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# *"Suppression of Cuticle Peeling by a Low Molecular Weight Amphiphilic Substance"*

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## 1. Introduction

It is well known that hair can be damaged by various external factors such as by chemical stimuli caused by perms and hair coloring, by environmental stimuli caused by ultraviolet rays, dryness and heat from blow-drying [1], and by physical stimuli caused by friction during washing, blow-drying wet hair, and brushing [2]. The progression of hair damage typically begins with the structural deterioration of the outermost layer, the cuticle, which becomes damaged and eventually peels off [3]. That process compromises the barrier function of the hair as a whole, thereby accelerating damage to the inner cortex layer, which decreases the hair's rigidity (firmness) and reduces its elasticity and strength. Conventional cuticle-repairing agents primarily function by forming a coating on the hair surface to suppress cuticle damage. However, the continued use of those agents often results in their buildup on the hair surface, which negatively affects hair texture [4].

It has been reported that 2-naphthalenesulfonic acid and p-toluenesulfonic acid can physically alter the internal hair structure and mitigate waviness [5, 6], which suggests that those compounds act within the hair interior. Additionally, the hair and skin are primarily composed of proteins, and their constituent amino acids are commonly used in various cosmetics as beauty and health agents for hair, skin, and more. In a previous study, we synthesized compounds by adding a tosyl group containing a benzene ring to amino acids and, through sensory evaluation screening, identified a component, Sodium Tosyl Valinate (TSV), which caused a significant improvement in hair firmness [7, 8]. TSV is a low-molecular-weight amphiphilic compound in which valine, an amino acid, is tosylated, thereby incorporating a hydrophobic tolyl moiety from the tosyl group. Although TSV lacks the strong coating ability that is characteristic of polymeric agents commonly used to suppress hair damage, its small molecular size suggests that it can readily penetrate hair fibers. Therefore, it is anticipated that TSV can enhance hair strength without causing a surface buildup, a common issue with coating agents. In this study, we demonstrate that TSV exerts a reparative effect against cuticle peeling without forming a surface coating or causing a buildup.

## 2. Materials and Methods

*2-1. Hair Bundle Processing Method*

Asian human hair (Beaulax, Saitama, Japan) was immersed in a 2.0% Sodium Laureth Sulfate (SLES) aqueous solution at 40°C for 30 minutes. After rinsing with water, the hair was dried and used as the untreated control sample, hereafter referred as "Untreated hair" (UH).

2-1-1. Bleached Hair

UH was immersed in a mixture of 6.0% hydrogen peroxide and 2.0% ammonia (weight ratio 1:1) at 30°C for 30 minutes, followed by rinsing with water. The hair bundle was then immersed in a pH 3 buffer solution (containing 0.1 mol/L citric acid and 0.2 mol/L disodium hydrogen phosphate) at room temperature for 5 minutes, after which it was rinsed with water and then dried. This process was repeated three times, and the treated sample is referred to as "Bleached hair" (hereinafter denoted as BH).

2-1-2. Bleached and Permed Hair

A 7% ammonium thioglycolate aqueous solution adjusted to pH 9.25 with monoethanolamine was applied to the BH and allowed to sit at room temperature for 15 minutes, followed by rinsing with water. The hair bundle was then treated with a 7% sodium bromate aqueous solution adjusted to pH 6.5 with phosphate buffer at room temperature for 15 minutes. After rinsing with water, it was dried. This sample is referred to as "Bleached and Permed hair" (hereinafter denoted as BPH).

*2-2. Cuticle Peeling Test*

BH was immersed in a 0.08% (w/w) TSV aqueous solution at 40°C for 60 minutes and then rinsed with running water at 40°C for 1 minute. The hair was then subjected to ultrasonic treatment at 40°C for 30 minutes (AS52TU, AS ONE). The number of cuticle layers in the hair cross-sections were counted using scanning electron microscope (SEM) images (JSM-6010LA, JEOL). As a control experiment, the sample was treated with deionized water instead of the TSV aqueous solution.

*2-3. Split End Test*

Approximately 300 strands of BH were immersed in a 0.08% (w/w) TSV aqueous solution or in a 10× diluted shampoo solution (Table 1) at 40°C for 10 minutes, followed by rinsing with running water at 40°C for 1 minute and drying. In the shampoo treatment, this process was repeated six times, and the samples were prepared. After treatment, split ends were induced by brushing (6,000 times), and the numbers of split ends were counted. For control experiments, the samples were treated with deionized water instead of the TSV aqueous solution, and with a shampoo without TSV instead of the TSV shampoo, and are referred to as the "control solution" and the "control shampoo (Table 1)," respectively.

**Table 1.** Formulations of hair shampoos.

| | w/w% | |
|---|---|---|
| **Ingredients** | **control shampoo** | **TSV shampoo** |
| TSV | - | 0.08 |
| Butylene Glycol | - | 0.12 |
| Polyquaternium-10 | 0.20 | 0.20 |
| Sodium Laureth Sulfate, Water | 30.0 | 30.0 |
| Cocamidopropyl Betaine, Water | 10.0 | 10.0 |
| Cocamide DEA | 3.00 | 3.00 |
| Citric acid | 0.10 | 0.10 |
| Preservative | 0.50 | 0.50 |
| Water | qs. 100 | qs. 100 |

*2-4. Observation of Surface SEM Images of Extended Hair*

Hair samples with cross-sectional areas of 5,000 ± 300 μm² from the BPH group were selected using a hair diameter measuring device (SK-2000, KatoTech). These hair samples were immersed in a 0.08% (w/w) TSV aqueous solution at 40°C for 60 minutes, then were rinsed with running water at 40°C for 1 minute and dried. The treated hair samples were then stretched using a high-sensitivity tensile testing machine (KES-G1-SH, KatoTech) under a tensile stress of 100 gf. The extended surface of each hair was observed using SEM at 800× magnification. The SEM images obtained were processed using Image J to binarize them and segment the regions where the cuticles were lifted and those where they were not, followed by image analysis to calculate the percentage of cuticles that had peeled off. As a control experiment, BPH treated with the control solution (water) instead of the TSV aqueous solution was also extended, and the surface was observed using SEM.

*2-5. Dynamic Friction Test*

Twenty BH strands were fixed onto a glass slide. The slide was immersed in 0.1% (w/w) or 0.2% (w/w) TSV aqueous solutions at 40°C for 10 minutes, followed by rinsing with running water at 40°C for 15 seconds and drying. The mean frictional coefficient (MIU) of the treated hair samples was measured using a friction tester (KES-SE, KatoTech).

## 3. Results

*3-1. Cuticle Peeling Test*

The ultrasonic treatment of BH resulted in a further reduction in the number of cuticle layers, but when TSV treatment was applied prior to the ultrasonic treatment, the number of cuticle layers remained comparable to that of the untreated BH (Figure 1). This result indicates that the TSV treatment has the effect of suppressing the cuticle peeling caused by ultrasonic treatment.

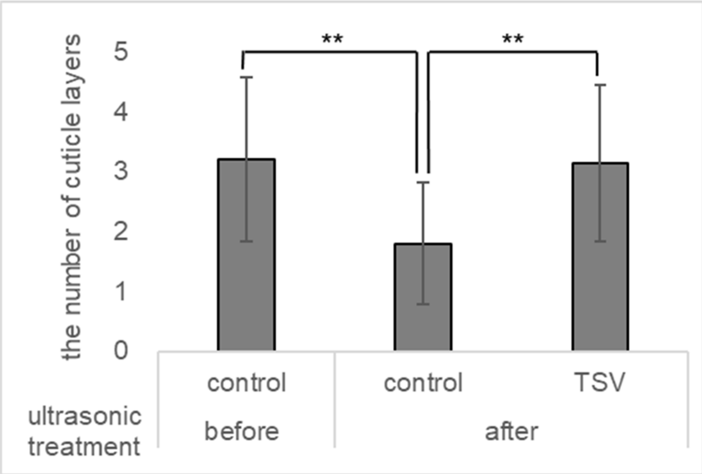


**Figure 1.** Relationship between the number of hair cuticle layers and ultrasonic treatment. The values of the bars represent means ± SE (n=15), and the asterisks indicate significant differences (***p*<0.01, Dunnett's two-tailed t-test) from the control.

*3-2. Split End Test*

TSV treatment successfully suppressed the occurrence of split ends. Additionally, by incorporating TSV into the shampoo formulation, the rate of split end occurrence significantly decreased (Figure 2).

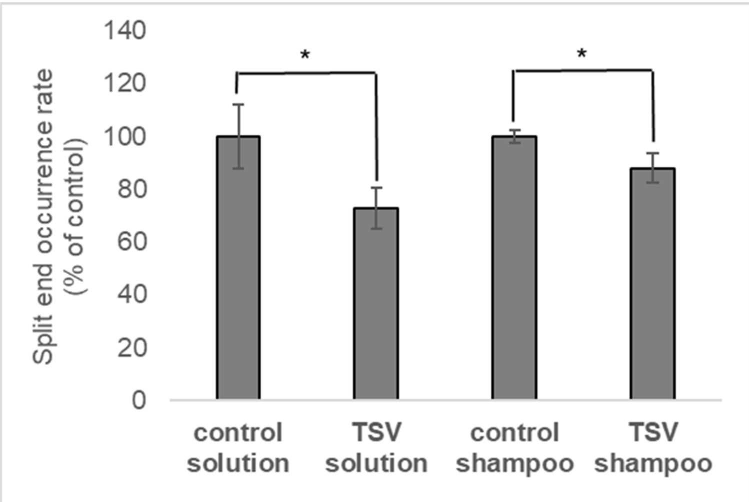


**Figure 2.** Relationship between TSV and the occurrence rate of split ends. The values of the bars represent means ± SE (n=3), and the asterisks indicate significant differences (**p*<0.05, Dunnett's two-tailed t-test) from the control.

*3-3. Surface SEM Images of Extended Hair*

In the BPH group, when treated with the control solution, the cuticles were severely lifted. However, in the stretched BPH after treatment with TSV, the lifting of the cuticles was suppressed (Figure 3). For comparison, UH was also stretched, and the surface was observed using SEM. In the UH group, the cuticles were mostly not lifted (Figure 4). Additionally, for the hair shown in Figure 3, the regions where the cuticles were lifted and those that were not were binarized and segmented, followed by image analysis. The results showed that TSV treatment significantly suppressed the percentage of cuticle lifting (Figure 5).

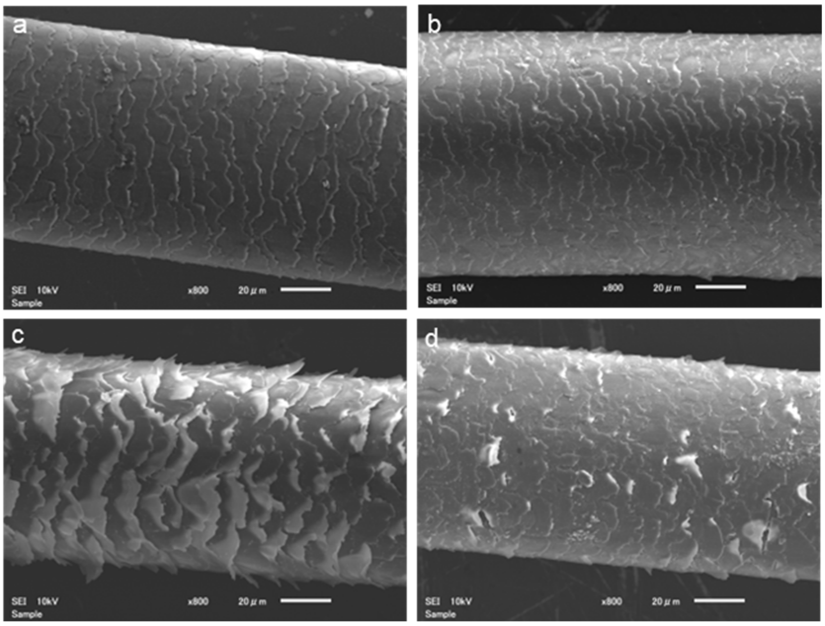


**Figure 3.** Surface SEM images of hair (BPH) before and after extension. **a:** BPH treated with control solution, **b:** BPH treated with TSV solution, **c:** Hair from panel a after extension, **d:** Hair from panel b after extension.

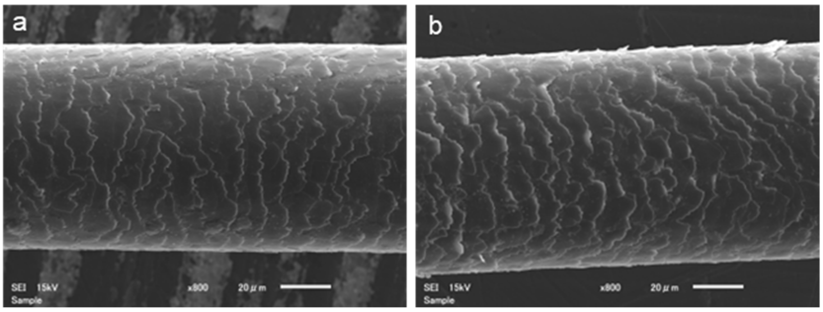


**Figure 4.** Surface SEM images of hair (UH) before and after extension. **a:** UH, **b:** Hair from panel a after extension.

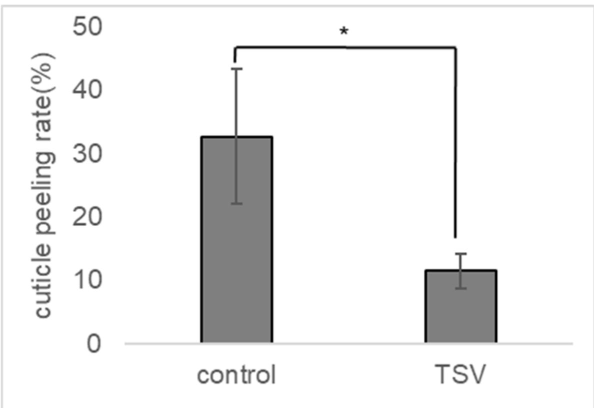


**Figure 5.** Cuticle peeling rate. The values of the bars represent means ± SE (n=3), and the

asterisk indicates a significant difference (*$p<0.05$, Dunnett' two-tailed t-test) from the control.

### *3-4. Dynamic Friction Test*

A dynamic friction test was conducted on the hair before and after TSV treatment. The results showed that the MIU values did not change significantly before and after treatment, and no friction reduction effect was observed (Table 2). Furthermore, increasing the concentration of TSV did not result in any change in the MIU.

**Table 2.** MIU Before and After TSV Treatment.

| | **control** | **TSV 0.1% (W/W)** | **TSV 0.2% (W/W)** |
|---|---|---|---|
| MIU | 0.131 ± 0.067 | 0.134 ± 0.092 | 0.131 ± 0.050 |

## 4. Discussion

It has been reported that hair can be damaged by various factors, including chemical, environmental, and physical stimuli [1, 2]. The progression of damage typically begins with the formation of a crack in the cuticle's CMC (cell membrane complex) and the relatively soft endocuticle, which is the outermost layer structurally, that leads to the peeling of the cuticle [3]. This reduces the overall barrier function of the hair, which in turn promotes damage to the cortex layer, reduces hair stiffness (body and resilience) and causes a loss of elasticity and strength. As previously reported, TSV penetrates the hair without coating it like conventional cuticle repair ingredients and improves its stiffness [7]. This study investigated whether TSV not only improves stiffness without causing a coating or buildup that could affect texture but also has a reparative effect on cuticle peeling.

It is known that ultrasonic treatment is an effective method for replicating the cuticle peeling phenomenon that occurs in real life in a laboratory setting [3, 9]. It is believed that TSV penetrates the CMC and the endocuticle, where damage occurs, and enhances stiffness [7]. Based on this, we hypothesized that TSV would suppress cuticle peeling by penetrating into the hair and we conducted experiments to test that hypothesis. As a result, while the number of cuticle layers was reduced by ultrasonic treatment in the control group, hair treated with TSV retained a similar number of cuticle layers as before treatment, indicating a suppressive effect on cuticle peeling. Furthermore, we focused on split ends caused by cuticle peeling. Hair is susceptible to physical and chemical stimuli from beauty treatments such as washing, perming, and brushing, which make it more prone to split ends [10-12]. In this study, an accelerated test was conducted under conditions similar to those experienced in daily life, such as brushing, to confirm the split end suppression effect of TSV [10, 12]. The results showed that the TSV-containing aqueous solution and shampoo significantly reduced the occurrence of split ends compared to the control. Additionally, since physical stress in daily life can cause hair elongation and can lead to hair breakage [10], we observed the surface of extended hair using SEM. After TSV treatment, the surface of the hair was observed to have significantly less cuticle peeling compared to the control. These results suggest that TSV is effective in suppressing cuticle peeling and can also prevent the occurrence of split ends.

Since it has been demonstrated that TSV has an inhibitory effect on cuticle detachment, the next step was to consider the cuticle repair mechanism of TSV. Existing cuticle repair ingredients often inhibit cuticle damage by coating the hair and/or reducing friction. However, SEM images of stretched hair did not show any coating of TSV on the hair surface. Therefore, an experiment was conducted to determine whether TSV inhibits cuticle damage by reducing friction. The results showed that the dynamic friction coefficient did not change after TSV treatment. Based on these results, we conclude that TSV, unlike the mechanisms of existing cuticle repair ingredients, penetrates into the hair and inhibits cuticle detachment. TSV is a compound where the amino acid valine is tosylated, forming a low molecular weight amphiphilic substance consisting of a hydrophilic part and hydrophobic parts like the tosyl group. These findings indicate that, unlike conventional cuticle repair agents, TSV penetrates into the hair structure and suppresses the cuticle detachment through a distinct mechanism. Based on the above results, TSV has emerged as a potentially novel and unique hair repair ingredient. In the next phase of this study, we intend to elucidate the repair mechanism of TSV upon its adsorption onto the delaminated surface of damaged hair. Particular focus will be placed on the ionic interactions with the CMC β-layer, as well as interactions between the hydrophobic moiety of the tosyl group and the CMC δ-layer on planar hydrophobic surfaces.

## 5. Conclusion

Based on the results of this study, we conclude that TSV possesses a mechanism of action distinct from conventional cuticle repair ingredients. By suppressing the progression of initial cuticle damage, TSV prevents cuticle peeling and the occurrence of split ends, while simultaneously enhancing the elasticity and stiffness of the hair. These results suggest that TSV can help maintain beautiful and healthy hair.

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