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Effects of Keratin Ingredients on Hair Damage Caused by Different Bleaching Times

Nobuyuki Fujiwara*, Nami Hosoi, Len Ito* and Junichiro Kamikado

Central Research Institute, Milbon Co., Ltd., Osaka, Japan

In recent years, hair bleaching has gained popularity. While bleaching can increase the brightness of hair compared to normal coloring and can produce pale and highly saturated hair colors that cannot be achieved by coloring alone, it puts a lot of strain on the hair, raising concerns about the damage caused by bleaching. Furthermore, there are limited reports on the relationship between the internal condition of hair and its shape, as well as the mechanism underlying complex damage to both the interior and exterior of hair caused by bleaching. Therefore, in this study, we measured the effect of different bleaching times on the internal structure of hair and the resulting changes in its cross-sectional shape, focusing on the thickness and internal condition of each bleached hair strand. Additionally, we investigated the effect of keratin ingredients in repairing the damage. The results showed that as the number of bleaching cycles increased, the hair became flatter and the cross-sectional shape of the hair approached an ellipse. The number and average size of voids in the hair increased owing to bleaching. It was also confirmed that as the hair flattened, its strength decreased. We found that 0.1% aqueous solutions of the keratin ingredients WK-F and KR-30 had a repairing effect against such damage. The repair effect of these keratins is related to the depth of damage and molecular weight, and each keratin has an appropriate molecular size to act on the internal structure of hair.

Key words: hair, bleaching, micro X-ray computed tomography, repair, hydrolyzed keratin, hair care, hair treatment, hair bleach damage, APDSC, SPring-8

1. Introduction

With the increase in hair fashion consciousness, many Japanese people have been coloring their hair. There are several types of hair coloring depending on the purpose, such as dyeing gray hair and bleaching. However, in recent years, hair bleaching has become popular, especially among young people, owing to the development of social media. Bleaching can increase the brightness of hair compared to normal coloring and can produce pale and highly saturated hair colors that cannot be achieved by coloring alone. However, while bleaching can express hair colors that have not been seen before, it puts a lot of strain on the hair, and customers are worried about the damage caused by bleaching.

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*Corresponding Authors: Nobuyuki Fujiwara (E-mail: nfujiwara@milbon.com), Len Ito (E-mail: lito@milbon.com)

Central Research Institute, Milbon Co., Ltd., 2-3-35, Zengenji-cho, Miyakojima-ku, Osaka 534-0015, Japan

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Active research has been conducted on hair damage caused by bleaching, and various reports have been published.[1–6] Hagihara et al.[1] reported that bleached hair has a significant loss of melanin during shampooing compared to healthy hair. Inoue[2] also reported that bleaching causes the disappearance of 18-methyleicosanoic acid present on the hair surface and the generation of cysteic acid. Kamikado et al.[3] proposed the following theory regarding the mechanism by which bleached hair becomes wavy: While washing, hair strands that become hydrophilic owing to bleaching stick together and become tangled. The authors reported that physical stress applied to the hair during detangling and drying compresses the amorphous intermediate filament-associated proteins of the hair, resulting in wavy hair.[3] It has also been reported that bleached hair has more cavities and is more porous than untreated hair.[4] Accordingly, bleaching causes changes both inside and outside the hair.[5] Hagihara et al. reported that the cross-sectional area of chemically treated hair decreases,[1] which in turn reduces its bending strength. However, Hagihara et al. study only covered hair coloring and perm treatment, and there have been few reports on the relationship between hair cross-section and damage in bleached hair. Additionally, Hagihara et al. study reported that when dodecylmethylammonium chloride is applied to hair that has been colored and permed, the cross-sectional area of the hair increases and the physical properties of the hair are improved, resulting in a repair effect.[1] Ishimori et al. reported that hair treated with glyoxylic acid showed a 10% reduction in oblateness, and its cross-sectional shape changed from elliptical to circular, thereby reducing the bilateral structure and improving the curvature of the hair.[6] Studies have reported that changing the cross-sectional shape of hair from elliptical to circular can make hair straighter and stronger. However, there are few reports on the relationship between the internal structure of hair and its shape, as well as the mechanism underlying complex damage to both the interior and exterior of hair caused by bleaching. In this study, we aimed to measure the effect of different bleaching times on the internal structure of hair and the resulting changes in its cross-sectional shape, focusing on the thickness and internal condition of each bleached hair strand. Additionally, we investigated the effect of keratin ingredients in repairing the damage and reported the results.

2. Materials and Methods

2.1. Hair samples

Black hair that had not been chemically treated, by bleaching or perming, provided by a Japanese woman in her 20s, was used as the experimental sample in this study. Informed consent was obtained from the donor.

The hair samples were washed with 10% (w/w) sodium polyoxyethylene lauryl ether sulfate. The washed hair was dried using a hair dryer, and then 1 g of hair bundles with a length of 21 cm were prepared. ORDEVE Addicthy High Bleach (Milbon, Osaka, Japan) was used as the bleach powder, and ORDEVE Addicthy Oxidant 6.0 (Milbon, Osaka, Japan) was used as the peroxide developer. For the bleaching treatment, bleach powder and peroxide developer were mixed in a 1:3 ratio, and the mixture was applied with a brush 5 times the weight of the hair and left at room temperature (25°C) for 30 min. The hair was thoroughly rinsed with running water. The prepared samples were black hair (BL0), hair bleached once (BL1), hair bleached 3 times (BL3), and hair bleached 5 times (BL5).

2.2. Method for processing hair raw materials

This study was conducted using hydrolyzed keratin raw materials, Promois WK-F (molecular weight [Mw]: 400) and Promois KR-30 (Mw: 30000) (Seiwa kasei, Osaka, Japan). A 0.1% (w/w) aqueous solution of each hydrolyzed keratin raw material was prepared, and each hair bundle was immersed in 100 mL of the aqueous solution for 1 h. The hair that was dried with a hair dryer after immersion was used as the raw material for hair treatment. In addition, an aqueous solution containing 0.1% (w/w) WK-F and 0.1% (w/w) KR-30 (total concentration of hydrolyzed keratin raw material: 0.2% (w/w)) was also prepared. As a blank, hair samples were prepared by immersing them in 100 mL of purified water for 1 h, followed by drying with a hair dryer (labeled: water).

2.3. Cross-sectional shape observation method

A device that measures 2 dimensions, TM-006R (Keyence, Tokyo, Japan), was used to measure the cross-sectional area of the hair. Measurement samples were made from single hair strands, and the diameter of each treated hair sample was measured (n = 20). A single hair sample was taken from the hair bundle, cut from the center to a length of 7 cm, and Scotch filament tape was attached to both ends to create the measurement sample. In addition, hair oblateness was calculated from the obtained hair diameter data using the following formula:

$$\text{Oblateness} = a/b$$

where a is the short diameter and b is the long diameter.

2.4. Swelling ratio measurement

Hair was fixed to a glass slide with Scotch filament tape, and the hair diameter (D0) in the dry state was measured using a BX51 Microscope (Olympus, Tokyo, Japan). Next, the hair sample was immersed in water along with the glass slide for 1 h, and the hair diameter (Dt) in the wet state was measured (n = 20). The swelling ratios were calculated using the following formula[3]:

$$\text{Swelling ratio} = (Dt - D0)/D0 \times 100$$

2.5. Micro X-ray computed tomography (CT) measurement

The hair was placed in a 0.2-mm diameter Kapton capillary (Furukawa eleectric, Tokyo, Japan) and measured at room temperature (25°C) and atmospheric pressure on a SPring-8 beamline 24XU (Hyogo, Japan). The X-rays used for the measurement were monochromatic light (7.0 KeV). CT reconstruction was performed from the image data of approximately 1200 projections to determine the 3-dimensional structure inside the hair.[7,8] The resolution of the CT scanner was 112 nm/pixel.

2.6. Tensile strength measurement

The tensile breaking tester FDAS770 (Dia-stron, Andover, UK) was used. The test was performed in the air (temperature 25°C, relative humidity 50%) with a test length of 30 mm and a tensile speed of 30 mm/min. The test was performed by stretching the hair to a certain length and then shrinking it until the stress reached 0. The strength–elongation curve was drawn using elongation against the force per unit cross-sectional area (N/m^2) as the stress. The mechanical parameters were then calculated from the strength–elongation curve (n = 20). In this study, the stress at the bending point of the hook and the yield regions was used as the yield stress.

2.7. Ambient pressure differential scanning calorimetry (APDSC)

A differential scanning calorimeter Q2000 (TA Instruments) was used to analyze the APDSC. The sample container (pan) was a Tzero Pan (TA Instruments), and the lid for the sample container was a Tzero Hermetic Lid (TA Instruments, Delaware, US). For the measurement samples, each hair sample was shredded until it was in a powder form. After shredding, the samples were conditioned at a temperature of 25°C and a relative humidity of 50% for 1 day.

The glass transition temperatures (Tgs) were measured using the method described by Miyake et al.[9] by using the following procedure: 5 mg of the shredded hair described above was placed in a pan, and the lid was sealed with a clamping machine. It was then set in a heating cell, and the measurement was taken in a nitrogen gas atmosphere under the following conditions: nitrogen flow rate of 50 mL/min, heating rate of 10°C/min, and temperature range of −10°C to 120°C (n = 3). The Tg and relaxation enthalpy (ΔHr) were obtained from the differential scanning calorimetry curve of the obtained glass transition region. Depending on the analytical method, various points, such as the start point, endpoint, and midpoint, are used for the glass transition temperature; however, in this case, the start point was used.

2.8. Statistical analysis method

Experimental results are shown as mean ± standard deviation. The Tukey–Kramer test was used to test for statistical significance.

3. Results and Discussion

3.1. Observation of hair damage

Hair bleaching was measured to understand the damage caused to the cross-sectional shape of hair. Figure 1 shows the calculation results of hair oblateness obtained from hair diameter measurements. The results showed that as the number of bleaching cycles increased, the hair became flatter, and the cross-sectional shape of the hair approached an ellipse.

Next, the swelling ratio of the hair was measured to examine its morphology under wet conditions. The swelling ratio measurements are shown in Fig. 2. The results showed that hair tended to swell with the number of bleaching cycles and became hydrophilic with bleaching. The oblateness and swelling ratio measurements showed that bleached hair tended to be flat when dry and swollen when wet. In other words, it is believed that bleach-damaged hair has a greater gap between dry and wet hair than healthy hair. The fact that bleached hair swells more when wet suggests that moisture penetrates the hair. It is considered that the space into which water infiltrates is larger in bleached hair than in virgin hair. This is due to factors such as the cleavage of disulfide (SS) bonds, the loss of proteins, and the degradation of melanin granules caused by bleaching. In such hair, water penetrates into the regions where SS bonds between intermediate filaments and intermediate filament-associated proteins have been cleaved, leading to swelling. During the subsequent drying process, the hair contracts, and this cycle is thought to cause greater disruption of the internal protein structure compared to healthy hair, resulting in a more flattened hair morphology. The results of the hair tensile

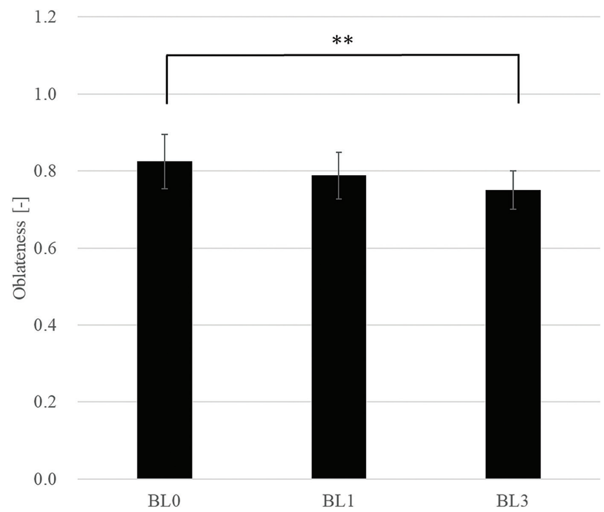

Fig. 1 Oblateness measurement results for each bleached hair (short diameter/long diameter). Data are expressed as means ± SE, $n = 20$, $**p < 0.01$ (Tukey–Kramer multiple comparison test).

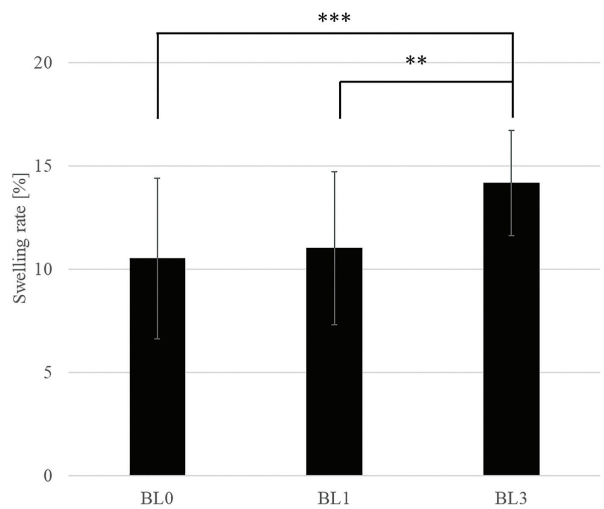

Fig. 2 Swelling rate results for each bleached hair. Data are expressed as means ± SE, $n = 20$, $**p < 0.01$ and $***p < 0.001$ (Tukey–Kramer multiple comparison test).

strength measurements are shown in Fig. 3. The results showed that hair strength decreased with increasing number of bleaching cycles.

To observe the internal structure of hair within the hair, the internal structure was measured using X-ray micro-CT. A CT image of the hair is shown in Fig. 4. The measurement results for the voids in each treated hair sample obtained by CT image analysis are listed in Table 1. The ratio of the data for each damaged hair was calculated with the data for the BL0 hair set to 1 (Table 1). As shown in Fig. 4 and Table 1, the number and average size of the voids increased with the number of bleaching cycles. In other words, the size of the existing voids increased with the number of bleaching cycles, and new voids were generated.

As shown in the above results, hair bleaching causes damage to various characteristics, such as thickness, strength, and internal structure. Moreover, these damages do not occur independently but are thought to be linked to each other, starting with an increase in hair voids. Bleaching treatment not only leads to the degradation of melanin granules but also causes the cleavage of SS bonds and the lifting of the cuticle layer. These changes are thought to occur in a coordinated manner, contributing collectively to the deterioration of hair integrity. In addition, proteins easily leak out of such hair, which results in a decrease in the physical properties of hair and a change in its appearance.[4,10] As a care approach

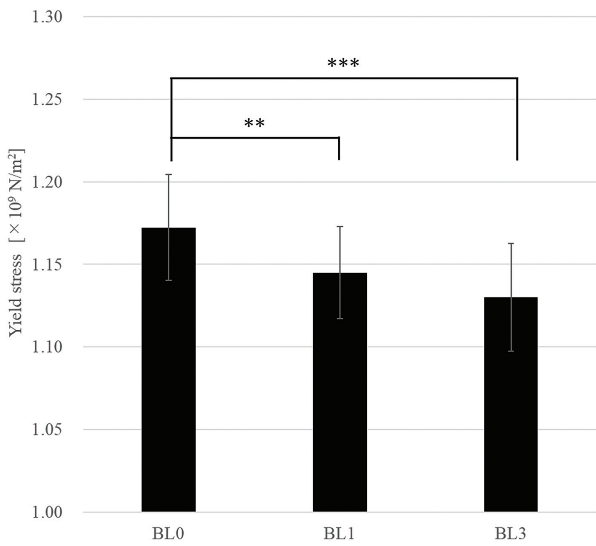

Fig. 3 Hair strength measurement results according to the number of bleaching treatments. Data are expressed as means ± SE, $n = 20$, **$p < 0.01$ and ***$p < 0.001$ (Tukey–Kramer multiple comparison test).

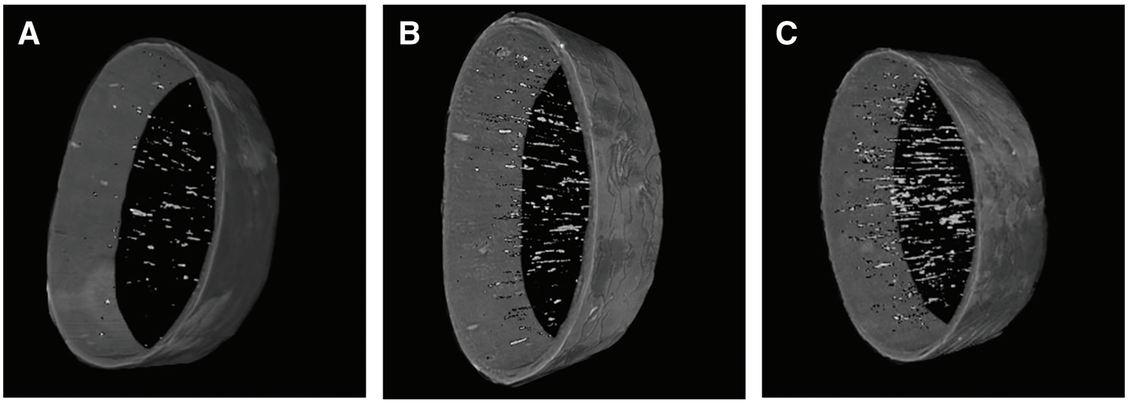

Fig. 4 3-Dimensional images of human hair fibers. (A) BL0, (B) BL1, and (C) BL3.

Table 1 Hair void data by the number of bleaching treatments.

	Number of voids [–]	Void size [–]
BL0	1.000	1.000
BL1	2.299	1.067
BL3	3.141	1.182

BL0, black hair; BL1, hair bleached once, BL3, hair bleached 3 times.

to this type of damage phenomenon, we thought that an effective approach would be to penetrate ingredients into the hair, fill in the sparse areas within the hair, and physically expand it to improve oblateness and fill the gap between dry and wet states, and we investigated the effects of keratin ingredients.

3.2. Effect of keratin ingredients on the recovery of bleached hair function

Table 2 shows the oblateness ratio of hair before and after treatment with a 0.1% aqueous keratin solution on hair that had been bleached 3 times. The results showed that oblateness tended to improve in BL3 hair treated with the keratin raw material aqueous solution.

Next, the tensile strength of hair treated with the ingredients was measured to confirm whether the ingredients improved the tensile strength. The results are shown in Fig. 5. The results showed that the tensile strength of hair was improved by treatment with keratin ingredients. If the keratin ingredients penetrate the hair, they also penetrate the

Table 2 Oblateness ratio measurement results of hair treated with keratin raw materials (before and after treatment).

Sample	Oblateness rate (after processing/ before processing) [−]	Standard error	p Value ($vs.$ water)
Water	0.9999	0.00227	—
WK-F	1.0077	0.00343	0.0576
KR-30	1.0073	0.00238	0.188
WK-F–KR-30	1.0124	0.00524	0.0013**

n = 20, ** $p < 0.01$ (Tukey–Kramer multiple comparison test).

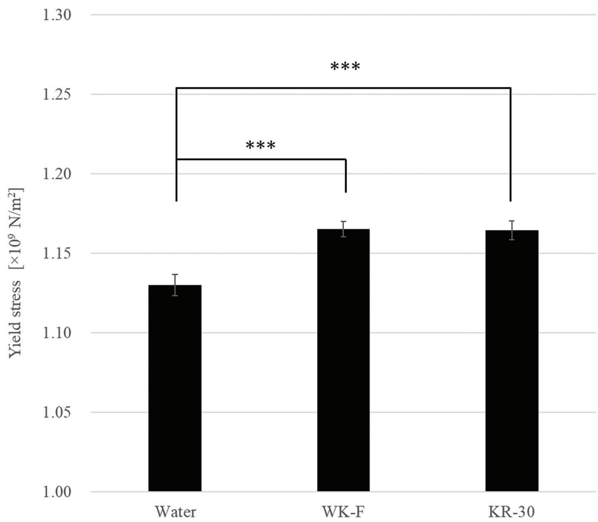

Fig. 5 Tensile strength measurement results of hair treated with keratin raw material. Data are expressed as means ± SE, $n = 20$. *** $p < 0.001$ (Tukey–Kramer multiple comparison test).

voids, and expand it, thereby improving hair functions such as oblateness, then the results of the tensile test should correspond to some extent to the Mw of the keratin ingredients used for treatment. However, the results of this study showed that both WK-F and KR-30 produced the same repair effect. Therefore, the repair effect of keratin ingredients on bleached hair could not be achieved by simply filling the voids inside the hair.

3.3. Mechanism of the effect of keratin ingredients

To confirm how each keratin acts on bleached hair, hair strands that had been bleached 1, 3, and 5 times, as well as those that had not been bleached, were treated with WK-F and KR-30, and to confirm the effect on the inside of the hair, Tg measurements were performed using an APDSC device to measure the effect of each keratin raw material on hair with different degrees of damage. The glass transition behavior measured by the APDSC device is thought to reflect information on the inside of the hair[9,11]; if this value changes owing to the keratin raw material treatment, it can be considered that the keratin raw material has penetrated the hair and changed its internal condition. The measurement results are presented in Fig. 6, which shows the ratio of Tg before and after treatment; the further the ratio is from 1, the more the keratin raw material is considered to penetrate the inside of the hair and affect its structure. An increase in the Tg suggests that the non-keratin protein aggregate structures become more thermally stable, thereby restricting the micro-Brownian motion of protein molecular chains and reducing their tendency to transition into a rubbery state.[12] The specific hair samples in which Tg increased varied depending on the type of keratin material used. This variation is believed to result from differences in the extent of structural disruption caused by bleaching, which leads to the formation of more loosely packed regions within the hair and alters the condition of protein aggregates. These changes, in turn, affect the penetration and binding behavior of the keratin materials. In hair with relatively minor damage, the areas surrounding the non-keratin protein aggregates remain mostly compact, though some less dense regions may exist. In such cases, WK-F, a low Mw keratin, is thought to interact effectively with the non-keratin proteins and influence the structure of the aggregates. On the other hand, KR-30, with its higher Mw, likely could not reach these compact regions and therefore showed limited effectiveness. In more severely damaged hair, the regions around the non-keratin

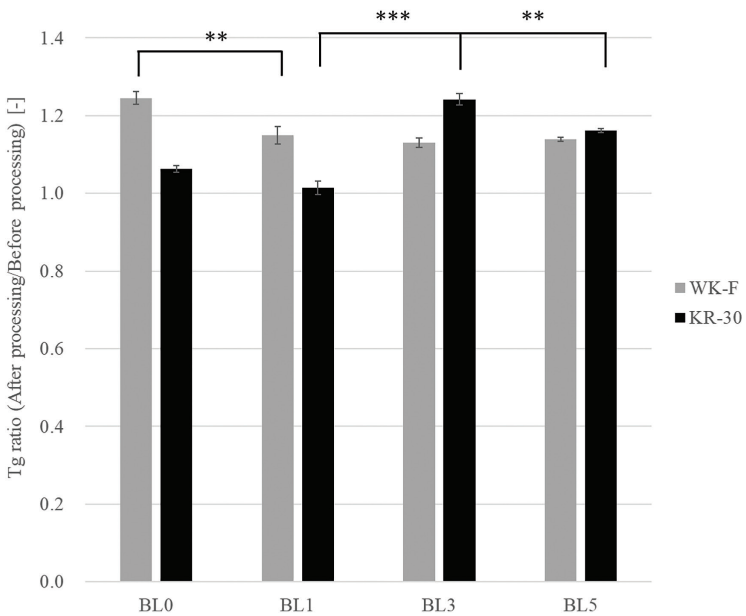

Fig. 6 Glass transition temperature measurement results for hair treated with each keratin material after bleaching several times. Data are expressed as means ± SE, n = 3. **p < 0.01 and ***p < 0.001 (Tukey–Kramer multiple comparison test).

protein aggregates are believed to become less densely packed compared to healthy hair or hair with only mild bleaching damage (e.g., BL0 and BL1). As a result, even the larger KR-30 molecules were able to interact with the non-keratin protein aggregates and alter their structural state. In contrast, although WK-F could still interact with the aggregates, the increased looseness of the surrounding structure may have limited its ability to induce significant changes, leading to a reduced rate of Tg increase as the severity of bleaching damage progressed.

This study has a limitation. We only focused on the differences in Mw. Therefore, it is not possible to consider the influence of factors such as the concentration and functional groups of keratin on the repair effect.

4. Conclusion

In this study, we investigated the effects of keratin ingredients on hair damage caused by bleaching. Owing to bleaching, the number and average size of voids in the hair increase, and the hair flattens and its strength decreases. We found that 0.1% aqueous solutions of the keratin ingredients WK-F and KR-30 had a repairing effect against such damage. Furthermore, the effectiveness of these keratin treatments was found to be dependent on the extent of hair damage and the Mw of the keratin. WK-F, with its lower Mw, was more suitable for modifying the internal structure of hair with relatively mild damage. In contrast, KR-30, with a higher Mw, was more effective in repairing severely damaged hair. The results of this study may contribute to proposing effective hair care methods for people with a complex hair bleaching history. Future studies should investigate the influence of the concentration and functional groups of keratin on the repair effect of hair to better understand the mechanism of damage caused by bleaching.

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Conflict of Interest: None.

Abbreviations: APDSC, ambient pressure differential scanning calorimetry; CT, computed tomography; Mw, molecular weight; SS, disulfide

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