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Research on the Evaluation Method of a Dual-Tube Facial Cleanser Product with Selective Cleaning Ability

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Abstract:Objective: To develop an evaluation method for the selective cleansing ability of a dual-tube facial cleanser on different sebum components in humans.**Methods:** In vitro tests used leather models to simulate human skin, quantifying squalene hydroperoxide clearance and cholesterol retention rates for four cleansers. Thirty oily-skin subjects underwent non-invasive evaluations, measuring skin oil content and transepidermal water loss (TEWL) before/after cleansing and after 7 days of use.**Results:** Cleansers showed significant differences in squalene hydroperoxide clearance and cholesterol retention, assessed by a selective cleansing coefficient. Post-cleansing, subjects exhibited a -93.26% oil secretion reduction and -11.77% TEWL decrease. After 7 days, oil secretion and TEWL dropped by -28.83% and -29.77%, respectively.**Conclusion:** The dual-tube cleanser demonstrated selective cleansing effects in both in vitro and human tests, balancing oil control and barrier protection. The proposed method, combining squalene hydroperoxide clearance, cholesterol retention, and non-invasive human evaluation, effectively assesses cleansers' selective performance, providing technical support for surfactant screening and cleanser evaluation systems.

Keywords: selective - cleaning; squalene peroxide; cholesterol; skin barrier

1. Introduction

Cleansing, as a fundamental and crucial step in skin care, is of vital importance for maintaining skin health. In modern life, waterproof, long-lasting, and smudge-proof sunscreens and cosmetics have indeed brought convenience, but they also pose challenges to skin cleansing. Consumers need facial cleanser products that can effectively remove makeup, oil, and lipid-soluble dirt. At the same time, they do not want to experience problems such as rough skin, itching, and tightness after cleansing. The long-term use of powerful cleansing products can lead to damage to the skin barrier[1].

Skin cleansing is a cornerstone of skincare. Yet, improper facial cleansers can harm the stratum corneum's lipid barrier via surfactants' physicochemical actions. Research indicates that surfactant - skin lipid interactions fall into three types: removing epidermal sebum, disrupting intercellular lipid lamellar structures, and dissolving lipid components directly[2].

Among these, the loss of intercellular lipids, especially cholesterol, is often overlooked, despite its cumulative damage to the barrier.

As a key 25% component of the stratum corneum's "brick - and - mortar" lipid structure, cholesterol maintains barrier integrity through hydrophobic interactions and regulates keratinocyte differentiation and lipid synthesis signaling. Studies show that sodium laurate and sodium lauroyl glutamate have high cholesterol elution rates in vitro and in vivo. Long - term use disrupts intercellular lipid ratios and weakens the skin barrier[3].. Thus, traditional cleansers, while removing sebum, may irreversibly damage structural lipids, worsening dryness and sensitivity.

Moreover, oxidative stress products like squalene peroxide in sebum threaten skin health. Squalene, a 10 - 12% sebum major component, oxidizes to squalene monohydroperoxide under UV or pollutant influence. Squalene peroxide can induce keratinocyte hyperkeratosis at follicular orifices and sebaceous gland hyperplasia, raising pore - blockage risk. It also activates lipoxygenase LOX, triggering keratinocyte inflammatory responses and increasing inflammatory factors, promoting acne progression, and potentially altering skin texture and causing wrinkles[4，5].Existing facial cleansers struggle to meet the dual needs of "removing oxidatively toxic lipids" and "retaining endogenous barrier lipids." Powerful surfactants effectively remove squalene peroxide but increase cholesterol loss, while mild surfactants reduce barrier damage yet lack cleaning efficiency. This contradiction is acute in oily and sensitive skin care.

Therefore, selectively removing squalene peroxide and minimizing cholesterol loss during cleansing is vital for skin health. We developed a dual - tube facial cleanser with different surfactant combinations and adsorptive clay to meet these lipid - related needs. This paper aims to establish an evaluation method using the residual ratios of sebum - derived squalene and epidermal cell - derived cholesterol as indicators to assess the selective cleaning ability of this dual - tube product, providing a theoretical basis for developing safe and effective facial cleansers.

2. Materials and Methods

2. 1. Materials

Experimental samples: C+ Dual-tube Facial Cleanser (Guangdong G Cosmetic Network Filing No. 2024144829), Guangzhou Xika Technology Co., Ltd.; Off-the-shelf Product A; Off-the-shelf Product B; Off-the-shelf Product C. Genuine leather; Squalene (purity 98%), Shanghai Macklin Biochemical Technology Co., Ltd.; Cholesterol (purity 95.0%), Shanghai Macklin Biochemical Technology Co., Ltd.; Ethanol (chromatographic grade), Anaqua, USA; Ethyl acetate (pesticide residue grade), CNW.

Ultra-high performance liquid chromatography-quadrupole tandem time-of-flight mass spectrometer 1290 Infinity/6540 UHD Accurate-Mass, gas chromatography-mass spectrometer 6890N/5973 inert, Agilent, USA; Vortex mixer XW-80A, Shanghai Jingke Industrial Co., Ltd.; High-speed centrifuge HT185, Xiangyi Testing Equipment Co., Ltd. in Changsha, Hunan Province.Sebumeter® SM815 skin surface sebum test probe,

Tewameter® TM300 transepidermal water loss rate test probe, Courage + Khazaka, Germany.

2.2. Ex vivo experimentation

Cut a 4.5 cm × 2.0 cm piece of genuine leather. Evenly apply squalene and cholesterol on it at a ratio of 12:2.2[6]. Expose the coated leather to an ultraviolet lamp at 365 nm and 254 nm wavelengths for 2 h.For the control group, immerse the UV - irradiated leather directly in an ethanol - ethyl acetate mixed solvent. For the cleaning group, clean the UV - irradiated leather following the usage methods of four products (C+ Dual - tube Facial Cleanser, Off - the - shelf Product A, Off - the - shelf Product B, Off - the - shelf Product C), dry it, then immerse in the same mixed solvent. Analyze the extraction liquids of each group by LC - Q - TOF/MS and GC - MS to quantify squalene peroxide and cholesterol, and compare the peak areas of these two substances between the control and cleaning groups.

Analysis of Squalene Peroxides:The analysis of squalene peroxides was performed using an Agilent 1290 Infinity/6540 UHD Accurate-Mass Q-TOF LC/MS system. Chromatographic separation was achieved on an Eclipase Plus C18 RRHD column (2.1 mm × 50 mm, 1.8 μm). The mobile phase consisted of 0.1% formic acid in water (phase A) and acetonitrile (phase B). The gradient elution program was as follows: 90–95% B from 0 to 3 min, followed by isocratic elution with 95% B from 3 to 23 min. The flow rate was set at 0.3 mL/min, the column temperature was maintained at 40 °C, and the injection volume was 1 μL.For mass spectrometry, the electrospray ionization (ESI) source was operated in the positive ion mode. The capillary temperature was set at 300 °C, the nitrogen flow rate was 8 L/min, the nebulizer gas pressure was 35 psi, and the capillary voltage was 4000 V.

Analysis of Cholesterol:Cholesterol analysis was conducted using an Agilent 6890N/5973 inert gas chromatography-mass spectrometry (GC-MS) system. Separation was carried out on a DB-5ms capillary column (30 m × 0.25 mm × 0.25 μm). The injector temperature was set at 250 °C, and helium was used as the carrier gas at a flow rate of 1.0 mL/min. The injection volume was 0.5 μL. The oven temperature program started at 100 °C, was ramped up to 280 °C, and then held at 280 °C for 15 min. The transfer line temperature was maintained at 280 °C.The mass spectrometer was equipped with an electron impact ionization (EI) source, and the ion source temperature was set at 230 °C.

2.3. In vivo trials

This study enrolled 30 adult participants aged 18 - 45 years with forehead sebum levels >100 μg/cm² and poor facial skin barrier function, as indicated by a transepidermal water loss rate of ≥ 15 g/㎡/h measured at the cheek area using Tewameter. Prior to measurement, participants acclimated for 30 minutes in a controlled environment (20°C, 40 - 60% humidity). Before using the product (D0), tests are carried out. The transepidermal water loss (TEWL) value is measured using the Tewameter® TM300 transepidermal water loss rate test probe, and the skin sebum secretion amount is measured using the Sebumeter® SM815 skin surface sebum test probe. After the tests, the subjects use the C Ka Amino Acid Orange Mud Pure and Radiant Dual-tube Facial Cleanser once (T1), and then use the corresponding probes again to measure the TEWL value and the skin sebum secretion amount.

The subjects receive the samples and try the facial cleanser for 7 consecutive days, once a day. During the test period, they are supervised by the testing laboratory regarding the usage situation. Seven days after using the product (D7), the efficacy measurement is conducted. When returning for the follow-up after 7 days, the subjects sit still and balance for 30 minutes in the constant temperature and humidity environment according to the procedure, and then the TEWL value and the skin sebum secretion amount are measured.

2.4. Statistical analysis

Statistical analysis was performed using IBM SPSS Statistics 17.0 software. P value less than 0.05 indicates a statistically significant difference. Among them, "0.01 ≤ P < 0.05" represents a significant difference; "0.001 ≤ P < 0.01" represents a very significant difference; and "P < 0.001" represents an extremely significant difference.

3. Results

3.1. Ex vivo experimentation

3.1.1 Clearance rate of squalene peroxide

The molecular formula of squalene peroxide is $C_{30}H_{50}O_2$, and its accurate mass number is 442.38. The mass spectrum under the electrospray ionization source (ESI) is shown in Figure 1. Among them, m/z 443.39 is the $[M+H]^+$ peak, m/z 465.37 is the $[M+Na]^+$ peak, and m/z 425.38 is the $[M+H-H2O]^+$ peak, which is consistent with the research of Mudiyanselage et al. In the experiment, the theoretical mass number m/z 443.3884 of the $[M+H]^+$ peak of squalene peroxide was extracted, and the obtained extracted ion chromatogram (EIC) was integrated. A typical EIC diagram is shown in Figure 2. The clearance rate of squalene peroxide in each sample was calculated using its peak area.

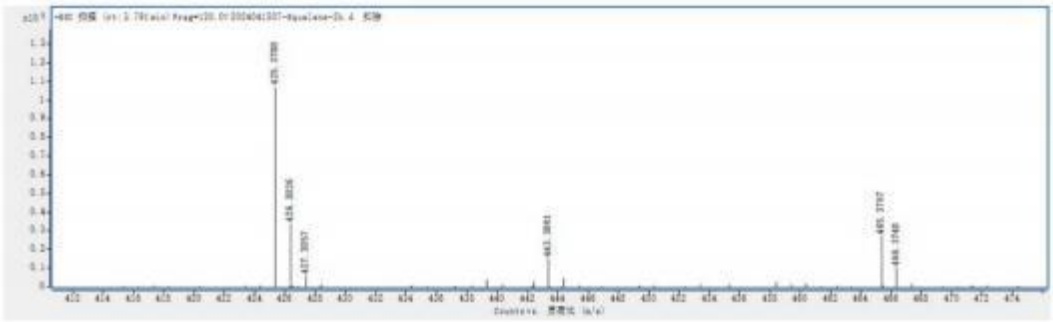

Figure 1. Mass spectrum of squalene peroxide

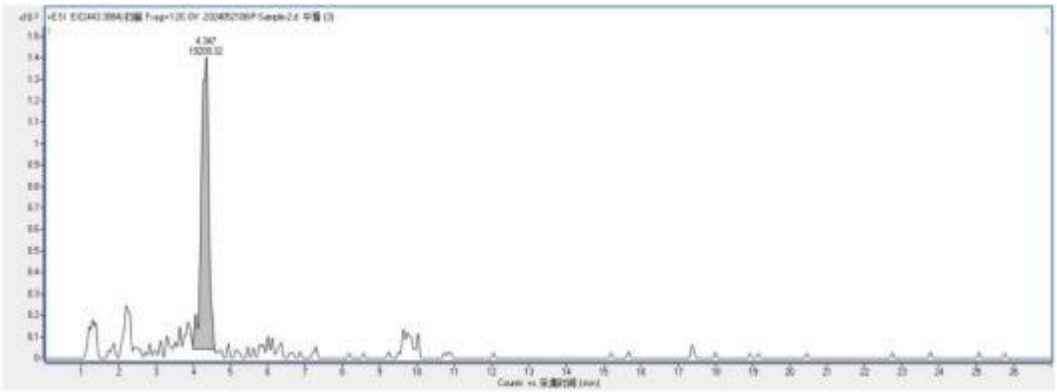

Figure 2. Typical extracted ion current chromatogram of squalene peroxide in the sample (m/z 443.3884)

The clearance rate of squalene peroxide(X) was calculated using the following formula:

$$X(\%) = (1 - \frac{A_1}{A_0}) \times 100\% \qquad (1)$$

where A_0 represents the peak area of the squalene peroxide control group; A_1 represents the peak area of the squalene peroxide cleaning group.The clearance rates of squalene peroxide for each product obtained are shown in Table 1:

Table 1. Squalene peroxide clearance rate

Sample	Squalene Peroxide (LC-Q-TOF/MS)		
	Peak Area of Control Group	Peak Area of Cleaning Group	Clearance Rate %
C+ Dual-tube Facial Cleanser	42935	24634	42.6
Off-the-shelf Product A		29252	31.9
Off-the-shelf Product B		32128	25.2
Off-the-shelf Product C		27819	35.2

3.1.2. Cholesterol retention rate

The molecular formula of cholesterol is $C_{27}H_{46}O$, and its standard mass spectrum under the electron impact ionization source (EI) is shown in Figure 3. In the experiment, the molecular ion peak of cholesterol at m/z 386 was extracted, and the obtained extracted ion chromatogram (EIC) was integrated. A typical EIC diagram is shown in Figure 4. The cholesterol retention rate of each sample was calculated using its peak area.

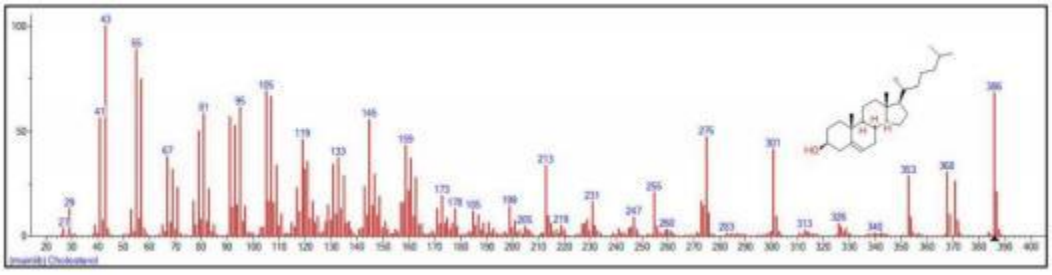

Figure 3. Standard mass spectrum of cholesterol

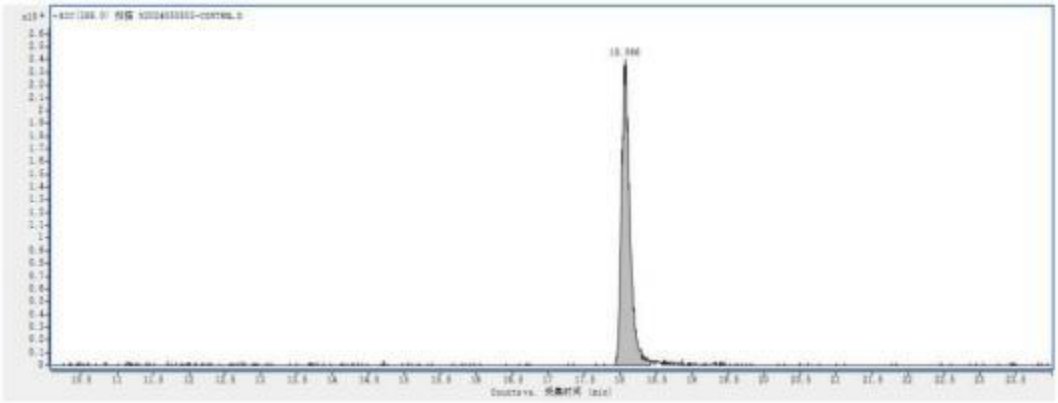

Figure 4. Typical extracted ion current chromatogram of cholesterol in the sample (m/z 386)
The cholesterol retention rate(Y) was calculated using the following formula:

$$Y(\%) = \frac{A_1}{A_0} \times 100\% \qquad (2)$$

where A_0 represents the peak area of the cholesterol control group; A_1 represents the peak area of the cholesterol cleaning group.The cholesterol retention rates of each product using genuine leather are shown in Table 2:

Table 2. Cholesterol retention rate

Sample	Peak Area of Control Group	Cholesterol (GC-MS) Peak Area of Cleaning Group	Retention Rate %
C+ Dual-tube Facial Cleanser	195270	124040	63.5
Off-the-shelf Product A		118963	60.9
Off-the-shelf Product B		111725	57.2
Off-the-shelf Product C		95489	48.9

3.1.3. Selective cleaning coefficient
The selective cleaning coefficient of the product, abbreviated as SCC, is calculated based on the clearance rate of squalene peroxide and the cholesterol retention rate. It is used to comprehensively evaluate the selective cleaning coeddicient（SCC） of each product. The calculation formula is as follows:

$$SCC = X \times Y \qquad (3)$$

The selective cleaning coefficients of each product obtained are shown in Figure 5 as follows:

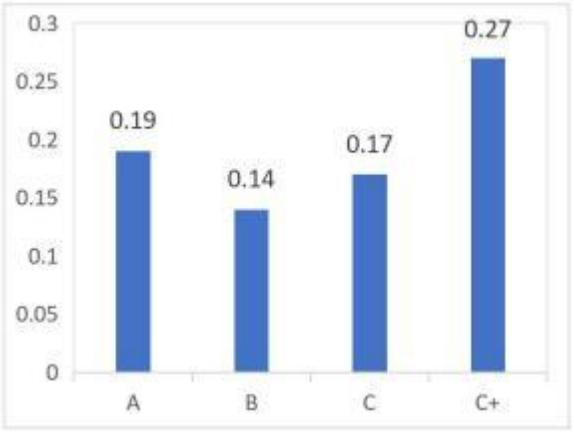

Figure 5. Selective cleaning coefficient.A represents Off-the-shelf Product A; B represents Off-the-shelf Product B; and C represents Off-the-shelf Product C; C+ represents C+ Dual-tube Facial Cleanser .

The squalene hydroperoxide clearance rate showed that the C+ Dual-Tube Facial Cleanser exhibited superior cleansing ability to remove squalene hydroperoxide compared to commercial skincare products A, B, and C, outperforming all comparators in oxidative lipid removal.

In cholesterol retention efficiency, the C+ Dual-Tube Facial Cleanser significantly surpassed products A, B, and C. As a critical component of the skin barrier, cholesterol retention by the cleanser helps maintain epidermal hydration and prevent irritant penetration, thereby supporting barrier integrity.

The selective cleansing coefficient (SCC) followed the order: C+ Dual-Tube Facial Cleanser > Commercial Product A > Commercial Product C > Commercial Product B. Specifically, the C+ cleanser's SCC was 1.42× (A), 1.93× (B), and 1.59× (C) higher, based on in vitro leather model testing.

As a key metric for selective cleansing, SCC reflects the balance between removing harmful substances (e.g., oxidized sebum) and preserving essential lipids (e.g., cholesterol). The C+ Dual-Tube Facial Cleanser's highest SCC indicates optimized barrier protection during cleansing, minimizing potential damage while ensuring effective detoxification.

In vivo trials

During the human efficacy verification, sebum clearance rate and transepidermal water loss (TEWL) served as key metrics to quantify post-cleansing skin lipid residue and assess cleanser-induced changes in stratum corneum barrier function, respectively. This dual-index system enabled comprehensive analysis of how facial cleansers impact skin barrier integrity alongside cleansing efficiency, providing a scientific foundation for developing products that balance cleaning power and mildness.

As shown in Figure 6, single application of the experimental sample (T1) significantly reduced skin oil secretion compared to baseline (D0) ($P < 0.001$), with a change rate of -93.26%. After 7 consecutive days of use (D7), oil secretion remained significantly lower than baseline ($P = 0.001$), with a change rate of -28.83%. These results indicate the sample reduces excessive sebum secretion while removing squalene hydroperoxide. Sustained use over one week mitigated sebum production and oxidative stress, protecting skin from free radical damage and supporting anti-aging effects.As shown in Figure 7, single use of the experimental sample (T1) significantly decreased TEWL values compared to baseline (D0) ($P < 0.001$), with a change rate of -11.77%. After 7 days of continuous use (D7), TEWL values further decreased by -29.77% ($P < 0.001$). Reductions in TEWL indicate improved barrier function. The sample demonstrated both immediate (T1) and sustained (D7) improvements, suggesting short-term efficacy and long-term maintenance of skin barrier health.

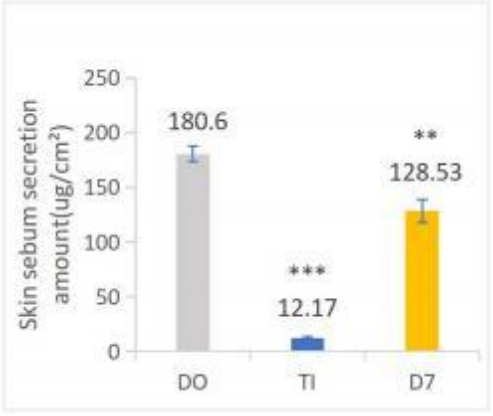

Figure 6. Comparison of oil secretion before and after product use（Significance labeling methods: “**”indicates a very significant difference, where 0.001 ≤ P < 0.01, “***” indicates an extremely significant difference, where P < 0.001）

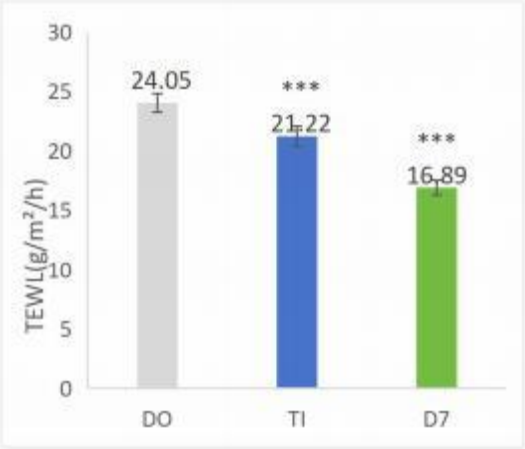

Figure 7. TEWL values at different time points（Significance labeling method: "***" indicates an extremely significant difference, where P＜0.001）

2 Discussion

The clinical characteristics of oily skin lie in excessive sebum secretion, which can lead to greasy skin, enlarged pores, and subsequently trigger skin problems such as acne vulgaris and seborrheic dermatitis[7]. In the sebum of normal skin, squalene can account for up to 12%. Compared with the sebum of normal skin, the most significant difference in sebum composition of acne - prone skin is the content of squalene, which is 2.2 times that of normal skin. Therefore, it can serve as a lipid marker for acne - prone skin[8,9] . Squalene has lubricity and high penetration efficiency, and its oxidation products may be related to skin oxidative damage, photoaging, and the production of inflammatory mediators. When selecting an indicator to measure the cleansing ability of facial cleansers on sebum in this experiment, squalene peroxide was specifically chosen as the marker. Cholesterol and its esters have a relatively low content in sebum, accounting for about 3.5%, while in intercellular lipids, they account for about 24%, playing a crucial role in maintaining skin function metabolism and barrier function[10]. In this experiment, cholesterol was selected as the marker to measure the ability of facial cleansers to maintain the skin barrier. A higher cholesterol retention rate indicates less damage to the barrier.

Related studies have shown [11]that different types of surfactants have different cleansing abilities for various types of sebum. By screening and combining these surfactants with selective cleaning abilities, different surfactant formula combinations can be obtained. Some can effectively clean squalene peroxide, while others can retain lipids derived from stratum corneum cells such as cholesterol. This is consistent with the design concept of the C+ Dual-tube Facial Cleanser. Based on the research findings, we adopted an original dual-tube design and combined amino acid surfactants with different carbon chain combinations to ensure moderate cleansing power and good foaming performance. One tube focuses on a formula combination for removing squalene peroxide, while the other aims to maximize the

retention of the skin's natural lipids and cholesterol. This effectively cleans the oil on the skin surface, avoids skin irritation, reduces sebum secretion, and repairs the skin barrier.

In this study, an in vitro leather cleaning test was conducted using the C+ Dual-tube Facial Cleanser to obtain the clearance rate of squalene peroxide and the retention rate of cholesterol for different facial cleansers, verifying the feasibility of selectively cleaning different sebum components. Conventional products typically exhibit either high or low cleaning rates for all sebum components. Through the calculation of the selective cleaning coefficient, it was found that the highest coefficient among commercially available products was 0.27. Compared with the other three commercially available products, the C+ Dual-tube Facial Cleanser has better abilities to remove squalene peroxide and retain cholesterol. Therefore, it is speculated that it can also achieve the same "selective" cleaning effect on the human body and maintain the permeability barrier function of the epidermis. The in vitro verification of the clearance rates of squalene and cholesterol by facial cleansers is mainly aimed at quickly screening surfactants or facial cleansers.

In human efficacy tests, results with the C+ Cleansing Milk showed that after 7 consecutive days of use, sebum secretion significantly decreased; meanwhile, the significant reduction in TEWL (transepidermal water loss) values indicated that the product does not damage the skin barrier and may have certain repairing effects. The human test results were consistent with in vitro test expectations, further validating the effectiveness of the C+ Facial Cleanser.

In conclusion, the C+ Dual-tube Facial Cleanser performs outstandingly in terms of selective cleaning ability and skin barrier protection, making it suitable for the daily cleaning needs of oily skin.

3 Conclusion

This study successfully established an evaluation method for a dual-tube facial cleanser with selective cleansing capability, which can selectively remove squalene hydroperoxide without over-cleansing cholesterol. Through in vitro evaluation of selective cleansing ability against squalene hydroperoxide and cholesterol, as well as human clinical trials, the feasibility and effectiveness of this method were demonstrated. The results showed that the tested C+ Dual-Tube Cleansing Milk exhibited superior selective cleansing ability against squalene hydroperoxide, protecting the skin barrier function while cleansing. This study provides a scientifically validated method for investigating the impact of surfactant raw materials and facial cleansers on skin component cleansing, offering references for subsequent related research.

However, this study has certain limitations. Factors affecting other aspects of skin health, such as the effects of cleansers on water-soluble moisturizing components (e.g., natural moisturizing factors, NMF), other lipids, and proteins[12], were not fully incorporated into the research scope. Future studies could further refine and expand this framework to comprehensively evaluate the overall impact of facial cleansers on skin health.

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