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*IFSCC 2025 full paper (IFSCC2025-368)*

## ***The “skin liquid rinse” concept: New technology for gently washing the skin***

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### **Introduction**

A wide range of cosmetic products are available for cleaning the skin, such as facial washes, shampoos, and cleansers, each of which involve specific cleaning methods [1, 2]. This variety may reflect a strong desire among consumers to gently remove unwanted contaminants from the areas targeted by cleaning, such as the skin and hair, while avoiding damage to these areas. Skin cleaning agents, for example, are expected to not just remove contaminants, but to do so without damaging the barrier functions and moisturizing factors of the skin. Moreover, in some cases, such products have to meet very specific needs, such as removing skin roughness that is not readily visible to the naked eye, in order to achieve more beautiful skin. To achieve maximal cleaning capability with minimal skin damage, a great deal of research has been conducted looking at types and formulations of surfactants and how to combine them with other supplementary ingredients [3]. However, the fact remains that improving cleaning power and reducing damage to the skin are notions that run counter to each other. Achieving both aims simultaneously is thus enormously difficult. We therefore set about developing a cleanser with the surfactant concentration kept to a minimum to reduce chemical damage to the skin, but with the power to remove old keratin and other contaminants without rubbing, to reduce physical damage to the skin. During the development process, we came up with the concept of a new liquid skin cleanser formulation, which, unlike the conventional lather or foam type, does not rub or chafe the skin. We believed that a liquid skin cleanser could reduce both chemical and physical damage to the skin. Taking the possible effects on the skin into consideration, for development we selected amino acid surfactants, which cause little irritation and are extremely safe [4], as the main component. In addition, we focused on oil-based formulations that can dissolve in water (water-soluble oils) to enhance the cleaning action of the surfactant [5]. We screened a range of water-soluble oils from the perspective of dynamic surface tension, after which we evaluated the ability of one water-soluble oil to remove sebum and keratin when used in combination with a surfactant.

## Materials and Methods

### Test substances

Sodium lauroyl aspartate (SLA) was purchased from Asahi Kasei Finechem Co. (Osaka, Japan). The water-soluble oils used in tests with SLA are shown in Table 1.

### Table 1

Components	Surface tension (mN/m)
Bis-ethoxydiglycol cyclohexane 1,4-dicarboxylate (BECD)	52
Bis-ethoxydiglycol succinate (BES)	46
Diethoxyethyl succinate (DS)	44
Ethoxydiglycol acetate (EDGA)	48
Ethoxydiglycol (EDG)	53

## Dynamic surface tension

The dynamic surface tension of SLA combined with each water-soluble oil was measured using a bubble pressure tensiometer (BP100; Krüss, Hamburg, Germany). Measurements were carried out with test substances at 25°C.

### Sebum cleaning ability test

The model for sebum contamination was artificial sebum containing 0.4 wt% carbon black, which was selected based on a previous report [6]. The model contaminant (3.0 mg) was applied to the skin of 6 subjects and spread to form a uniform circle 1.2 cm in diameter. Color measurements were taken using a spectrophotometer (CM-700d; Konica Minolta, Tokyo, Japan). The model sebum was washed off only by rinsing with 10 ml of the test substance at a fixed rate, after which color measurement was repeated. Cleaning ability was evaluated by calculating the cleaning rate (%) from the measurements before and after cleaning. The cleaning rate was calculated as described below.

Cleansing rate (%) =  $\Delta ES^*/\Delta EO^* \times 100$

$\Delta ES^*$ : difference in skin color between before and after cleaning

$\Delta EO^*$ : difference in skin color between before and after application of model sebum

#### Keratin cleaning ability test

Donor chamber (parts of a Franz Cell) was affixed to the inner arm and 1 ml of each test substance dripped on the skin with a micropipette. Using a dropper, pipetting was carried out at a rate of approximately 1 drop/sec for 2 min to clean the skin by rinsing with the test substance alone. The fluid from rinsing was collected in a microcentrifuge tube and centrifuged at 15,000 rpm for 10 min, and the supernatant was removed. 30  $\mu$ l of Phosphate-buffered saline (PBS) was added, and the suspension was thoroughly agitated, then which 10  $\mu$ l was dispensed into a 96-well plate. To 10  $\mu$ l of this suspension, 10  $\mu$ l of 0.4% trypan blue solution (Thermo Fisher Scientific, Tokyo, Japan) was added and the suspension was agitated once again. The suspension was added to a hemacytometer, and dead skin cells counted under electron microscopy.

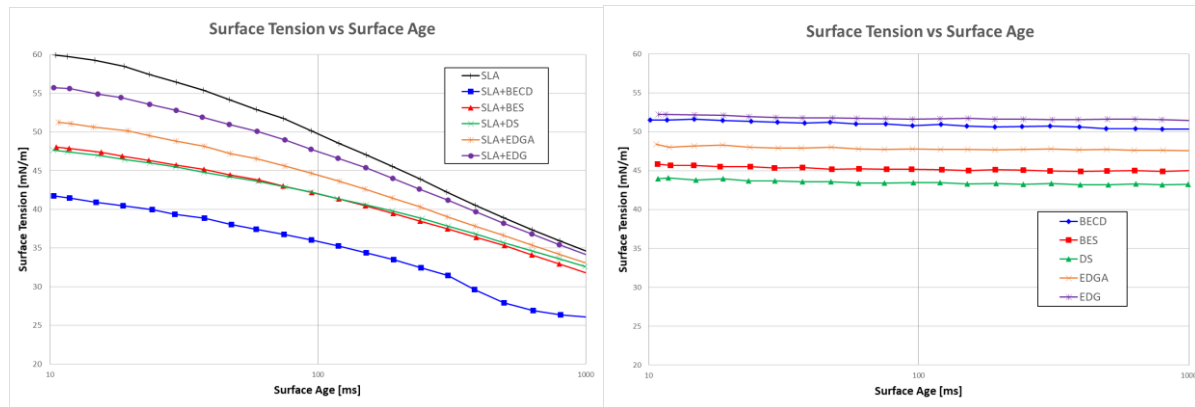
#### Clinical tests

Keratin plugs removal from the nose was evaluated in 6 female subjects aged in their 20s and 30s. The skin liquid rinse that was developed was placed in a dedicated cup, and perinasal skin was washed by holding the dedicated cup against the tip of the nose and squeezing it with gentle movements for 30 s to generate movement of the fluid. This washing method was carried out once daily for 2 weeks, and changes in keratin plugs on the nose were observed (Fig. 3). Observations were carried out using pore photographs from a Visia facial imaging system (Canfield Scientific, New Jersey, United States) and a microscope (50 $\times$ ; Moritex, Kanagawa, Japan). The improvement rate of Keratin plugs was evaluated as the change in the size of the keratin plug in the same pore. For comparison, similar images were obtained from 6 female subjects in their 20s and 30s who used only regular face wash in the normal way, without using the skin liquid rinse. This study was conducted in accordance with the principles of the Declaration of Helsinki.

## **Results**

### **Dynamic surface tension reduction with SLA and BECD used together**

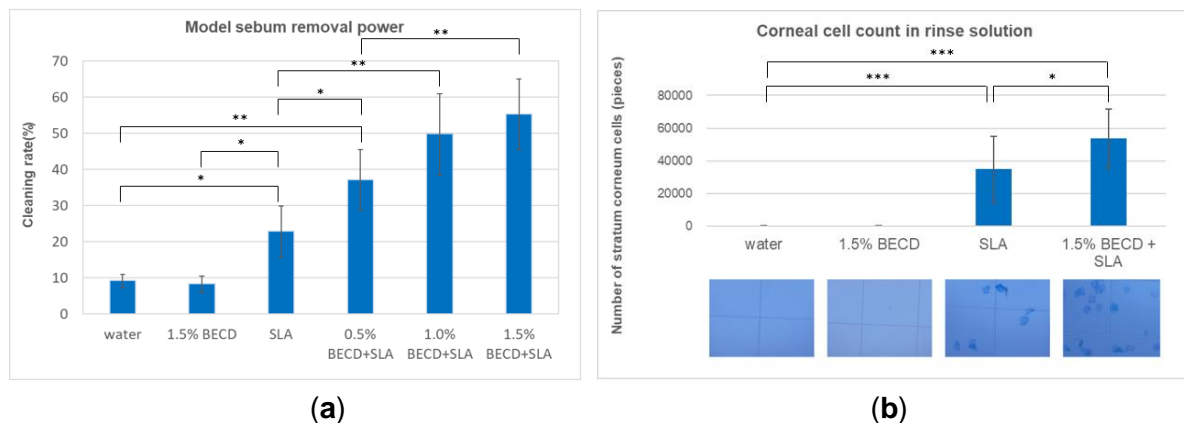
To examine the effects of surfactant and water-soluble oils on the interface, mixtures of 1% SLA and each water-soluble oil (1:1, w/w) were prepared. Following incubation at 25°C, dynamic surface tension was measured. Interestingly, the results showed that using BECD and SLA together causes a notable reduction in dynamic surface tension (Fig. 1).



**Figure 1.** Dynamic surface tension at 25°C. a) Using 1% SLA in combination with 1% water-soluble oils. b) Using 1% water-soluble oils.

### Model sebum and keratin cleaning tests

To examine the cleaning ability of surfactant combined with water-soluble oil, tests were carried out in which the contaminant was washed off by rinsing with the test substance alone (Fig. 2). While BECD itself showed no cleaning action against the model sebum, the combination of BECD with SLA increased the sebum removal rate in a dose-dependent fashion (Fig. 2a). Similar results were obtained in the cleaning test in which keratin was washed off the skin (Fig. 2b).

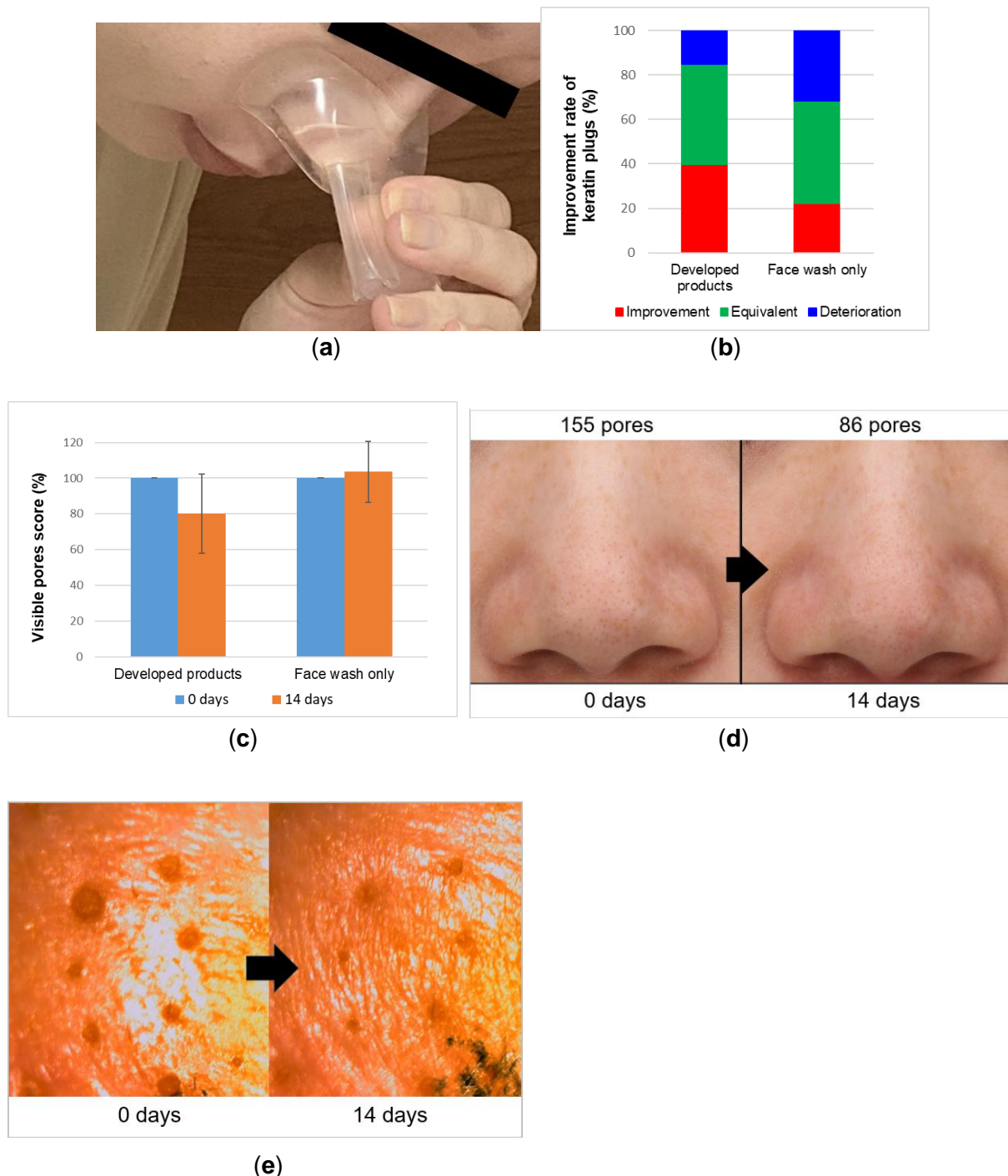


**Figure 2.** Cleaning power test results. a) Test on model sebum. \* $P < 0.05$  \*\* $P < 0.01$  b) Stratum corneum removal test. Cell counts were assessed on a hemocytometer using electron microscopy. \* $P < 0.05$  \*\* $P < 0.01$  \*\*\* $P < 0.001$

### Clinical tests

A number of issues must be addressed before the skin liquid rinse can be commercialized. In particular, while foam-based cleansers can readily be spread over a wide area, liquid rinse would require a greater quantity of the liquid for the same area. Liquids are also prone to problems such as running and splashing. Given these problems, along with developing the skin liquid rinse, we also developed a dedicated cup that covers the nose from the tip to the wings (nostrils) (Fig. 3a) and examined application with a cleanser that can remove dirt from

pores in this area of the nose through the generation of fluid movement in the cup. The results of tests to evaluate the usefulness of the cleanser and cup showed a greater proportion of smaller keratin plugs after continuous use of the liquid skin cleanser compared to baseline (Fig. 3b). Image analysis also showed a trend toward a decreased number of noticeable pores (Figs. 3c–e), suggesting that continued use may be expected to improve keratin plugs on the nose.

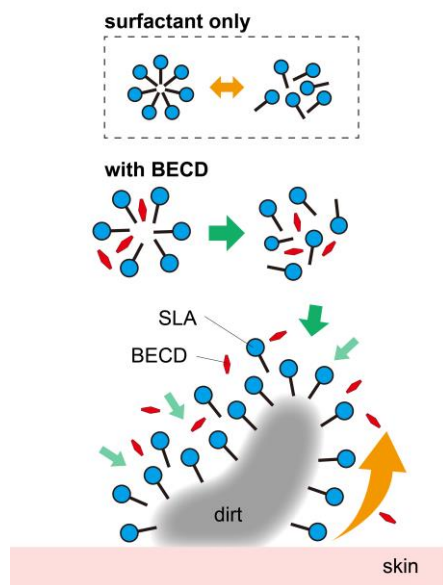


**Figure 3.** Clinical trial results and case report of marked responses in 6 patients in one group. a) The prototype cleaning cup. b) Observations made with a microscope of the same pores before and after continuous use. Improvement rate of keratin plugs was evaluated at three levels, results were compiled, and proportions were calculated for each group. c) In images

taken by Visia, the score before use was taken as 100 and the score after 14 days was compared to this. d) Image taken by VISIA, showing marked improvement of pores. e) Microscopy shows marked improvement of pores.

## Discussion

Cleaning ability comes from the physical action of scrubbing and the chemical action of the cleanser [7]. Keeping the concentration of surfactant as low as possible while maintaining the ability to clean off dirt such as old keratin without rubbing requires the cleanser itself to have the chemical action of rapidly reducing the surface tension of contaminants. We therefore examined the dynamic surface tension of various water-soluble oils in combination with surfactant to screen for materials that increase surfactant activity. The results show that all of the water-soluble oils decreased surface tension when used in combination with SLA (Table 1). BECD achieved particularly noticeable reductions in dynamic surface tension. The behavior of BECD clearly differs from that of other water-soluble oils, so the cleaning action was investigated. The cleaning ability test focused on the ability to clean the skin by rinsing with the test solution, with no rubbing. The results show that while BECD alone has no cleaning ability, use in combination with 1.0 wt% SLA improved the cleaning rate of SLA significantly (Fig. 2). The same result was found in the sebum cleaning test and the keratin cleaning test. Conventional skin cleansers are formulated with anionic surfactants in excessive concentrations to improve detergency and foam retention, but we discovered that combination with a water-soluble oil allows for a cleanser with a low concentration of surfactant and sufficient cleaning ability to remove dirt without rubbing. Based on this result, we developed a liquid skin cleanser that combines SLA and BECD, and evaluated its action on dirt in pores. As shown by the results of clinical tests (Fig. 3), dirt in pores was improved by the addition of a 30-sec cleaning action once a day, with no skin irritation. Looking at the results of dynamic surface tension measurement, the addition of BECD may be conjectured to change the adsorption behavior on dirt of the surfactant, resulting in increased cleaning ability. BECD exhibited different behaviors from other water-soluble oils, probably due to differences in the molecular structure. In aqueous solution, surfactants form micelles in which the molecules constantly coalesce and separation. The polarity and structural arrangement of the cyclohexane skeleton of BECD that is taken into SLA micelles is believed to weaken the aggregation force of the micelles. We hypothesize that as a result of this, SLA molecules can disperse more readily, so the rate of adsorption of SLA into the contaminant increases (Fig. 4). We intend to test our hypotheses and clarify the mechanism of cleaning in future studies.



**Figure 4.** Hypothesized cleaning mechanism

## Conclusion

The surface tension of a chemical solution itself does not correlate with its actual cleaning ability [8]. However, when combining two components together results in increased cleaning ability, this can be explained and discussed by comparing dynamic surface tension. Using this method of evaluation, we identified a new cleansing technology that keeps the amount of surfactant to a minimum but can still wash away old keratin.

The cleanser that we developed is a clear, non-viscous liquid containing an amino acid surfactant at a low concentration and a water-soluble oil. We named this skin liquid rinse. The distinguishing feature of this cleanser is that, unlike conventional cleansers applied in foam or gel form and scrubbed off, this highly fluid, low-viscosity liquid can be applied as is to the skin. Neither foam stabilizers nor thickening agents are needed, the skin liquid rinse formulation can be designed with a minimal amount of surfactants and other types of stabilizers.. By capitalizing on these advantages, it will be possible to provide a cleanser that does not irritate sensitive skin prone to inflammation from chemical components or rubbing the skin. This new concept of skin liquid rinse, in which the liquid itself cleanses the skin, holds great potential as a new skin cleansing technology for consumers.

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