



# Complex Nanoemulsions Delivering Dipropylene Glycol/Squalane for Scalp Care and Hair Repair

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## Introduction:

Consumers nowadays attach more importance to multifunctional demands of hair wash products, including damage repair and healthy scalp, which is determined by the skin moisture content. Nanoemulsion can enhance permeability and has good drug storage performance in cuticles and dermis and promotes scalp care. Dipropylene glycol has low skin irritation and toxicity and often serves as coupling reagent and moisturizer in cosmetics and wash protect products. [1,2] Squalane is a biological moisturizer and has good skin affinity, used as a spice fixator and skin lubricant in various cosmetics. Trimethylpropane tricaprilate in shampoo can significantly improve dry hair softness while providing fine dry and wet combing. [3] The combined application of nanoemulsion technology and active ingredients can make wash care products have better moisturizing and permeability promoting effects. [4]

As a unique ecology, scalp health is closely related to scalp micro-ecology. When scalp micro-ecology is destroyed, the homeostasis of microbial community is broken, which will lead to the breeding of scalp and hair diseases. Clavaud [5] and other scholars used PCR technology to quantitative detection over scalp microorganisms and found out that the dominant bacteria flora over scalp is staphylococcus epidermidis, and the main fungus flora is malassezia restricta. Staphylococcus epidermidis is gram positive coccus and plays an important role in maintaining skin micro-ecological balance as well as skin self-purification [6]. Staphylococcus epidermidis can produce lipase, decompose excessive grease produced by scalp, make the hair look less greasy, and adjust skin cutin to promote cell growth and wound healing [7]. Malassezia restricta is a kind of fungus that closely associated with seborrheic dermatitis and dandruff [8], which may result in epidermal cell metabolism disorder over head and destroy the scalp micro-environment [9].

This study produced adipropylene glycol/squalane/trimethylpropane tricaprilate nanoemulsion(MER-SCEX) to assess its physicochemical properties and stability, evaluate its efficacies in scalp moisturizing and caring, hair repair and influence in scalp micro-ecology.

## Materials & Methods:

### MER-SCEX's preparation and stability test

Dipropylene glycol, squalane, trihydroxymethylpropane trioctanoate and polyglycerin-10 diisostearate, caprylic acid/capric triglyceride were added into a beaker as oil phase according to the prescribed amount, heated at 70°C and stirred for 20 min, until completely dissolved. Distilled water was weighed as the aqueous phase, heated to 70°C, and then added into the oil phase. The colostrum was homogenized under high pressure at 800 bar for three times to obtain MER-SCEX. The prepared MER-SCEX was stored under normal temperature, high temperature, and low and high temperature cycling conditions.

### Evaluation on hair scalp moisturizing effect

The test sites were applied equal amounts of blank cream, free cream and MER-SCEX cream respectively. Among them, the blank cream is the cream matrix, adding the active ingredient on the basis of the blank cream is free cream, and adding 10% of MER-SCEX nanoemulsion in the blank cream is MER-SCEX cream. Then, TEWL and skin water content were detected in the test area when the subjects did not apply the cream in the test area and when the cream was used for 15 min, 30 min, 1 h, 2 h, 4 h, 6 h and 8 h respectively.

### Hair cuticle damage repair and hair combing

Two hair strands with the same quality were collected, one was used as blank control, the other was washed with MER-SCEX sample, and both groups of hair strands were treated under UV for 48 h and removed. The hair sample of small sections is fixed on the sample platform with conductive adhesive. After spraying gold and a suitable shooting multiple is selected to observe the tidiness and warping degree of the hair scales and evaluate the damage and repair of the hair. Take two hair strands with the same hair quality, one as a blank control, and one with MER-SCEX sample washing. Dry them under constant temperature environment, hang the hair bundle in load slots, install the comb in the movable part of the tensile tester, and then determine the force produced by moving from top to bottom.

### Staphylococcus epidermidis growth detection and malassezia inhibition test

Weigh 0.512 g of MER-SCEX sample, add 2 mL of sterile water to dissolve, filter with 0.22 μm filter membrane, and use it as sample mother liquor. Staphylococcus epidermidis was inoculated on an LB plate and cultured at 37°C for 24 hours. A single colony was picked and cultured in LB broth overnight. The OD600 was adjusted to 0.8 ~ 1.0, and then diluted 1000 times for later use. Add 100 μL of LB into a 96-well plate, then add 100 μL of samples with concentrations of 1, 4, and 16 μg/mL, and set a blank group at the same time. The 96-well plate was placed in an ultraviolet/visible full wavelength microplate and its OD value was determined at 600 nm.

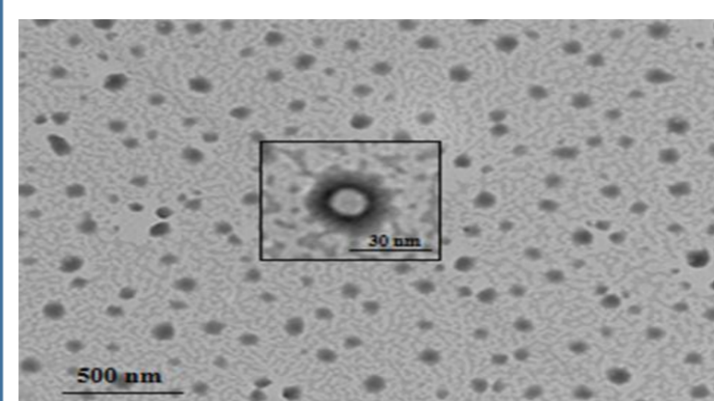
## References:

[1] Lovecchio F , Nourani C , Watts D J , et al. Acute renal failure, neuropathy, and myopathy after ingestion of dipropylene glycol fog solution[J]. American Journal of Emergency Medicine, 2008, 26(5):635.e1-635.e2.  
[2] Patel A, Iliopoulos F, Caspers PJ , et al. In Vitro-In Vivo Correlation in Dermal Delivery: The Role of Excipients. Pharmaceutics. 2021,13(4):542.  
[3] Li Zhonghua, Tan Huifen, Meng Juguang, etc. Application of trimethylol propane trioctanoate/tridecanoate in shampoo[J]. China Cleaning Industry, 2018, 08: 75-79.  
[4] Kakizawa Y, Miyake M. Creation of New Functions by Combination of Surfactant and Polymer - Complex Coacervation with Oppositely Charged Polymer and Surfactant for Shampoo and Body Wash. J Oleo Sci. 2019, 68(6):525-539.  
[5] Clavaud C, Jourdain R, Bar-Hen A, et al. Dandruff is associated with disequilibrium in the proportion of the major bacterial and fungal populations colonizing the scalp[J]. PLo S One, 2013, 8(3): e58203.  
[6] Pothmann A , Illing T , Wiegand C , et al. The Microbiome and Atopic Dermatitis: A Review[J]. Am J Clin Dermatol. 2019, 20(6):749-761.  
[7] Apollo Stacy, Yasmine Belkaid. Microbial guardians of skin health[J]. Science, 2019, 363(6424): 227-228.  
[8] Theelen B, Cafarchia C, Gaitanis G et al. Malassezia ecology, pathophysiology, and treatment[J]. Med Mycol, 2018, 56 (1):10-25.  
[9] VuLlo D, Prete SD, Nocentini A, et al. Dithiocarbamates effectively inhibit the β-carbonic anhydrase from the dan druff-producing fungus Malassezia globosa[J]. Bioorg Med Chem, 2016, 25 (3):1260-1265.  
[10] Huang H C, Chang T M. Ceramide 1 and ceramide 3act synergistically on skin hydration and the transepidermal water loss of sodium lauryl sulfate-irritated skin[J]. International Journal of Dermatology, 2008, 47(8): 812-819.

## Results & Discussion:

### MER-SCEX's characterization and stability

MER-SCEX is a light yellow clear liquid with a particle size of 30.2 nm, a PDI of 0.218 and a Zeta potential of 22.3 mV. The microscopic morphology of MER-SCEX observed by transmission electron microscopy is shown in Figure 1. The test results of time and temperature stability are shown in Table 1. After three cycles of high and low temperature, the particle size of MER-SCEX is 27.7 nm, PDI is 0.274, and Zeta potential is 28.7 mV.



	room temperature			4℃			45℃		
	PDI	grain size/nm	Zeta/mV	PDI	grain size/nm	Zeta/mV	PDI	grain size/nm	Zeta/mV
7天	0.218	30.2	-29.2	0.222	28.2	-28.7	0.258	30.2	-25.9
15天	0.234	31.1	-25.4	0.279	30.7	-26.3	0.247	33.2	-21.2
30天	0.254	28.1	-21.5	0.281	29.6	-21.5	0.275	36.1	-13.7

Sample	Inhibition zone diameter (mm)
Control	0
2.5 mg/mL MER-SCEX	7.6
5 mg/mL MER-SCEX	9.2
10 mg/mL MER-SCEX	13.7

Fig. 1 MER-SCEX's Transmission electron microscopy Table 1 MER-SCEX's stability Table 2 Inhibition zone diameters of different concentrations to Malassezia

### Influence on hair scalp moisturizing effect

As shown in Figure 2, after the application of MER-SCEX cream, the moisturizing trend of MER-SCEX cream and free cream was basically the same, but the moisturizing effect of MER-SCEX cream was better than that of free cream with the same concentration of active substance. After 15 min, the skin moisture content of free cream increased by 13.2%. The skin moisture content of MER-SCEX cream was increased by 24.4%, indicating that the active ingredients in MER-SCEX could penetrate the skin surface quickly and effectively enhance the skin moisturizing function. As shown in Figure 3, after the application of MER-SCEX cream for 6 h, the transdermal water loss rate TEWL decreased rapidly, and the transdermal water loss rate TEWL decreased by 24.2%, indicating that the skin barrier was repaired and the amount of ceramide in the skin was replenish [10], indicating that the moisturizing active ingredients in MER-SCEX could enter deep skin tissues.

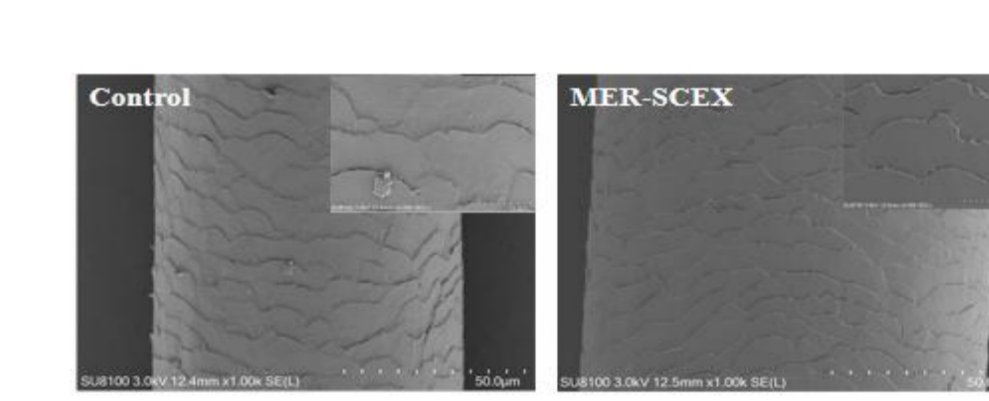
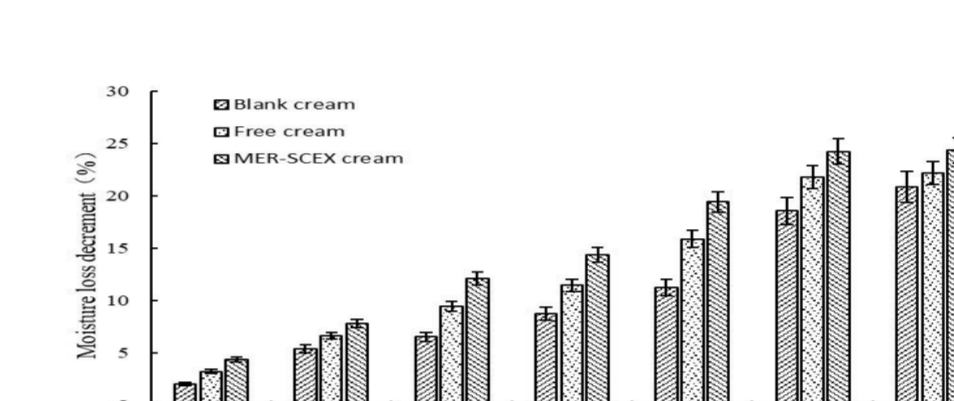
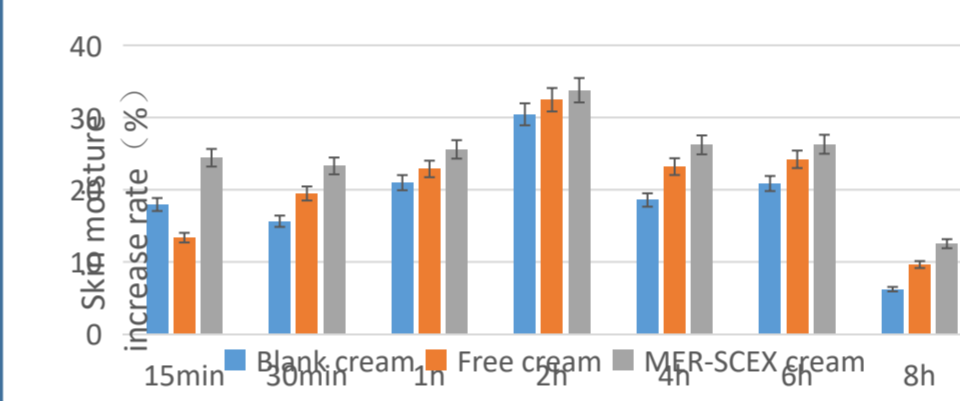


Fig. 2 Skin water content test after using each cream Fig. 3 Skin moisture loss rate test after applying each cream Fig. 4 MER-SCEX's repair effect on hair cuticles

### Influence on hair cuticle damage repair

After 48 hours of treatment under UV irradiation, the microscopic morphology of hair was observed by scanning electron microscope (SEM), as shown in Figure 4. As can be seen from Figure 4, hair in the control group showed partial epidermal surface cracking, while hair scales treated with MER-SCEX had smooth surface.

### Effects on hair combing

The dry combing work measured according to the experiment is shown in Figure 5, and the feedback results of skin sensation evaluation of volunteers are shown in Figure 6. As can be seen from Figure 5, the dry combing work of the hair bundle after blank control treatment decreased from the initial 101.7 to 65.7, with a reduction rate of 35.4%; the dry combing work of the hair bundle after MER-SCEX treatment decreased from the initial 101.9 to 54.5, with a reduction rate of 46.5%. Compared with the blank control group, the combing work of hair bundle treated by MER-SCEX decreased and showed a significant difference, making it easier to combing. Combined with the results of the volunteers' feedback on the dry hair combing experiment in Figure 6, the evaluation of simple dry hair combing and dry hair smoothness of MER-SCEX treatment was significantly better than that of the blank control group.

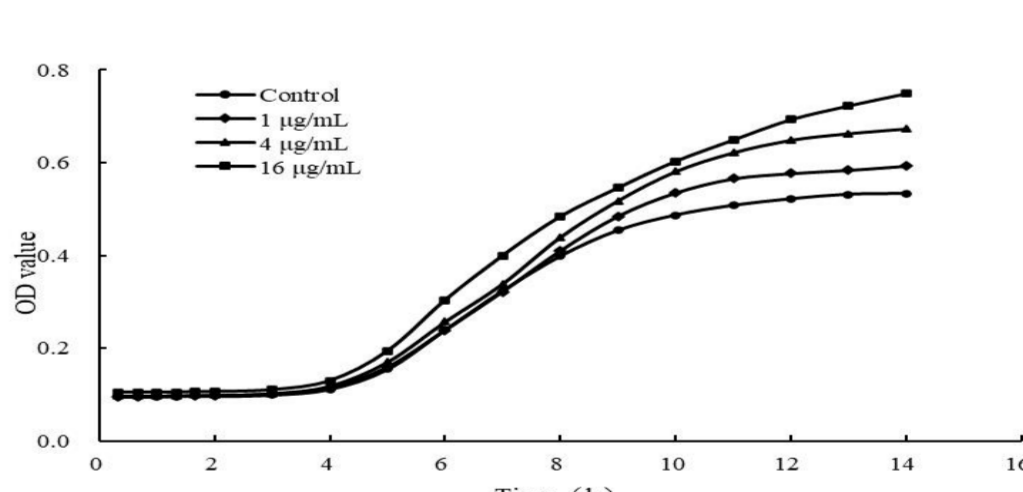
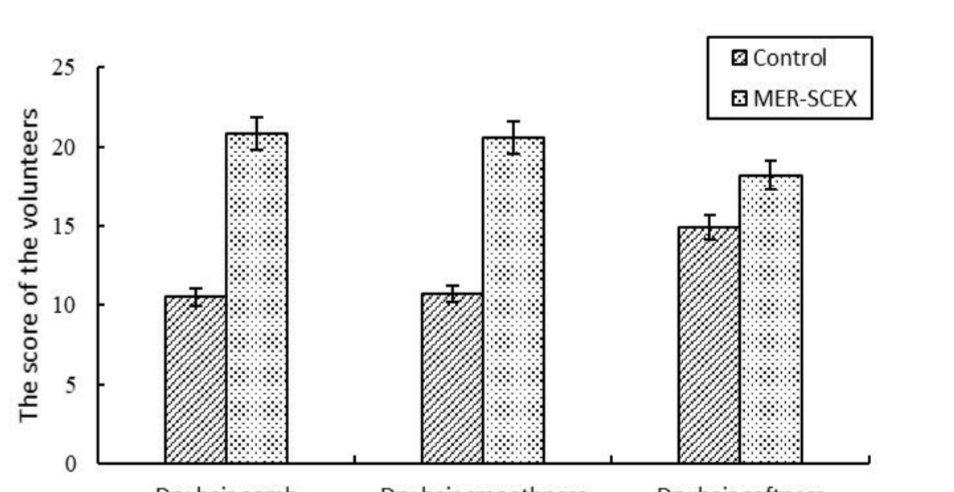
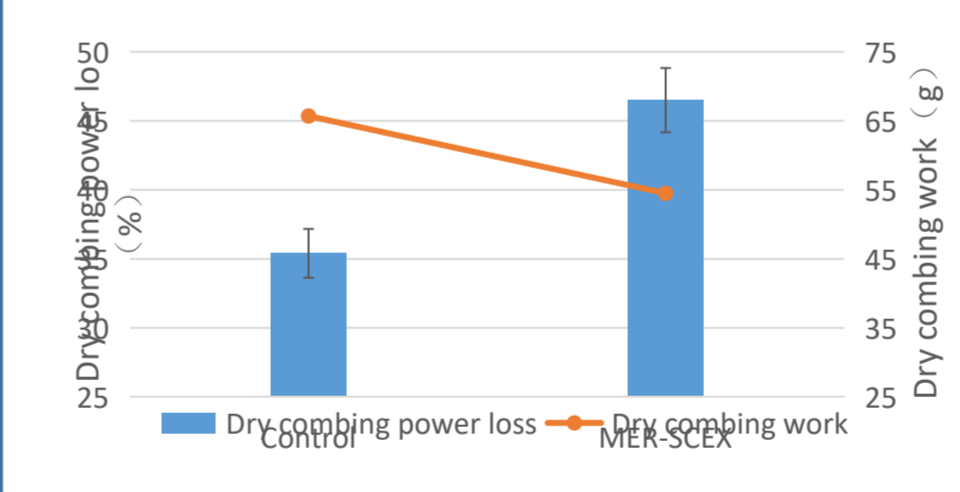


Fig. 5 MER-SCEX's effect on dry hair combing Fig. 6 Volunteers' assessment on MER-SCEX's dry hair combing Fig. 7 MER-SCEX's influence on the growth curve of staphylococcus epidermidis

Measurement on staphylococcus epidermidis' growth curve and malassezia' antibacterial activity It can be seen from Figure 7 that when the concentration of MER-SCEX was 1-16 μg/mL, the growth curve showed that MER-SCEX could promote staphylococcus epidermidis to enter the logarithmic proliferation phase and stable phase earlier, and the promotion effect was concentration-dependent. From Table 2, in the antibacterial activity test, the diameter of the antibacterial ring in the control group containing only culture medium was 0, and there was no antibacterial phenomenon. The diameters of the antibacterial ring in different concentrations of MER-SCEX were all greater than 7 mm, indicating that MER-SCEX had an inhibitory effect on malassezia.

## Conclusions:

In this study, the prepared dipropylene glycol/squalane nanoemulsion MER-SCEX has uniform particle size, regular microstructure, and good storage stability under different temperature conditions. MER-SCEX cream has significant moisturizing and repairing effects on skin as well as good maintenance effects on hair scale repair, hair combing and scalp microflora.