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"Revolutionizing Pore-care

A Breakthrough Approach for Effective and Gentle Removal of Keratotic Plugs for Healthier Skin"

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1. Introduction

In recent years, significant advancements in camera technology—particularly the development of high-resolution imaging driven by the widespread use of photography and social media sharing—have brought increased attention to the visual appearance of skin. Among various factors influencing perceived skin quality, the presence of keratotic plugs has emerged as a critical concern, as they visibly affect the condition of pores. The appearance of pores plays a crucial role in the evaluation of visual attractiveness but also in their own perception of hygiene and cleanliness. Consequently, improving pore-related skin concerns has become an important objective for individuals seeking enhanced skin aesthetics.

In response to this growing demand, the present study proposes a novel method for the effective and gentle removal of keratotic plugs, defined herein as a general term for solid substances that obstruct the pores.

Conventional methods for the removal of keratotic plugs include mechanical exfoliation using scrubs, chemical exfoliation through peeling agents, and physical extraction via pore strips. While these approaches can be effective to some extent, they often impose mechanical or chemical stress on the skin, potentially leading to irritation or barrier disruption. Keratotic plugs are primarily composed of proteins and lipids, with reported ratios ranging from approximately 50–70% protein to 30–50% lipid content [1,2]. Accordingly, removal strategies have been developed that target these components using surfactants to disperse proteins, oils to solubilize lipids, or combinations thereof [3]. However, despite these efforts, the prevalence of individuals

experiencing pore-related concerns continues to increase, and current methods often fall short of delivering satisfactory results in terms of both efficacy and skin tolerability.
In this study, we began by conducting a detailed structural analysis of keratotic plugs, revealing their composition and morphology with a level of resolution not previously reported. Building upon our findings, we investigated novel approaches for their effective removal, including the screening of unconventional ingredients not typically used in cleansing formulations. Through this process, we identified Tris(hydroxymethyl)aminomethane (commonly referred to as TRIS) as a promising candidate capable of promoting the spontaneous disintegration of keratotic plugs. We subsequently confirmed the efficacy of TRIS as a functional cleansing agent and formulated a prototype facial cleanser incorporating TRIS. This formulation was then subjected to a series of efficacy evaluations to assess its performance in keratotic plug removal.

2. Materials and Methods

2-1. Collection of Keratotic Plugs and Preparation of Sections

Keratotic plugs were collected from the nasal area of males in 20s and 30s using Pore strip . Sections of the keratotic plugs were prepared using a Cryostat HM550 (Thermo Fisher Scientific). Continuous sections with a thickness of 10 μm were cut at -20°C.

2-2. Comprehensive Analysis of Lipids and Proteins in Keratotic Plugs

Lipids were extracted from the keratotic plugs using chloroform. The extracted lipids were analyzed using an Agilent 6460 triple Quadrupole MS system (Agilent Technologies). Quantification was performed using correction factors derived from sensitivity differences with internal standards for Fatty acids (FA), Wax esters (WAX), Cholesterol (ChE), Squalene, and Triglycerides (TAG). After lipid extraction, the keratotic plug samples underwent pretreatment using the PTS method, followed by LC-MS/MS analysis with an Ultimate 3000 RSLCnano System (Thermo Fisher Scientific) and a TripleTOF 5600+ system (Sciex). Protein identification and quantification were achieved by matching the obtained data with the SwissProt database.

2-3. Microstructural and Compositional Distribution Analysis of Keratotic Plugs

Keratotic plug sections sliced to a thickness of 10 μm were observed under reflected light using a KH-8700 digital microscope (HiROX). For the same sections, component distribution analysis was performed using IR imaging. The wave number regions of amide I (1690-1620 cm^{-1}) and NH stretching vibrations (3500-3200 cm^{-1}) were utilized for proteins, while the CH_2 and CH_3 stretching vibration wave number regions (3150-2750 cm^{-1}) were used for lipids. The peak areas of the IR spectra were processed as signal intensity for imaging.

2-4. Microscopic Observation of Morphological Changes in Keratotic Plugs and Sections

Keratotic plugs or sections were placed on a glass slide and covered with a cover slip. After adding 0.05 mL of the sample to the edge of the cover slip, the sample entered the gap between the slide and cover slip due to capillary action, facilitating contact between the keratotic plugs and the sample. The morphology of the keratotic plugs or sections was then observed and scored over time using the digital microscope VHX-5000 (Keyence).

2-5. Confirmation of Miscibility with Solid FA (Lauric Acid)

A ternary phase diagram was constructed using lauric acid, a pH 10 buffer solution composed of 0.26 % sodium carbonate and 0.21 % sodium bicarbonate, and various agents (Sodium POE alkyl ether sulfate (SLES), L-Arginine, Triethanolamine, and TRIS). A total of 35 ternary mixtures were formulated with lauric acid and base components at concentrations ranging from 0 to 20%. The mixtures were stirred at 60°C, and equilibrium was reached when the phase state remained unchanged for over one week at 25°C. Visual inspection, polarized microscopy, small-angle X-ray scattering, and differential scanning calorimetry (DSC) were performed to assess the phase state.

2-6. Swelling Test of Stratum Corneum Proteins

20 mg of pulverized and dried stratum corneum from the soles of the feet, 1.8 mL of 1 M TRIS solution and 2 % SLES solution were mixed. The mixture was then placed in test tubes with a diameter of 5 mm and allowed to stand for 12 hours. The height of the swollen stratum corneum was measured.

2-7. Changes in the Keratotic Plug with Continuous Use of Facial Cleansers

A study was conducted with 22 Japanese women aged 30s to 40s who were asked to use either a TRIS-containing facial cleanser* or a conventional facial cleanser** (approximately 2 g) twice daily (morning and night) for 4 weeks. UV-illuminated facial images were captured before and after the 4-week period. Image analysis was conducted to calculate and compare the area of keratotic plugs before and after 4 weeks of continuous use for the forehead, cheeks, and nose. Statistical significance was assessed using paired *t*-test.

The study protocol was approved by the ethics committee of Kao Corporation in Japan, in accordance with the recommendations of the Helsinki Declaration. Informed consent was obtained from all volunteers prior to the study.

*TRIS-containing Facial Cleanser ; Sorbitol, glycerin, mannitol, trehalose, TRIS, arginine, propylene glycol, laureth-21, steareth-13, laureth-4 carboxylic acid, (acrylate/acrylic acid alkyl (C10-30)) crosspolymer, EDTA-2Na, phenoxyethanol, fragrance.

**Conventional Facial Cleanser ; Water, glycerin, stearic acid, myristic acid, laureth-6 carboxylic acid, palmitic acid, potassium hydroxide, lauric acid, sorbitol, polyquaternium-7, propylene glycol, sodium benzoate, EDTA-2Na, fragrance.

3. Results

3-1. Composition of Proteins and Lipids in Keratotic Plugs

The protein composition of keratotic plugs collected from the nasal area is shown in Figure 1a [4]. The results indicate that Keratin 17 (KRT17), which is predominantly derived from hair follicle cells, is present in keratotic plugs in significantly greater quantities compared to KRT10, a major protein expressed exclusively in epidermal cells. This finding suggests that the proteins in keratotic plugs are primarily derived from hair follicles rather than the epidermis. Figure 1b [4] shows the lipid composition obtained from the keratotic plugs. As indicated by these results, the majority of the constituent lipids were derived from sebum. Reports indicate that the FA

ratio in sebum account for 7.9-39.0 %, with an average of 16.4 % [5]; however, it was found that keratotic plugs contain a slightly higher quantity of FA. Additionally, solid FA constitute over 30 % of the total lipids. Considering reports that estimate solid FA in human skin surface sebum to be approximately 6 % of the total lipids [6], it can be concluded that the amount of solid lipids in keratotic plugs is considerably higher.

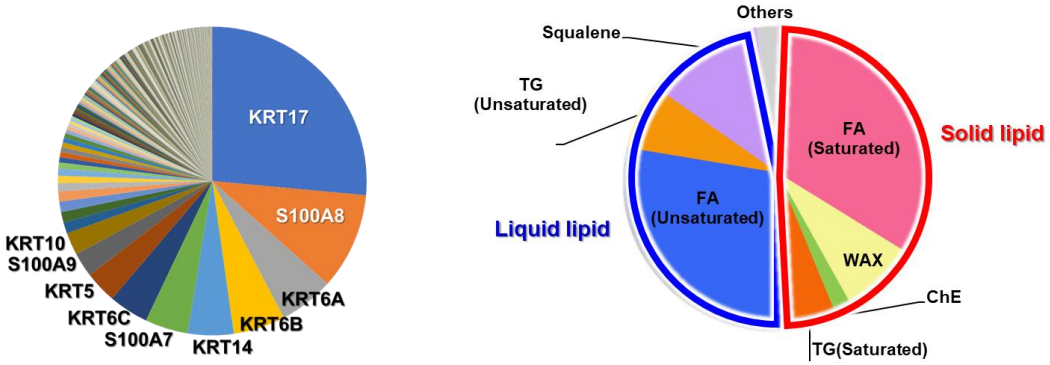


Figure 1. Composition of Keratotic Plug: (a) Proteins, (b) Lipids

3-2. Microstructural and Compositional Distribution Analysis of Keratotic Plugs

Observation of the keratotic plug sections obtained from the nasal area using a digital microscope revealed a layered structure resembling natural geological strata (Figure 2a [4]). Furthermore, by comparing the distributions of proteins and lipids as shown in Figures 2b and 2c [7], it was found that lipids and proteins coexist in certain regions, with lipids forming distinct domains, while proteins were structured around the lipids.

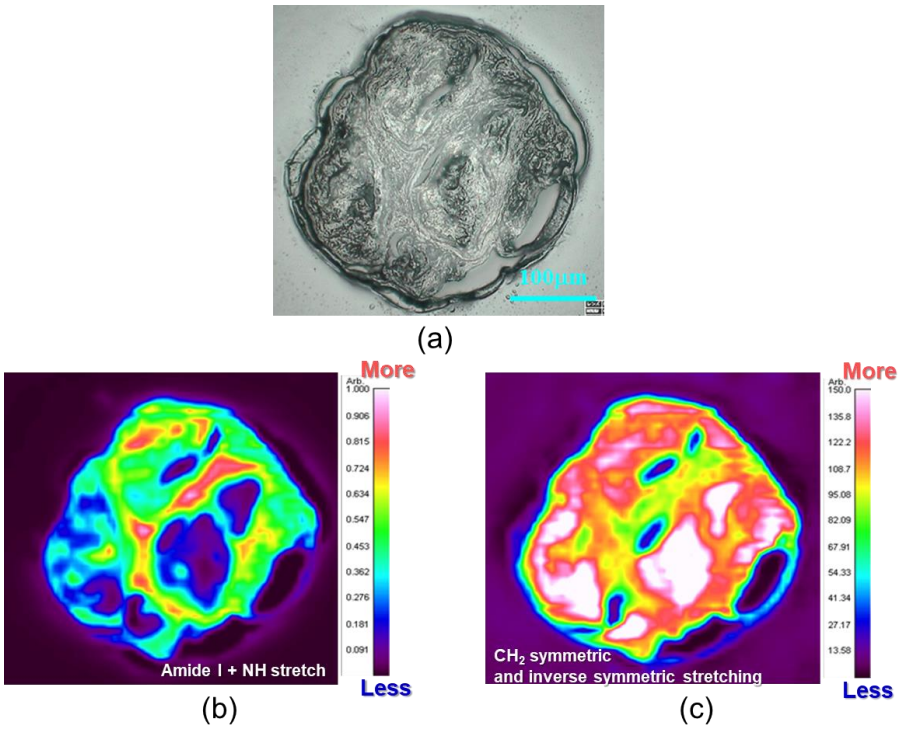


Figure 2. Keratotic Plug sections: (a) Microscopic image, (b) IR imaging of Proteins, (c) IR imaging of Lipids.

3-3. Microscopic Observation of Morphological Changes in Keratotic Plugs and Sections

When various oils and solutions of cleansing bases, including urea, were applied to the keratotic plugs collected from the nasal area, almost no change-only slightly swelling on the outside were observed, with minimal overall change (Figure 3-4 [4]).

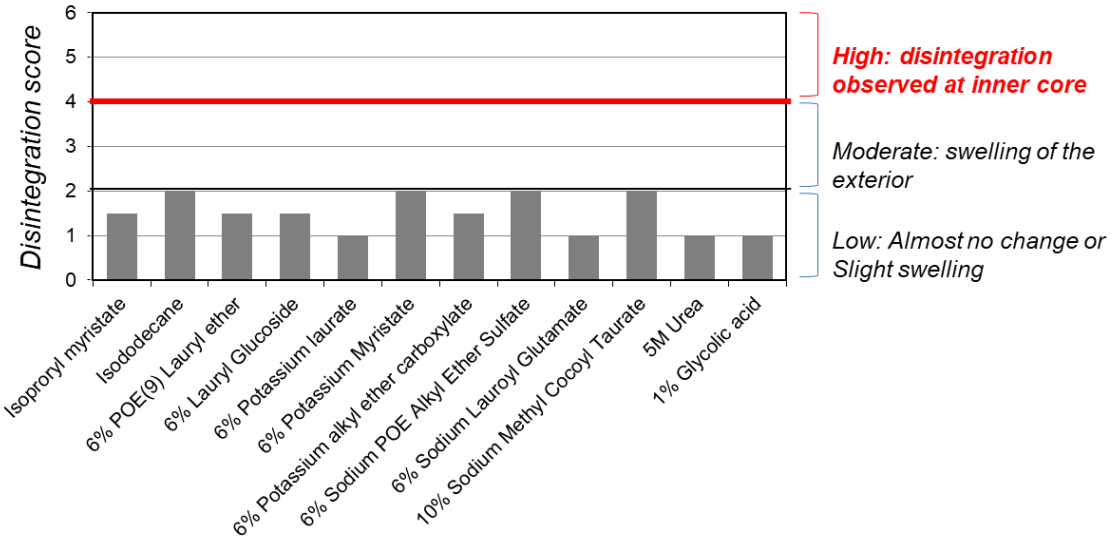
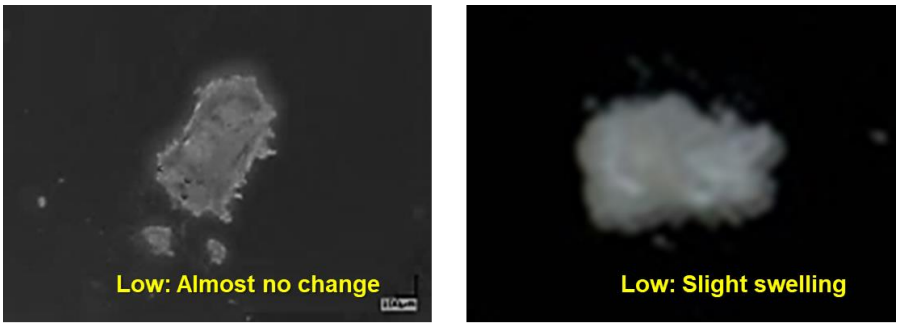


Figure 3. Disintegration score of keratotic plugs when immersed in each sample.



(a) (b)

Figure 4. Images of keratotic plugs when immersed in (a) AEC (Potassium alkyl ether carboxylate) and (b) SLES for 1 minute.

To investigate further, potassium fatty acid salt solutions were applied to the keratotic plug sections. Although the outer rim swelled slightly, there was no sign of disinteratration, and the overall state remained unchanged (Figure 5 [4]).

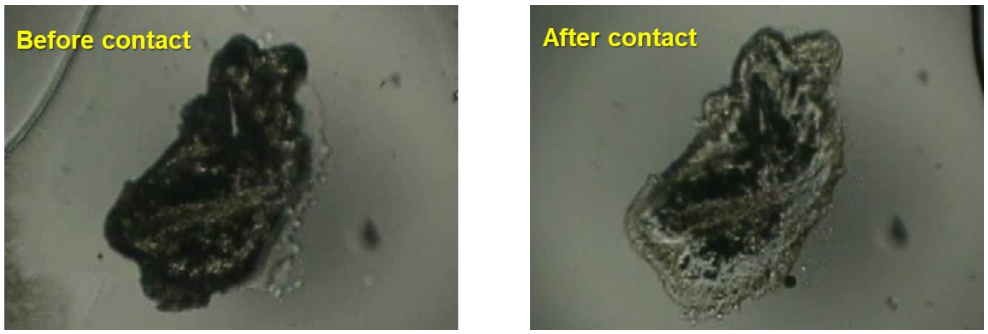


Figure 5. Keratotic Plug Section Treated with 6% of Potassium -Neutralized Mixed Fatty Acid Solution (lauric acid (2.5%), myristic acid (1.9%), and palmitic acid (1.6%), pH10)

A synergistic effect was anticipated using a 1:1 mixture of AEC (Potassium alkyl ether carboxylate) and SLES in a 6% solution with a pH of 10, because, AEC was known for its strong lipid removal capabilities and SLES was known to excell at dispersing solid contaminants like proteins. However, while a slight increase in swelling was observed compared to the use of each component on its own, no significant change in state was noted (Data not shown).
Focusing on the high presence of fatty acids among the lipids constituting the keratotic plugs, basic cleansing agents such as Sodium bicarbonate and alkaline pH adjusters were tested as well. Upon contact with solutions of these bases, it was found that Sodium bicarbonate did not alter the keratotic plugs, either. However, Triethanolamine and L-Arginine demonstrated the ability to cause moderate swelling of the exterior, while Amino methyl propanol and TRIS exhibited high levels of disintegration that reached the inner core (Figure 6). Notably, TRIS exhibited the highest disintegration score for keratotic plugs. When keratotic plug sections were immersed in TRIS solution, spontaneous infiltration of the solution into the interior of the keratotic plugs was observed, causing the surrounding proteins to rupture (Figure 7 [4]).

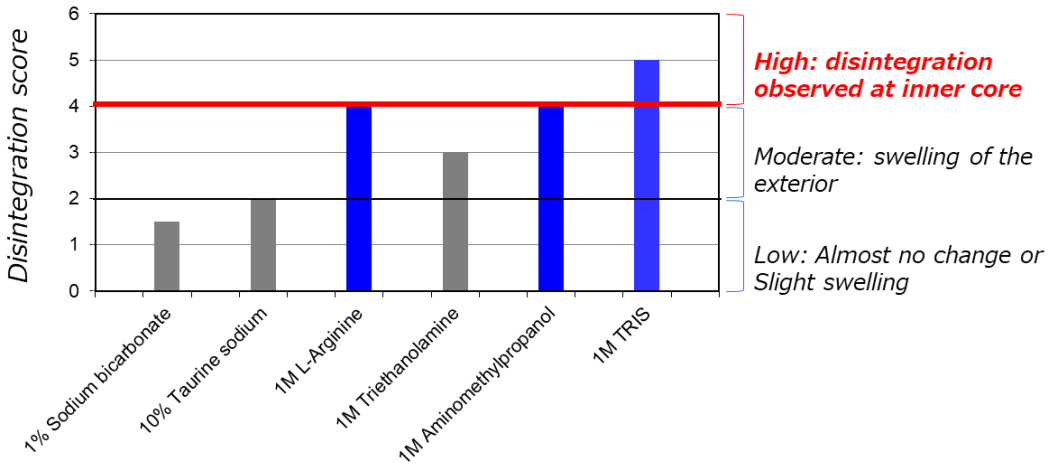


Figure 6. Morphology change of keratotic plugs when immersed in various alkali solution.

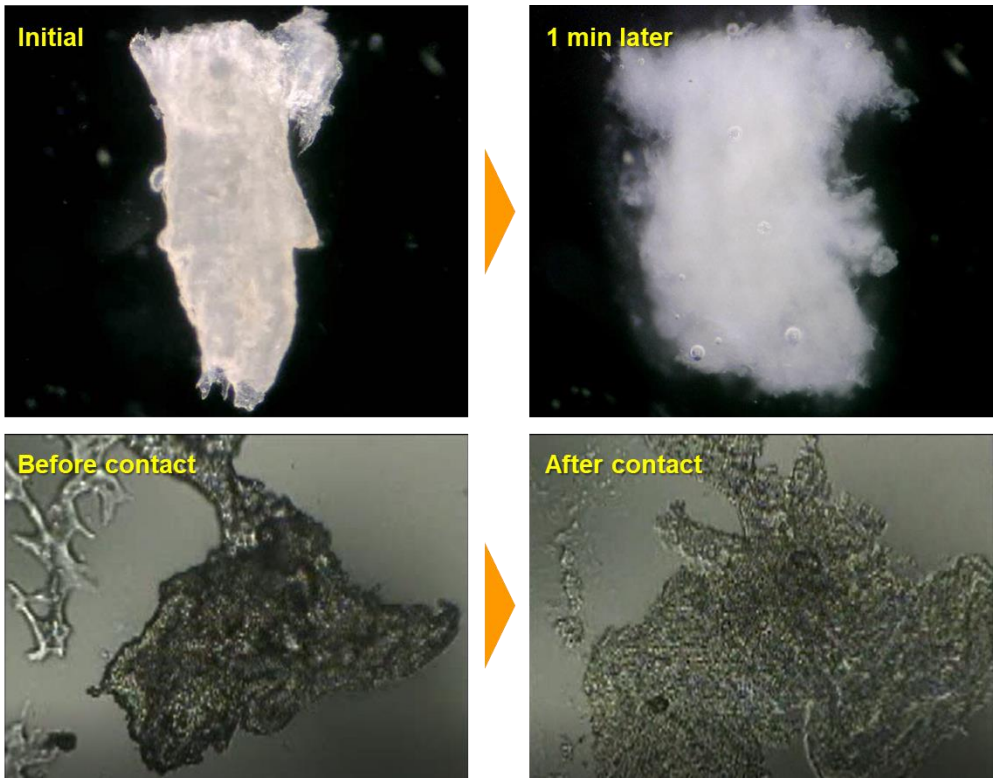


Figure 7. Morphological changes in the Keratotic plug (above) and its section (below) upon exposure to TRIS solution for 1 minute, respectively.

3-4. Miscibility with Solid Fatty Acids

As shown in Figures 8a-d, three distinct regions exist for each agent: isotropic solution, liquid crystal formation, and solid precipitation. The regions demonstrating miscibility expanded in the order of SLES, L-Arginine, Triethanolamine, and TRIS. Notably, TRIS exhibited the widest region of miscibility with lauric acid, indicating its strong ability to disrupt keratotic plugs.

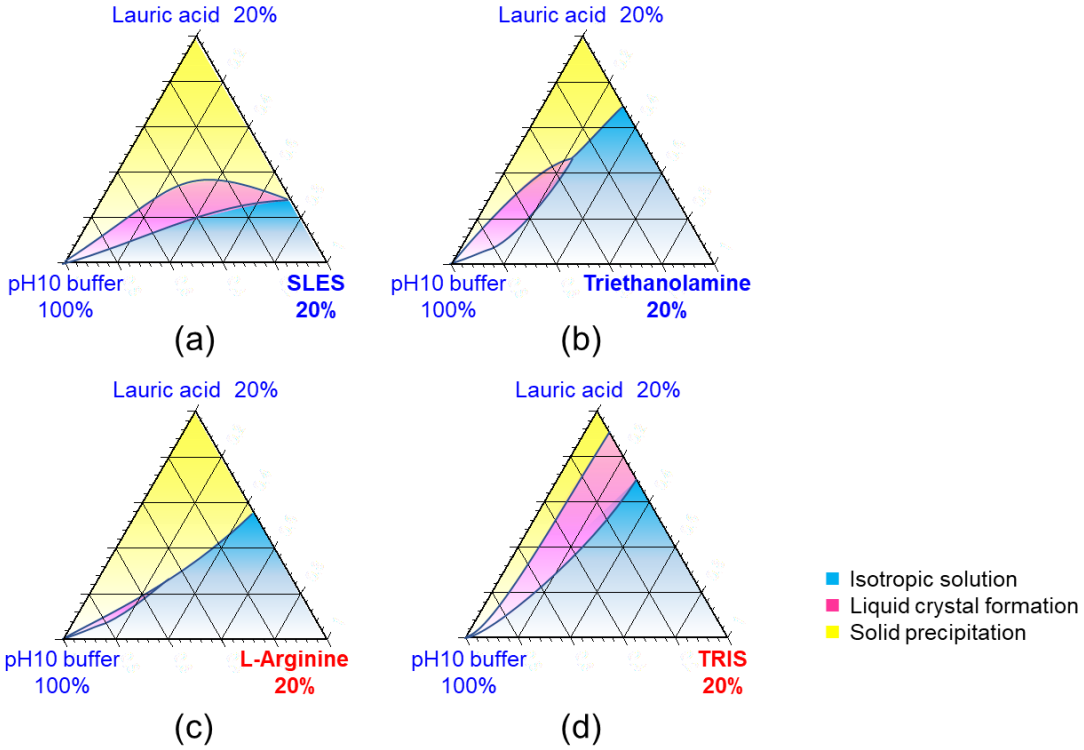


Figure 8. Ternary phase diagram of each sample with Lauric acid and pH10 buffer.

3-5. Effect of TRIS solution to stratum corneum

The degree of swelling of the stratum corneum, an indicator of skin mildness, was assessed for the TRIS solution. The results showed that the TRIS solution exhibited significantly higher swelling comparable to that of water (Figure 9 [4]).

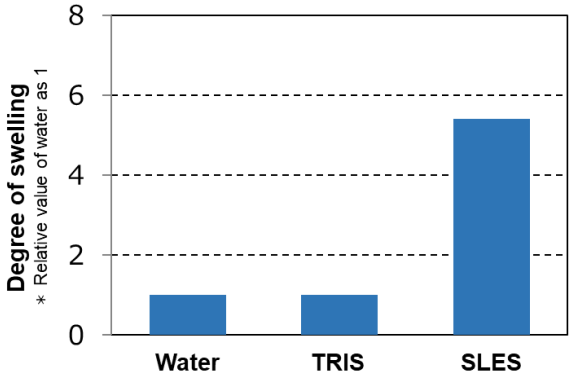


Figure 9. Swelling degree of Stratum corneum protein by TRIS solution.

3-6. Efficacy of Keratotic Plug Removal by TRIS-Containing Facial Cleanser

A facial cleanser incorporating TRIS was then developed, and a 4-week continuous use study was conducted. Upon use, a significant reduction in keratotic plugs on the entire face was observed upon use of the TRIS-containg facial cleanser after 4 weeks of use (a), while the conventional facial cleanser without TRIS did not show a significant change afer use (b). (Figure 10 [7]).

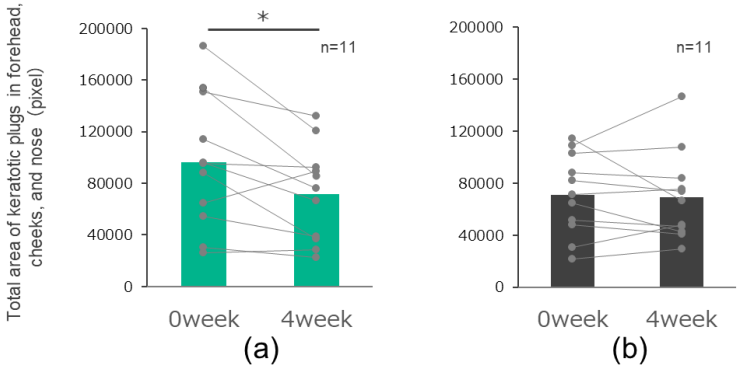


Figure 10. Changes in keratotic plugs due to 4 weeks of continuous use of the TRIS-containing facial cleanser (a) and a conventional facial cleanser without TRIS (b)

4. Discussion

4-1. Difficulty in Cleaning Keratotic Plugs

Keratotic plugs have long been regarded as difficult to clean due to their complex nature, consisting of lipids and proteins, and their location, settled within the pores where it is difficult to physically touch putting a limit to the effectiveness of conventional cleansing agents. Through this detailed analysis, it has become evident that keratotic plugs are aggregates of solid lipids, encased in a layered shell of hair follicle-derived proteins. This new understanding reveals that the difficulty in cleaning keratotic plugs is largely attributed to the presence of solid lipids, which are challenging to cleanse, and their interconnection with difficult-to-remove proteins.

4-2. Mechanism of TRIS-Induced Disintegration of Keratotic Plugs

For keratotic plugs to spontaneously disintegrate upon contact with TRIS solution, changes must occur from within. All base agents demonstrating keratotic plug disintegration in this study contained amino groups, suggesting a potential neutralization reaction with the fatty acids present in the plugs. The interaction between these amino groups and solid fatty acids is expected to yield a fluid solution. The ternary phase diagram created with lauric acid as the solid fatty acid and the base agent mixed with water showed that TRIS could dissolve lauric acid in the widest area (Figure 8). This indicates that TRIS neutralizes lauric acid, converting it from a solid to a fluid water-soluble component, specifically a lauric acid TRIS salt. As shown in Figures 7, applying TRIS solution to the keratotic plugs resulted in increased volume, indicating water infiltration, likely due to osmotic pressure created by the generation of water-soluble fatty acid TRIS salts within the plugs. Figure 11 [4] visually illustrates this process, demonstrating how TRIS disintegrates keratotic plugs.

Keratotic plugs consist of solid lipids functioning as cores or binders, enveloped in a layered shell of proteins. It is hypothesized that TRIS molecules forms of fatty acid TRIS salts with poorly soluble solid lipids, converting them into water-soluble components. The creation of these water-soluble fatty acid TRIS salts induces osmotic pressure, facilitating water entry and the removal of the entire plug. This mechanism is believed to explain the spontaneous disintegration of keratotic plugs [4].

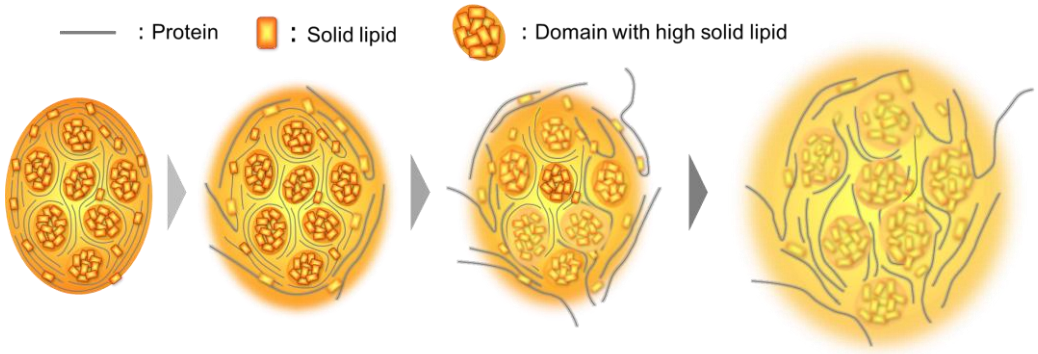


Figure 11. Image of process of spontaneous disintegration of keratotic plug by TRIS

4-3. Utility of Cleaning Techniques Using TRIS

TRIS is widely used as a buffering agent known to enhance protein and peptide solubility, stabilize tertiary structures, and increase resistance to thermal denaturation [8]. Effective cleansing formulations typically exert a greater stress on the skin, creating a trade-off between cleansing efficacy and mildness [9]. TRIS's ability to remove keratotic plugs without surfactant properties is therefore a revolutionary finding.

Traditionally, keratotic plugs were viewed as a localized issue primarily affecting the nose; however, recent findings indicate that keratotic plugs exist across the entire face, including the forehead and cheeks [7]. Cleansing products utilizing this novel TRIS technology are expected to offer a gentle yet effective solution for keratotic plug removal.

5. Conclusion

In this study, we elucidated the structure and composition of keratotic plugs through detailed analysis, demonstrating the effectiveness of tris(hydroxymethyl)aminomethane (TRIS) as a novel approach that surpasses traditional methods for removing keratotic plugs. Keratotic plugs are primarily composed of hair follicle -derived proteins and solid lipids. TRIS interacts with these components to promote their spontaneous disintegration through a neutralization reaction between its amino groups and fatty acids, generating water-soluble fatty acid TRIS salts. This process facilitates osmotic water influx into the keratotic plugs, resulting in their effective removal.

We are currently marketing a facial cleanser that utilizes this TRIS technology, enabling customers to remove keratotic plugs daily without burdening the skin. This innovation aims to address concerns regarding rough skin texture and visible pores. Moving forward, we will continue to enhance cleansing formulas that incorporate this mechanism, beginning with TRIS, and assess their efficacy in real-world applications. Our goal is to develop more effective and

skin-friendly skincare products, contributing to the realization of beautiful skin for our customers.

6. References

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