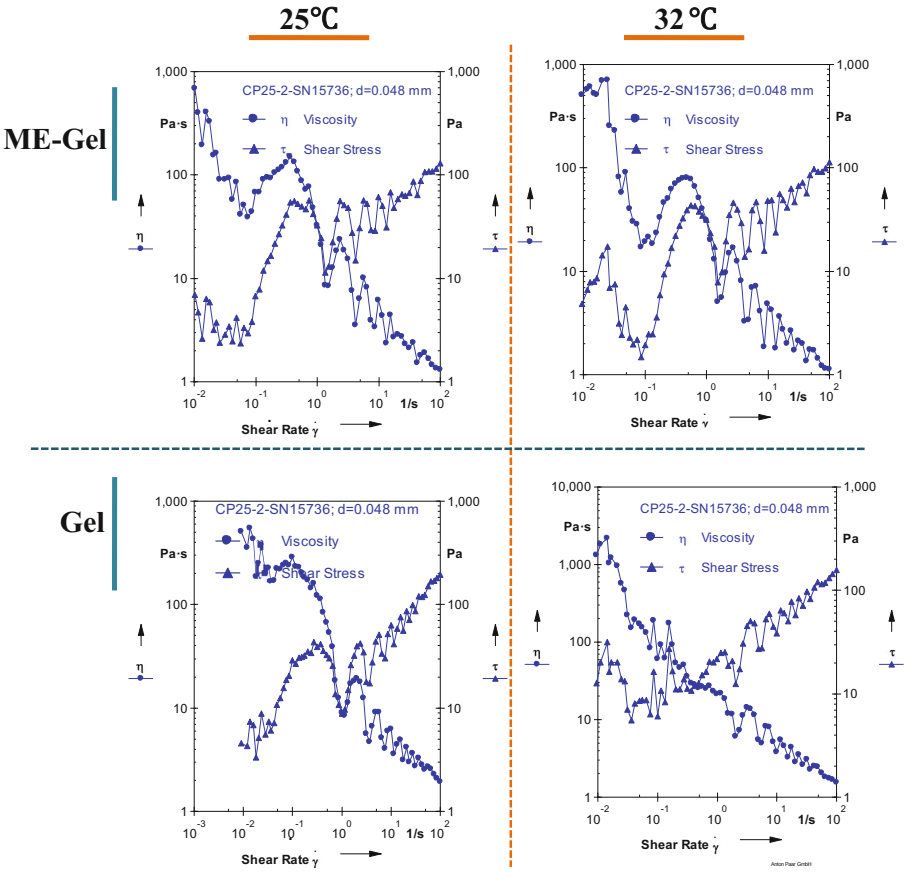


**Fig. 2.** Rheogram of the ibuprofen-loaded microemulsion (ME) and ibuprofen-loaded microemulsion-based hyaluronic acid hydrogel (ME/Gel)

of drug molecules from the preparation to the skin surface, thereby reducing transdermal drug delivery (29). The rheological test results showed that the prepared Gel and ME/Gel had moderate viscosity and pseudoplastic flow properties (Fig. 2). The hydrogel is composed of long-chain HA molecules and is hooked and entangled at a static or low flow rate. It also has a large viscosity and is viscous. When the shear rate increases, the scattered chains are rolled and contracted into a group, thereby reducing mutual hooking and the apparent viscosity, which leads to the phenomenon of shear thinning (30). There was no significant difference in the rheology of the gel at room temperature (25°C) versus normal skin surface temperature *in vivo* (32°C). In addition, the prepared hydrogel exhibited certain viscoelastic characteristics, and the distribution of the microemulsion had no significant effect on its rheology, suggesting that the microemulsion droplets were distributed in the gel network without a reaction with the HA molecules. The rheological characteristics exhibited by ME/Gel will facilitate its storage and application in clinical applications.

Polyacrylic acid (Carbomer®) is a commonly used hydrogel matrix, but drug precipitation from nanoemulsion-based Carbomer® hydrogel has been reported during storage (31). Our previous studies showed that the Carbomer® hydrogel loaded with microemulsion changed the pH of the system, thereby destroying the cross-linking of the Carbomer®, and changing the gel system from transparent to milky white (32). By using HA as a gel matrix, a gel network could be spontaneously formed after water absorption. Besides, it has a high tolerance to pH changes and shows great stability. Because HA has excellent water absorption and moisturizing properties, it does not require the addition of a moisturizer to build a hydrogel. As a result, the HA formulated ME/Gel prepared in this study showed good stability and was stored at room temperature for 3 months without turbidity, discoloration, and drug precipitation. In addition, no significant changes ($p > 0.05$) in microemulsion droplet size distribution and drug concentration were found during the 3 months of storage.

The *in vitro* transdermal delivery of ibuprofen in the tested preparations conformed with the first-order kinetics equation. Compared with Gel and commercial cream, the transdermal permeation of ibuprofen in the ME/Gel group was the largest (Fig. 3a, $p < 0.01$), and the cumulative permeation in 24 h was 1.42-fold and 2.52-fold greater than that found in the Gel group and the cream group, respectively; transdermal flux was also 1.42-fold and 2.52-fold greater than those of the above preparation groups (Fig. 3b, $p < 0.01$). As a natural product with excellent biocompatibility, HA promotes the transdermal penetration of drugs by increasing the water content of the skin and the hydration of the stratum corneum. Transdermal delivery of ibuprofen in the Gel group was larger than that of the commercial cream; this penetration-promoting effect was further enhanced with the assistance of microemulsion, which could assist in the achievement of the same therapeutic effect at a smaller dose than the tested commercial cream. Because ibuprofen is poorly soluble

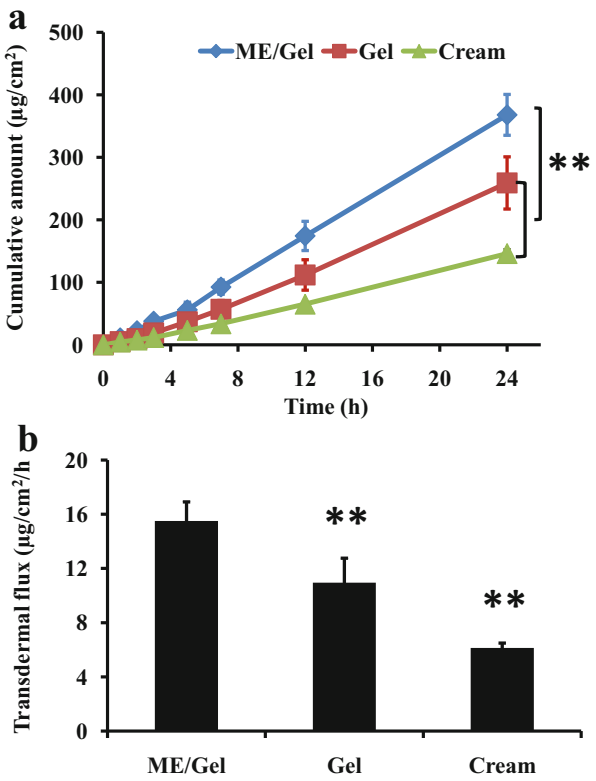


**Fig. 3.** *In vitro* transdermal permeation profiles (**a**) and transdermal fluxes (**b**) of ibuprofen from ibuprofen-loaded microemulsion (ME) and ibuprofen-loaded microemulsion-based hyaluronic acid hydrogel (ME/Gel). Compared with ME/Gel, $^{**}p < 0.01$. ($n = 3$)

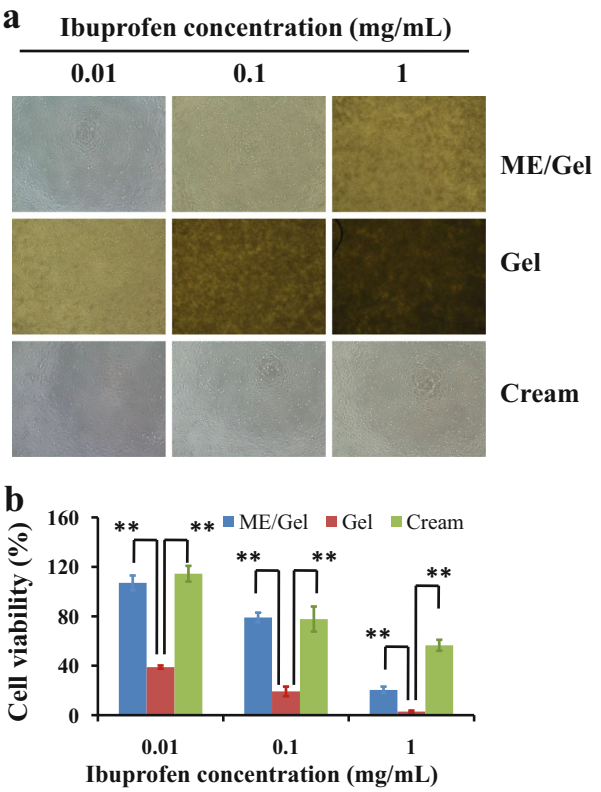


**Fig. 5.** Morphology (**a**) and cell viability (**b**) of *in vitro* cultured HaCaT cells after incubation with the tested ibuprofen preparations for 24 h (ME/Gel, ibuprofen-loaded microemulsion-based hyaluronic acid hydrogel; Gel, ibuprofen-loaded hyaluronic acid hydrogel; Cream, commercial ibuprofen cream). $^{**}p < 0.01$. ($n = 3$)

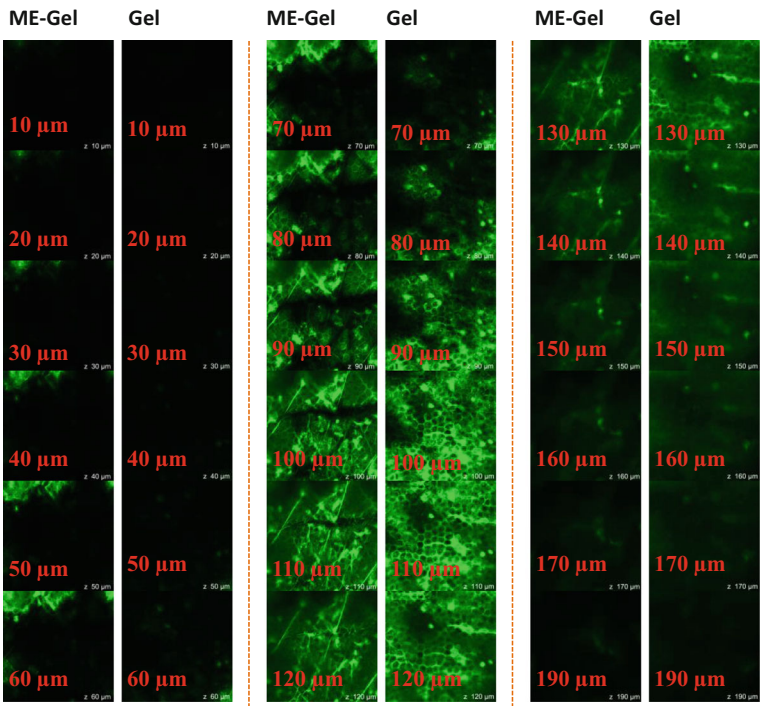


**Fig. 4.** Laser confocal tomography of the percutaneous penetration of Coumarin 6-labeled formulation over 6 h (ME/Gel, ibuprofen-loaded microemulsion-based hyaluronic acid hydrogel; Gel, ibuprofen-loaded hyaluronic acid hydrogel)

**Table II.** Cytotoxicity Grading Standards

| Grade | Reaction | Cell morphology |
|---|---|---|
| 0 | No | Cell morphology is normal; adherent growth is good; discrete particles exist in the cytoplasm; no cell lysis. |
| 1 | Extremely light | Up to 20% of the cells are round, loose, and adherent, without cytoplasmic particles; occasionally, cell lysis occurs. |
| 2 | Slight | Up to 50% of the cells are round; no cytoplasmic granules exist; cytolysis and intercellular space are clearly visible. |
| 3 | Moderate | Up to 70% of cells are round or dissolved. |
| 4 | Severe | Cells are almost completely destroyed. |

in water, has low thermodynamic activity in the Gel group, and a larger droplet size in the commercial cream group, the transdermal permeability of the drug in both preparations was worse than that in the ME/Gel group. The enhancement of microemulsion penetration mainly occurred through the following processes: the high concentration and thermodynamic activity of drugs accumulated in the skin first formed a high concentration gradient. Subsequently, with the help of this gradient, rapid penetration of

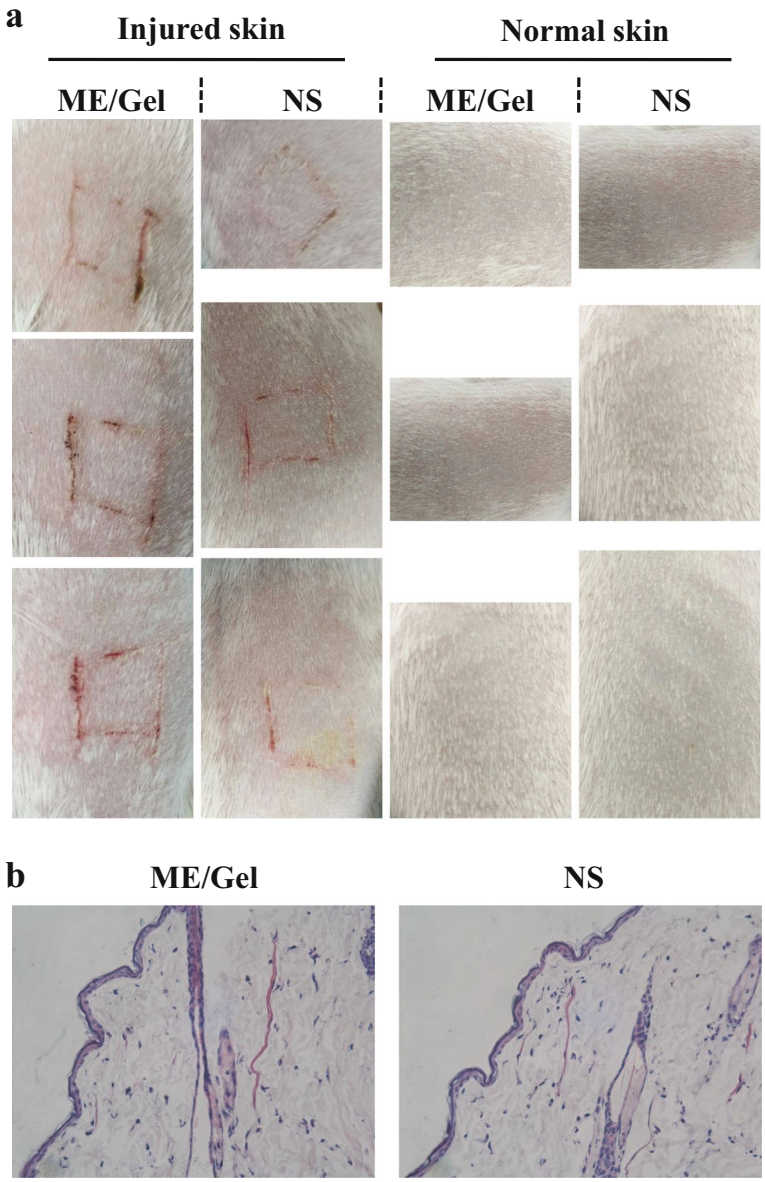


**Fig. 6.** Primary skin irritation (**a**) and hematoxylin and eosin staining of skin slices (**b**) of rat administered a transdermal administration of the ibuprofen-loaded microemulsion-based hyaluronic acid hydrogel (ME/Gel) once per day for 3 consecutive days

drugs was promoted, creating a high penetration rate. Microemulsion has low interfacial tension and high thermodynamic activity in the oil-water interfacial film. In this system with high activation energy, the drug can be transported freely between the phases through the flow of the interfacial film. With the penetration of drugs into the skin in the outer phase, the concentration decreases, and the drug in the internal phase is used as a reservoir to provide continuous high concentration gradient drug penetration. Microemulsion can also wet the stratum corneum by reducing the interfacial tension, change the structure, and destroy the structure of the lipid bilayer of the stratum corneum, thereby reducing its hindrance and enhancing transdermal drug delivery (8,33). The permeability of the microemulsion is related to the properties of the surfactant, drug, and oil phase. The surfactant and co-surfactant contained in the microemulsion act as transdermal enhancers and thus can expand sweat glands and open hair follicles, and is beneficial to drug penetration (34). In this study, Transcutol® P was used as the co-surfactant for the microemulsion. It has an affinity for the hydrophilic groups of the stratum corneum, affecting specific molecular fragments of the stratum corneum lipids and proteins, causing a significant change in skin capacitance, weakening the skin's barrier function against the drug, and ultimately promoting percutaneous penetration of the drug (35–37).

Under the laser confocal microscope, the fluorescently labeled ME/Gel group had strong fluorescence distribution in the shallow and deep layers of the skin, indicating that the microemulsion could form a drug depot in the skin (Fig. 4). Because Coumarin 6 is a hydrophobic fluorescent probe, fluorescence quenching is fast in water and is loaded into the microemulsion to improve stability. Indeed, the results of the study revealed that the microemulsion can walk freely in the gel network formed by HA; therefore, it could successfully carry the cargo to reach the stratum corneum, and then penetrate into the skin. The depot formed in the skin continues its release of the fluorescent substances to the deep skin (38–40).

Transdermal administration requires the evaluation of skin irritation. HaCaT cells are normal human skin cells and are commonly used to evaluate the biosafety of transdermal formulations. The cell morphology after treatment with the tested preparations for 24 h is shown in Fig. 5a. According to the ISO10993-5: 2009 (Table II), when the concentration of ibuprofen was 0.01 mg/mL, the cell state of the ME/Gel group and the commercial cream group was good; however, the Gel group had many cell death (grade 2) (41). At the 0.1-mg/mL drug concentration, the Gel group had more cell death (grade 3) while the other two groups displayed 1st-grade response. At high concentrations (1 mg/mL), all cells in the Gel group died (grade 4), a small amount of cells survived in the ME/Gel group (grade 3), and the commercial preparation group experienced slight changes (level 2 reaction). The above results indicate that the ibuprofen released from the dissolved hydrogel in the Gel group was toxic to cells, and after microemulsion encapsulation, drug release slowed and cytotoxicity was reduced (Fig. 5b, $p < 0.01$) (42). However, due to the small droplet size and high permeability, cell uptake of the drug was increased by microemulsion; hence, the cytotoxicity in the ibuprofen high concentration group was higher than that in the commercial cream group ($p < 0.01$). Hence, microemulsion exhibits strong permeability as is reflected in the results of *in vitro* percutaneous penetration experiments.

The primary skin irritation test results showed that there was no obvious irritation of the injured skin and intact skin in the ME/Gel group after continuous administration, no difference relative to the normal skin group (Fig. 6a), and no evident lesions in the skin tissue (Fig. 6b), suggesting that the ME/Gel has good biocompatibility with the skin.

## CONCLUSIONS

The microemulsion system consisting of ethyl oleate/Transcutol® P/Cremophor® EL had a small size distribution and high solubility for fat-soluble drugs. In addition, the excellent penetration-promoting effect of Transcutol® P greatly improved transdermal drug delivery. The hyaluronic acid–formed hydrogel could thus be used as a skeleton for transporting the microemulsion. As this microemulsion gel system is stable in nature, it displays significant improvements for transdermal delivery and biosafety of the drug.

**CONTRIBUTIONS** ALL AUTHORS CONTRIBUTED TO THE EXPERIMENTAL STUDIES, DATA COLLECTION AND STATISTICAL ANALYSIS, AND THE STRUCTURING AND WRITING OF THE ARTICLE.

**Availability of Data and Materials** The data used to derive Fig. 3 and Fig. 5b in this work can be obtained upon reasonable request from the corresponding author.

## FUNDING INFORMATION

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## COMPLIANCE WITH ETHICAL STANDARDS

**Ethics Approval and Informed Consent** The study protocol was approved by the Experimental Animal Ethics Committee of Shanghai University of Traditional Chinese Medicine (License No. SYXK (HU) 2014-0008).

**Consent for Publication** All authors accept the submission conditions.

**Competing Interests** The authors declare that they have no conflicts of interest.

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