



# OPEN Biomimetics through bioconjugation of 16-methylheptadecanoic acid to damaged hair for hair barrier recovery

Sang-Hun Song<sup>✉</sup>, Hyun Sub Park, Byung Tack Lim & Seong Kil Son

The primary component of the lipid barrier on human hair, which is essential for defense against aging and environmental stresses, is 18-methyleicosanoic acid (18-MEA), which provides hydrophobic properties and protective benefits. Since 18-MEA cannot be regenerated once damaged, developing technology that can permanently bind alternative materials to hair is critical. Once 18-MEA was removed from hair via X-ray photoelectron spectroscopy (XPS), pentaerythritol tetraisostate (PTIS) was hydrolyzed and observed via gas chromatography/mass spectrometry (GC/MS) to confirm that it mimicked 18-MEA, and 16-methylheptadecanoic acid (16-MHA) was obtained at pH 4 or lower. 16-MHA was bioconjugated to damaged hair from which 18-MEA was removed via a carbodiimide reaction using polycarbodiimide. Time-of-flight secondary ion mass spectrometry (TOF-SIMS) confirmed that 16-MHA remained on the surface of the bioconjugated hair even after washing. Observation of the endothermic reaction of moisture in hair via a differential scanning calorimetry (DSC) and evaluation of the moisture content confirmed that the physical properties of hair enriched with 16-MHA were similar to those of virgin hair. This biomimetic approach has been shown to restore both external structural integrity and internal moisture homeostasis.

**Keywords** 18-MEA, Hair surface, Biomimic, Pentaerythritol tetraisostate, 16-methylheptadecanoic acid

The lipid barrier of human hair, which is crucial for resilience against aging and environmental stressors, relies on 18-methyleicosanoic acid (18-MEA) for hydrophobicity and protective function. 18-methyleicosanoic acid (18-MEA) is key to hair health, because it results in the hair hydrophobicity, making the hair smooth and shiny. 18-MEA is a covalently bound lipid approximately 1.1 nm thick that is located in the top layer of each cuticle, including the outermost cuticle of the hair<sup>1,2</sup>. Lipid studies revealed 60% protein and 36% 18-MEA in the top 3 nm of fibers. Computational modeling studies have shown that 18-MEA is an ideal material that has the highest hydrophobicity on the hair surface compared with other eicosanoic acids<sup>3</sup>.

18-MEA can be damaged by chemical processes such as dyeing or perming, resulting in weakened hair from external factors<sup>4</sup>. The amount of 18-MEA in hair strands naturally decreases by more than 10% each year<sup>5</sup>, and the overall amount of 18-MEA has been reported to decrease since the age of 50<sup>6</sup>. Although 18-MEA is not completely removed by dyeing<sup>7</sup>, through observation under an atomic force microscope, the surface of 18-MEA hair partially damaged by dyeing exhibited changes in flatness and an increase in friction<sup>8</sup>. The interaction and arrangement of 18-MEA molecules also affect the adsorption of hair conditioning molecules<sup>9</sup>. Since 18-MEA is related to dehydrogenase, it is also relevant from a pathological perspective, as studies have shown that it is not detected in the hair of patients with maple syrup urine disease<sup>10,11</sup>. Therefore, 18-MEA is an important lipid in the human body, and its recovery is meaningful from a cosmetic and health perspectives.

Many attempts have been made to recreate the cuticle surface, which has changed from hydrophilic to hydrophobic as a result of damage to 18-MEA. Polymers, oils, waxes, hydrolyzed amino acids, and cationic molecules used as conditioners provide hydrophobicity by lubricating the surface of hair<sup>12</sup>. However, imparting hydrophobicity via cosmetics has the disadvantage of adding unnecessary textures, such as the stickiness of raw materials.

LG Household and Health Care, Gangseo-gu, Seoul 07795, Republic of Korea. ✉email: sanghun@uw.edu

18-MEA has the disadvantage of being difficult to obtain naturally. There have been attempts to induce hydrophobicity in healthy hair through the adsorption of substances with similar physical properties or structures to 18-MEA as much as possible. For example, the thioesterase activity of palmitoyl proteins has been found to increase the hydrophobicity of hair<sup>13</sup>. There have also been attempts to synthesize 18-MEA through enzymatic hydrolysis or to synthesize ceramide and adsorb it to hair<sup>14,15</sup>. The use of cationic surfactants allows the conjugate of 18-MEA and stearypropyldimethylamine to adhere to human hair, enhancing hair conditioning and rejuvenating the damaged surface of the hair<sup>7,16</sup>. Moreover, other researchers have attempted to attach various substances mimicking 18-MEA to hair and 19-methyleicosanoic acid (19-MEA)<sup>17</sup>. Using electrostatic and polar forces between proteins, it was possible to increase the hydrophobicity of the lipophilic layer by adsorbing protein lipids to the sulfonate of the 18-MEA layer<sup>18</sup>.

Many of these studies provided damaged hair with hydrophobicity, but the adsorbed substances were easily washed away. To increase durability after washing, we aimed to covalently bind mimic 18-MEA to the surface of the hair to increase its hydrophobicity. This study was conducted with the goal of easily obtaining and efficiently covalently combining mock 18-MEA, which is difficult to synthesize.

## Results

### Physical properties of 18-MEA damaged hair

18-MEA on the hair surface was removed via a 0.1 M potassium *t*-butoxide/*t*-butanol solution. To compare the degree of damage to hair 18-MEA quantitatively, the hair surface was observed via X-ray photoelectron spectroscopy (XPS). Two sulfur S2p peaks were observed, as shown in Fig. 1a. The peaks from virgin hair observed at 163 eV and 167 eV can be assigned to the disulfide bond of S2p(II) and the sulfonate bond of S2p(IV)<sup>19</sup>. When the hair was left in a potassium *t*-butoxide/*t*-butanol solution for 1 min and 5 min, the disulfide peak gradually disappeared over time. The area/electron density of the S2p(II) peak decreased to 1.66 (virgin), 0.48 (1 min), and 0 (5 min). The area/electrons of S2p(II) atoms lower at the hair tips, where more damage occurs, than at the roots<sup>20</sup>. Therefore, a decrease in the S2p(II) peak indicates impaired binding of disulfide components that make up the hair, including 18-MEA.

The cuticle surfaces of the hair were evaluated via atomic force microscopy (AFM), as shown in Fig. 1b. AFM scans were performed on the same hair at different treatment times. The cuticle height was evaluated as normal at 200–400 nm<sup>1</sup>, and it was confirmed that there was no change in the hair cuticle structure before and after 18-MEA damage.

The lateral force, which measures the force exerted along the lateral axis as the cantilever scans, represents friction. Red color indicates that the lateral force microscope (LFM) has greater friction values, with values increasing significantly at the cuticle edge. The LFM values at the filled triangular mark positions were 3.1 V, 4.7 V, and 5.2 V for the virgin sample and after 1 and 5 min, respectively. As the time for treating the potassium *t*-butoxide/*t*-butanol solution increased, the friction of the cuticle surface increased. A decrease in LFM indicates that changes are occurring on the hair surface.

The concentrations of non-covalently bound lipids in hair were determined via gas chromatography/mass spectrometry (GC/MS). The methods for extraction and chromatography are described in Figure S1. Since 68.5% of total fatty acids are myristic acid (C14), palmitic acid (C16), and stearic acid (C18)<sup>1</sup>, these lipids were used as representative lipids in Fig. 1c. There was no statistically significant difference in the hair lipid concentration before and 5 min after the hair was immersed in the solution. The concentration of hair lipids decreased significantly after incubation for 30 min. This finding suggests that hair treatment for 5 min had no effect on other lipids, with the exception of 18-MEA.

The results in Fig. 1 show that 18-MEA on the surface of hair treated with a potassium *t*-butoxide/*t*-butanol solution for 5 min was sufficiently removed. Further experiments were conducted under the assumption that the internal lipids of the hair prepared were the same.

### Obtaining 16-MHA from PTIS via hydrolysis

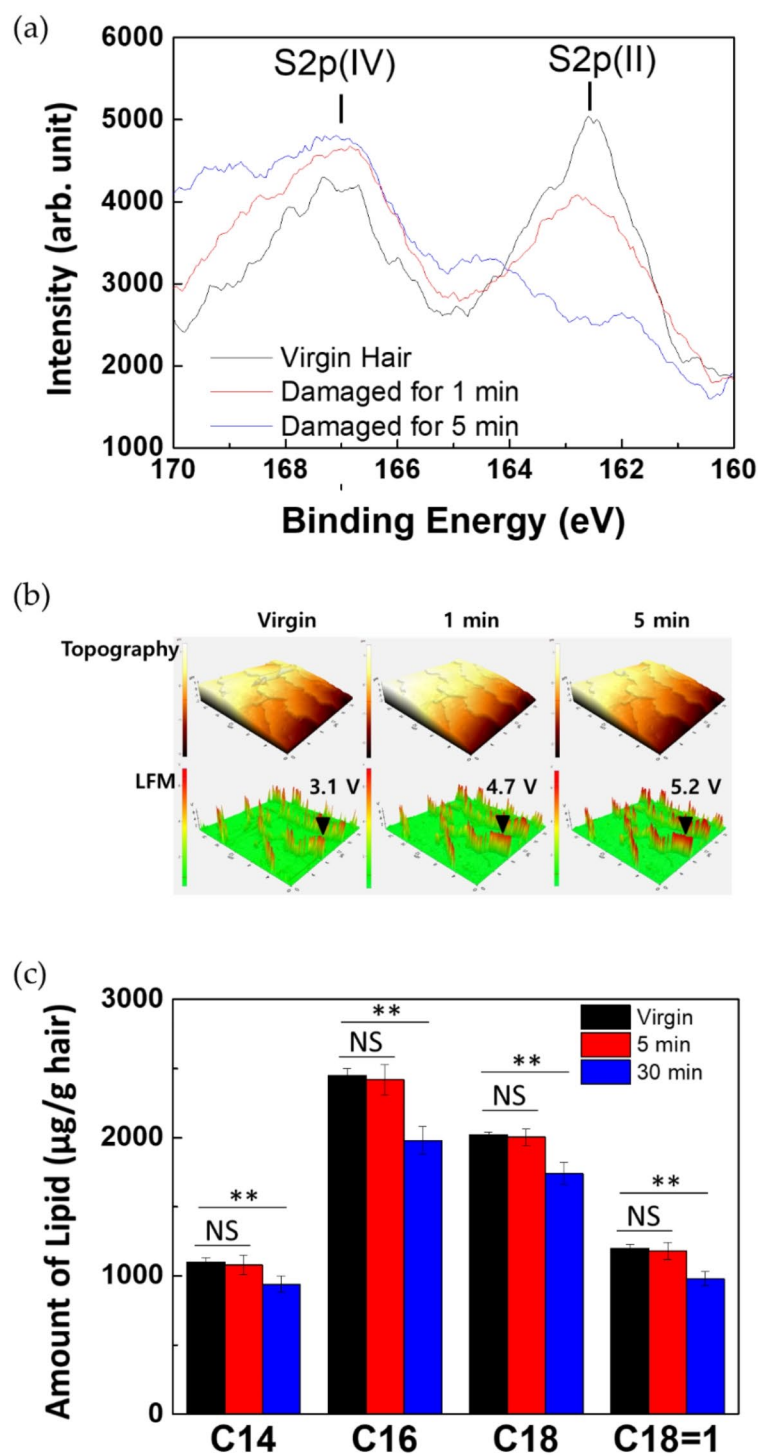
18-MEA and 16-methylheptadecanoic acid (16-MHA) are similar in that they contain carboxylic acids and are hydrophobic. If 16-MHA were bound to the outermost surface of the hair, it would be expected to have a similar arrangement and physical properties similar to 18-MEA. Pentaerythritol tetraisoosterate (PTIS), which is used as a moisturizer, viscosity modifier, and dispersant, contains four 16-MHA bonds. The following experiment was conducted to obtain 16-MHA by hydrolyzing PTIS, which is already widely used in personal care products.

Figure 2 shows the GC/MS results of PTIS dissolved in isopropyl alcohol (IPA) and hydrolyzed at various pH values. In the GC spectrum, a peak for 16-MHA appeared at 30.2 min (Fig. 2a). Figure S2 shows the mass spectrum of 16-MHA at 30.2 min. A 30.2 min-peak was observed at pH 1.5 and pH 4, as shown in Fig. 2a. The addition of PTIS to ethanol resulted in aggregation due to transesterification. When PTIS was dissolved in *t*-butanol, no 16-MHA was produced, as shown in Figure S3. Instead, butyloctadecanoate was generated at 32.5 min. Since butanol is a linear chain, highly reactive butyl octadecanoate appeared to be generated between the linear chains.

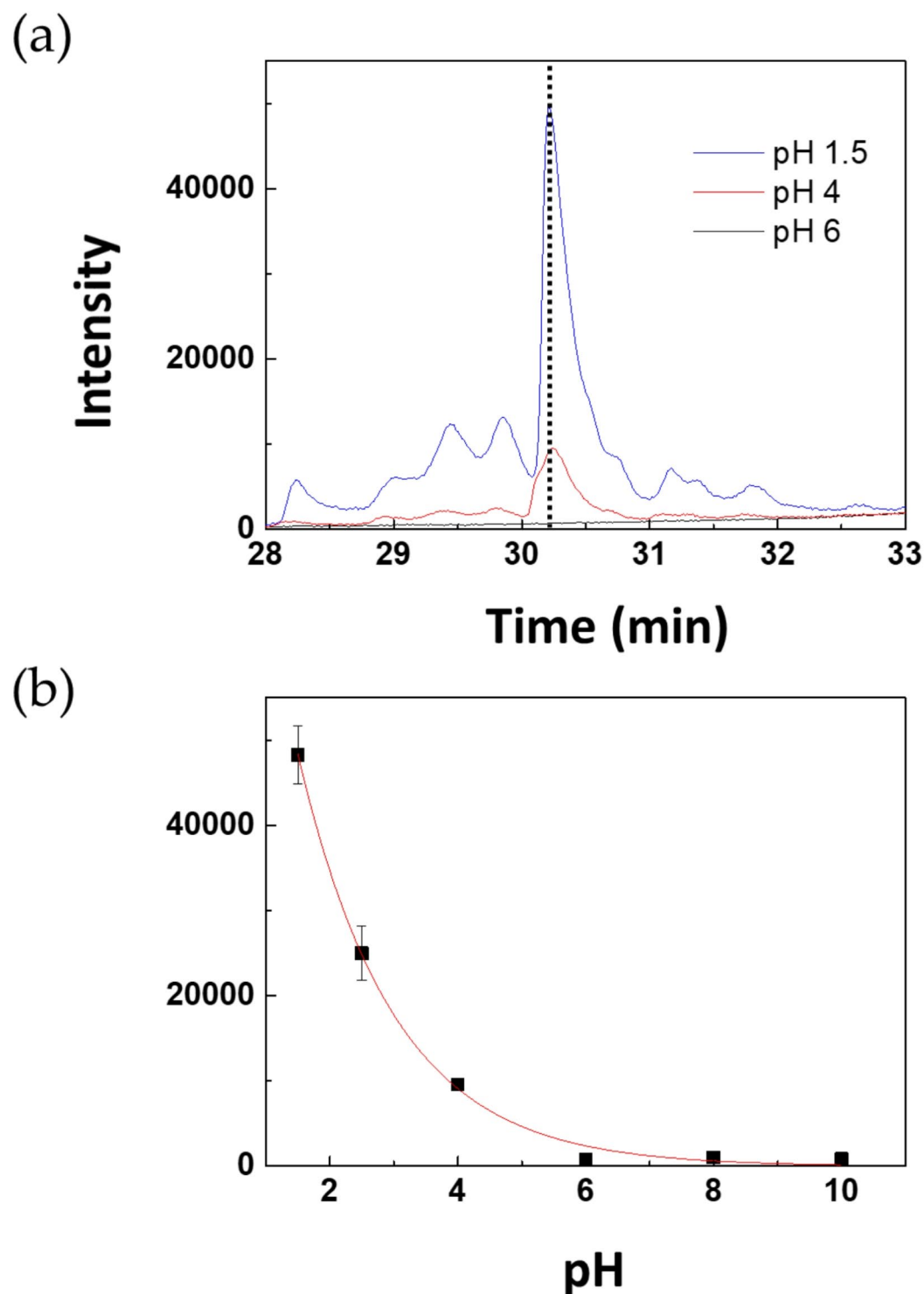
Figure 2b shows the intensity of the 30.2 min peak evaluated at various pH values. The peak was not observed above pH 6, indicating that 16-MHA was hydrolyzed below pH 4. pH 1.5 is highly acidic and not suitable for use in personal care products. Therefore, a pH of 4 in IPA was used as a condition for hydrolyzing PTIS to obtain 16-MHA.

### Bioconjugation of 16-MHA to damaged hair

The reaction of 16-MHA obtained from the results in Fig. 2 was performed by reacting with the primary amines of hair amino acids. The carbodiimide reaction produces urea as a by-product and binds 16-MHA to the hair as shown in Fig. 3. A reaction occurred in which carboxylic acid components and the primary amines in the hair



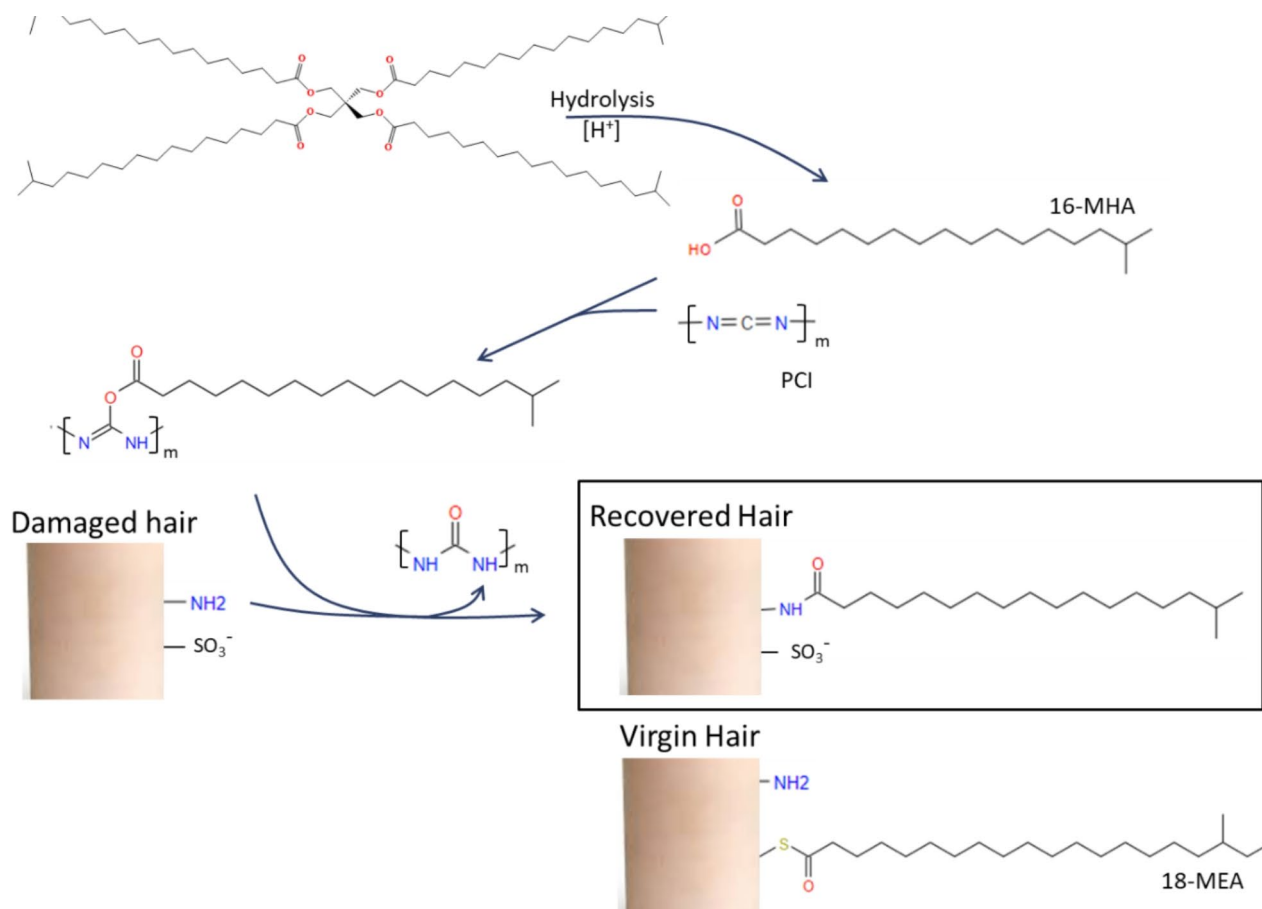
**Fig. 1.** Surface measurements of hair with 18-MEA removed. **a** XPS spectra of hair. Time refers to the length of time the hair is soaked in the *t*-butoxide/*t*-butanol solution to remove 18-MEA. S2p(II) and S2p(IV) represent the disulfide and sulfonate,  $\text{SO}_3^-$ , respectively, of the surface of the hair. **b** Topographical images obtained via AFM (top) and LFM (bottom). **c** Hair lipid composition after treatment. The lines above the bar graph denote a significant difference ( $n=6$ ) calculated via Student's *t* test. NS, Not significant;  $**P < 0.01$ .



**Fig. 2.** GC/MS spectrum confirming that 16-MHA can be obtained by hydrolyzing PTIS. The solvent was IPA, and the samples were tested at various pH values. **a** Chromatogram of the sample at different pH values. **b** pH dependence of the GC/MS intensity at 30.2 min ( $n = 3$ ).

were combined. 16-MHA obtained from PTIS undergoes a reaction with polycarbodiimide (PCI), resulting in the formation of primary amine and amide bonds within the hair structure. Compared with the disulfide bond present in 18-MEA from virgin hair, structurally similar covalent bonds are formed.

After bioconjugation, to confirm whether 16-MHA actually remained in the washed hair, the hair surface was analyzed via time-of-flight secondary ion mass spectrometry (TOF-SIMS). To determine how homogeneously



**Fig. 3.** Chemical formula for the binding of 16-MHA hydrolyzed from PTIS to damaged hair. PTIS, containing four 16-MHA bonds, was separated into 16-MHA under various pH conditions. 16-MHA was bioconjugated to the amine groups of damaged hair through a carbodiimide reaction. This reaction was intended to bioconjugate 16-MHA to the hair surface, which lacks 18-MEA due to disulfide breakage, thereby recovering the physical properties of the hair to those of healthy hair.

16-MHA was conjugated to the hair surface, the hair surface was evaluated via TOF-SIMS rather than XPS. Figure 4a shows TOF-SIMS spectra for virgin hair, hair bioconjugated with 16-MHA and PCI, and hair with only 16-MHA applied without bioconjugation. The 18-MEA peak was expected to appear at  $m/z$  341 (reverse triangle) but was not present in the results of this experiment. This finding is consistent with the disulfide peak reduction results in Fig. 1, indicating that 18-MEA was successfully removed.

The 16-MHA on the hair surface was detected as the negative molecular ion peak at  $m/z$  312.3 (filled circle). A 16-MHA peak was observed in hair bioconjugated with 16-MHA. The 16-MHA peak did not appear in hair that had not bioconjugated to the damaged hair.

Figure 4b shows the TOF-SIMS images of 16-MHA for the hair samples. 16-MHA was detected uniformly in the bioconjugated hair, but in the hair with only 16-MHA applied, all of the 16-MHA was removed and was almost undetectable in the imaging analysis. This finding indicates that non-bioconjugated 16-MHA can be easily washed away.

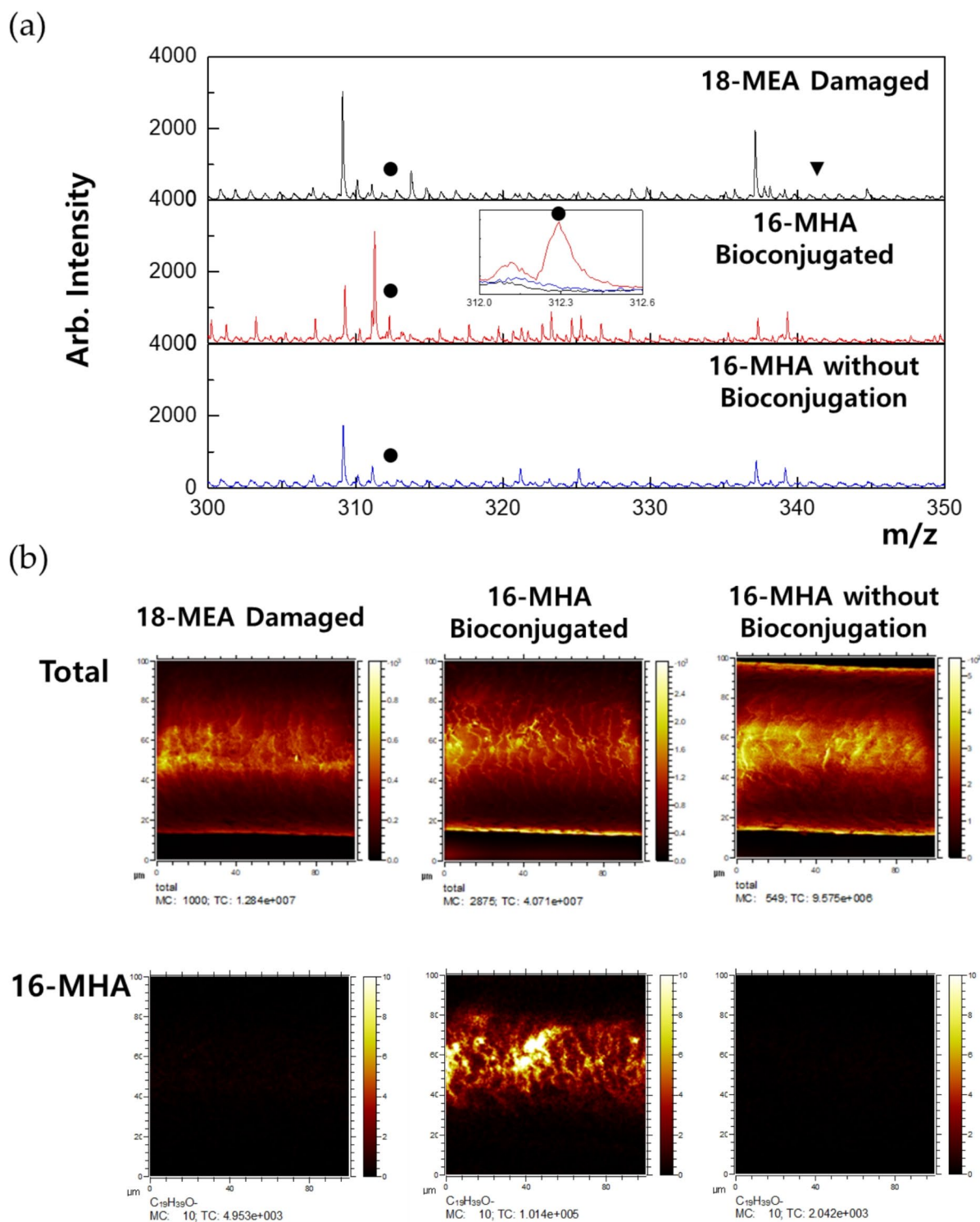
When the hair surface is damaged, phenomena such as increased friction, increased breakage due to combing, and loss of shine occur. The physical properties also change to hydrophilicity, which is observed as a change in the contact angle. A contact angle experiment was conducted to measure the change in hydrophobicity of hair when 16-MHA obtained from hydrolyzed PTIS was applied to the surface of the hair.

The best linear fit (line) with the contact angle changing over time is plotted in Fig. 5a. The contact angle slope for the hair without bioconjugation decreased significantly from  $-0.26$  before washing to  $-1.89$  after washing. On the other hand, for hair bioconjugated with 16-MHA, the slope before and after washing was  $-0.43$ , which was not different.

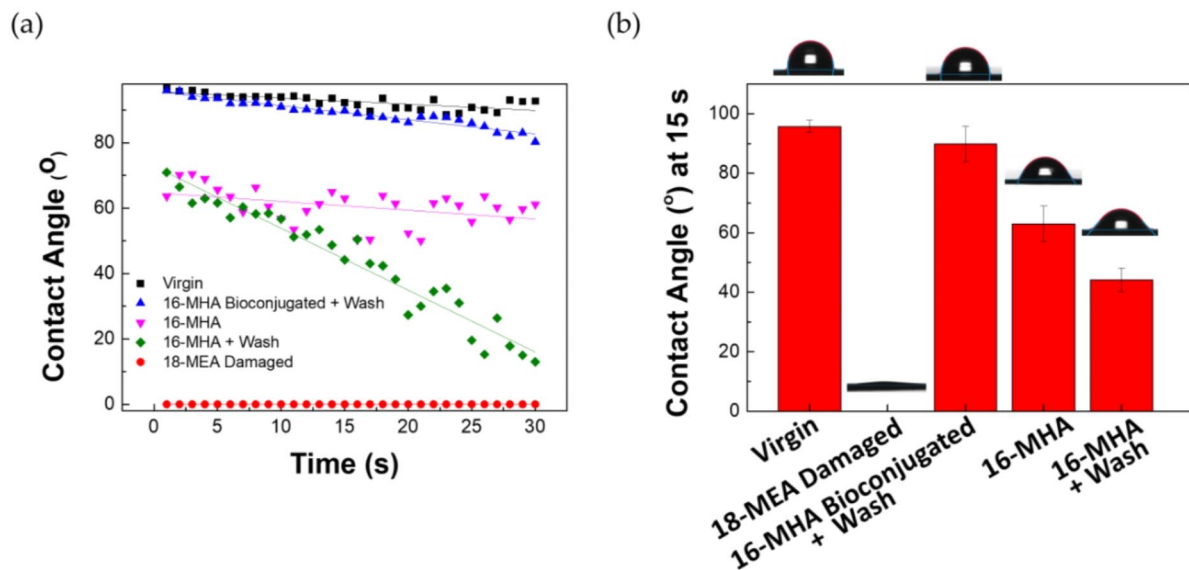
Figure 5b shows the contact angles at 15 s. The contact angle of virgin hair was  $95.75^\circ$  at 15 s. For hair without 18-MEA, the moment a water drop was placed on the hair, the droplet lost its shape, making it impossible to measure. The contact angle of the hair in which 16-MHA was covalently bound to 18-MEA damaged hair was  $90.19^\circ$ , with no difference before and after washing.

When only 16-MHA was applied to 18-MEA damaged hair, the angle of the hair without bioconjugation showed was  $62.98^\circ$  at 15 s. When the hair was washed with surfactant, the contact angle was reduced to  $51.65^\circ$ ,





**Fig. 4.** TOF-SIMS measurements. **a** TOF-SIMS spectra in negative ion mode for 18-MEA damaged hair, 16-MHA bioconjugated hair upon 18-MEA damaged hair, and 16-MHA applied without bioconjugation to 18-MEA damaged hair. All hair samples were washed 5 times with surfactant after treatment. Filled circle: 16-MHA; filled reverse triangle: 18-MEA. The inset is a magnified portion of the  $m/z$  312.3 region reflecting 16-MHA. **b** TOF-SIMS images of total ions (top) and 16-MHA in the hair samples (bottom). The bright color indicates the investigated ions of 16-MHA.



**Fig. 5.** Evaluation of the hydrophobicity of the hair surface by 16-MHA. **a** Contact angle measurements versus time measurements from images of a water droplet on virgin hair (black), hair with 18-MEA removed (red), bioconjugated hair with 16-MHA added to the damaged hair followed by one wash (blue), and hair coated with 16-MHA without a carbodiimide reaction on the damaged hair (violet) and washed once (green). The solid lines indicate the fitted linear regression lines. **b** Contact angle at 15 s. Images are water droplets (0.3  $\mu$ L) suspended on the different hair surfaces at 15 s.

which was significantly lower than the contact angle of bioconjugated hair. Once the samples were washed, the contact angle decreased rapidly over time, probably due to the disappearance of 16-MHA. The water droplets on the surface of the hair that were bioconjugated with 16-MHA maintained their shape, as did the virgin hair. Therefore, the durability of the bioconjugation after washing was confirmed.

After absorbing water equivalent to 1.2 times its own weight, the hair was horizontally shaken at 90 rpm to release the water between hair fibers (Fig. 6). As the water between the hairs is released, the volume of the hair tress increases, as shown in Fig. 6a. When the weight of hair after water release was measured, the water release rate of the 18-MEA damaged hair was lower than that of virgin hair (Fig. 6b). The percentage of hair bioconjugated with 16-MHA was similar to that of virgin hair at 90 min.

### Recovery of the lipid barrier

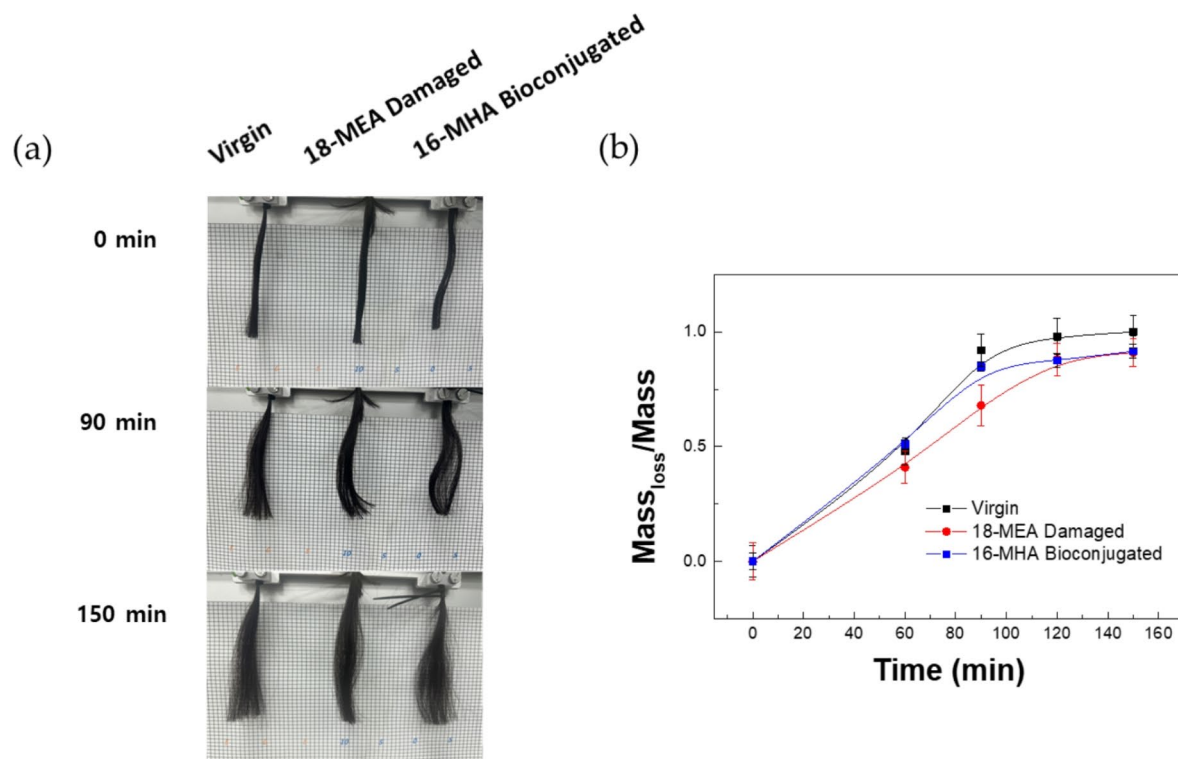
By bioconjugating 16-MHA, recovery of the lipid barrier of the hair was observed based on moisture content. The moisture content is an important indicator of hair health<sup>21</sup>. According to the contact angle results, the bioconjugated 16-MHA remains on the surface of the hair even after washing. After recovery of the hydrophobicity of the hair surface with 16-MHA, the effect of strengthening the hair barrier is shown in Figs. 7 and 8 through moisture behavior evaluation.

The moisture binding capacity and moisture behavior of hair covalently bound to 16-MHA were evaluated via differential scanning calorimetry (DSC), as shown in Fig. 7. The first reaction peak at approximately 110 °C was attributed to the reaction of moisture molecules inside the hair<sup>22</sup>. The peak that occurred approximately 230 °C was a result of bonds during the decomposition of  $\alpha$ -helix proteins<sup>23</sup>.

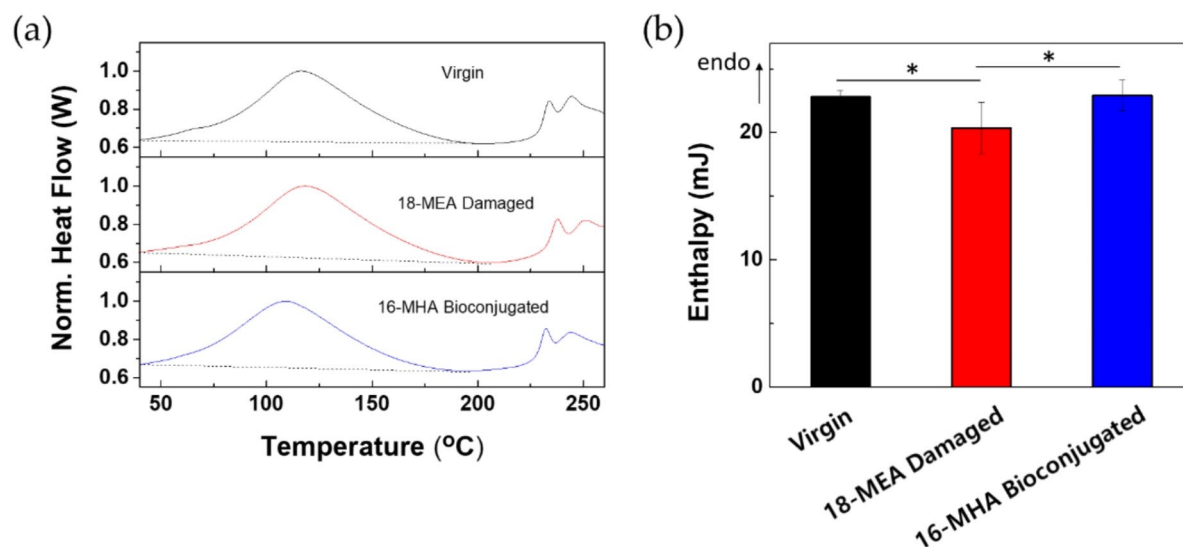
Virgin hair produced a moisture peak at approximately 118 °C. However, the maximum moisture peak value of hair damaged by 18-MEA occurred at 108 °C. The lower reaction temperature of the maximum moisture peak is consistent with the lower moisture response values of bleached hair<sup>24</sup>. The maximum moisture peak for hair bioconjugated with 16-MHA to 18-MEA-damaged hair reacted at 115 °C. Treating hair with hyaluronate increased the hair moisture peak reaction temperature of DSCs<sup>25</sup>. The temperature at the maximum peak and the enthalpy for the moisture endothermic reaction increased to the level of virgin hair, indicating that the moisture content inside the hair was also at the level of virgin hair.

Changes in the moisture content inside the hair were observed as shown in Fig. 8a. A change in moisture content occurs when hair is heated to a certain temperature. Since hair protein reacts at approximately 230 °C<sup>23</sup>, the change in mass temperatures below 165 °C used in this experiment can be attributed to moisture.

During the heating process, the moisture inside the hair evaporates over time, but after approximately 20 min of heating, the moisture content reaches its maximum without changing. Figure 8b shows the final moisture contents obtained after 20 min of heating and after equilibrium was reached. The moisture content of hair from which 18-MEA was removed was lower than that of virgin hair. At a heating temperature of 75 °C, the moisture content of virgin hair was 10.13%, but the moisture content of hair damaged by 18-MEA was low at 8.19%. The hair bioconjugated with 16-MHA had a moisture content of 9.98% at a heating temperature of 75 °C, which was similar to that of virgin hair. Hair damaged and bioconjugated with 16-MHA to 18-MEA presented

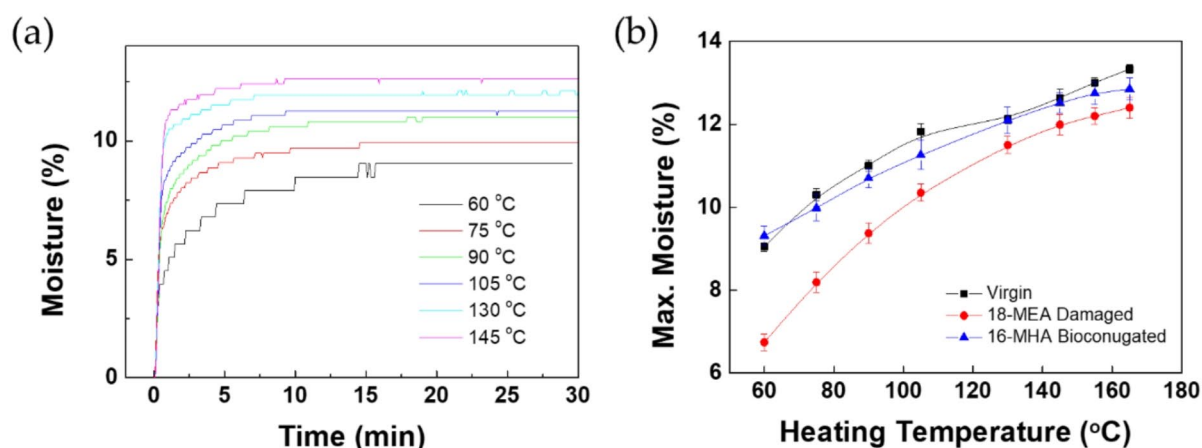


**Fig. 6.** The change in weight of the water after the hair was immersed in water was 1.2 times greater than the weight of the hair, and the hair was shaken at 90 rpm. **a** Changes in hair volume were observed. The more hydrophilic the hair is, the less volume it has because the hair strands stick together. **b** Mass ratio of water removed.



**Fig. 7.** DSC measurement curves showing the moisture binding energy inside hair. **a** DSC spectra measured to determine hair moisture behavior patterns ( $n=6$ ). The dashed line in the DSC spectrum is the area reference line for the enthalpy calculations. **b** Enthalpy due to the endothermic reaction of moisture when hair is heated. The lines above the bar graph denote a significant difference ( $n=6$ ) calculated via Student's  $t$  test.  $*P < 0.05$ .





**Fig. 8.** Observation results of the hair barrier strengthening effect of 16-MHA binding to 18-MEA damaged hair. **a** Moisture extraction content compared with total hair weight obtained via mass change after heating hair at various temperatures and changes in moisture behavior inside hair. **b** Final moisture content according to the heating temperature of virgin, 18-MEA damaged, and 16-MHA bioconjugated hair to 18-MEA damaged hair ( $n=5$ ).

a moisture content similar to that of virgin hair over the entire heating temperature range. The number of hairs whose surface was rejuvenated with 16-MHA appeared to have increased, and moisture binding inside the hair was maintained. The amount of released water did not follow an exponential function but decreased linearly. However, after 120 min, the weight of damaged hair was similar to that of undamaged hair.

## Discussion

The content of 18-MEA varies even in healthy hair depending on the environment and personal history of the individual. 18-MEA is not completely damaged even when oxidized<sup>7</sup>. Since it is difficult to make and test hair with the same amount of residual 18-MEA, we decided to completely remove 18-MEA by damaging virgin hair with chemicals.

One method involves the use of liquid chromatography/mass spectrometry (LC/MS) to measure the amount of 18-MEA, but this method is time-consuming and requires the complete elution of hair lipids from hair<sup>26</sup>. Other researchers have used TOF-SIMS to detect 18-MEA in hair<sup>27–30</sup>. However, because we focused on quantitative comparisons, we chose XPS over TOF-SIMS for superior quantitative analysis<sup>31</sup>.

In the XPS experiment, as the treatment time for hair damage increased, the area representing disulfide decreased significantly, whereas the  $\text{SO}_3^-$  area slightly increased. As the disulfides on the hair surface break, the sulfur undergoes several reactions, some of which are converted to sulfonates. Therefore, as the degree of damage increased, the disulfide peak decreased, whereas the area of sulfonate increased slightly. Comparing hair before and after bleaching via atomic force microscopy (AFM) revealed that nonionic spaces existed at intervals of 10 to 30 nm in the area where 18-MEA was removed<sup>32</sup>. Thus, not all 18-MEA is converted to sulfonate when it is removed from the surface of the hair.

It has yet to be determined exactly where the sulfur in 18-MEA is bound to hair. Since the sulfur of 18-MEA binds to hair protein, it is assumed that it is most likely a thiol bond that can bind to the sulfur of 18-MEA. Therefore, the disulfide bond detected at 163 eV represents the bond between the sulfur of 18-MEA and cystine on the hair surface. In other words, the disappearance of the disulfide peak in Fig. 1 indicates that the disulfide bond in the hair protein has been removed, suggesting that almost all of the 18-MEA has been removed. Considering the 1–2 nm thickness of the 18-MEA layer<sup>2</sup>, the reduced disulfide signal in XPS is closely related to 18-MEA damage on the surface of the hair.

We attempted to achieve a permanent bond by bioconjugating 16-MHA to hair through a carbodiimide reaction. The carbodiimide reaction is nontoxic and one of the few that can be used under mild conditions in household products that react with water<sup>33</sup>. On the basis of this carbodiimide chemistry, it is assumed that carboxylic acids form cross linkages with the amine compounds of the hair, as depicted in Fig. 3. They can be toxic because of their high reactivity, but their irritant toxicity has been reduced by the use of PCI. As shown in Figure S4, as a result of conducting a skin irritation test by causing a PCI reaction on actual skin, it was confirmed that it did not cause skin irritation. Hair is composed of 18 amino acids, and all amino acids, with the exception of proline contain primary amines, so there is a high possibility that carbodiimide reactions will occur.

Through evaluation of the internal binding force of hair with polypeptides, it was confirmed that a minimum PCI concentration of 0.4% was needed for bioconjugation (Figure S5). As shown in Fig. 1c, when 18-MEA was removed from hair via a potassium *t*-butoxide/*t*-butanol solution, the lipid content, which is the main component of hair, did not change. This finding indicates that 18-MEA damage did not affect the inside of the hair. Young's modulus and tensile strength measurements revealed that there was no change before or after the reaction (Figure S6). Therefore, the 0.4% PCI concentration used in this experiment appears to be suitable for

bioconjugation and affects only the hair surface. In the TOF-SIMS spectrum, the peak at  $m/z$  312.3, representing 16-MHA, appeared only in the bioconjugated hair. When the hair treated with only 16-MHA was washed, no 16-MHA peak was observed, indicating that the bioconjugation attempted in this study was successful.

The contact angle of hair bioconjugated with 16-MHA was similar to that of virgin hair, unlike the contact angle of 18-MEA-damaged hair. The contact angle was measured, which revealed that the bioconjugation had high durability against washing. The covalent bonding of the bioconjugated hair was stable, but the measured contact angle slightly decreased over time. A decrease in the contact angle over time was also observed in virgin hair, whereas a slightly greater decrease was observed in bioconjugated hair. This appears to be due not to the loss of hydrophobicity from broken bioconjugation, but to the uneven distribution of charge on the hair surface<sup>32</sup>. Since additional washing is likely to cause changes in hair properties such as hydrophobicity, the number of washings for durability testing to confirm bioconjugation was limited to five. Even after washing, the hydrophobicity of the hair did not change significantly over time, indicating that covalent bonds were maintained.

The DSC results revealed that the enthalpy of the moisture reaction peak inside the hair where 16-MHA was bioconjugated was greater, indicating that the moisture inside the hair was more strongly bound. As the 18-MEA on the hair surface was removed, the moisture binding pattern inside the hair changed because SLES (sodium lauryl ether sulfate) penetrated the hair during washing. Moreover, washing with surfactants caused a loss of lipoproteins inside the hair<sup>34,35</sup>. Additionally, pores appeared inside hair washed with surfactants<sup>36</sup>. The enthalpy of the hair moisture reaction peak increased when 16-MHA was bioconjugated because the hydrophobicity of the hair surface was restored, preventing the internal penetration of surfactants during washing. Water molecules penetrating into hair disrupt the internal structure of hair<sup>34</sup>.

Once the hydrophobicity of the hair surface was restored, the penetration of moisture or surfactants from the external environment during the washing process decreased. The change in moisture binding by making the hair surface hydrophobic with 16-MHA prevented the structural perturbation of the hair when the hair was washed with surfactant.

As shown in Fig. 6, the hydrophobicity of the 16-MHA bioconjugated hair was confirmed once again. The water content of the 16-MHA-bioconjugated hair initially decreased to that of virgin hair but later remained at a level similar to that of damaged hair. The failure of the exponential process to remove water between the hairs also causes the 16-MHA to not align properly. 16-MHA has a methyl group at position 18, so it is well aligned on the hair surface<sup>37</sup>. Structurally, 18-MEA is the optimal lipid for the hair surface. Future studies are needed to properly align 16-MHA to the hair surface.

In this study, PTIS was hydrolyzed to produce 16-MHA, the structure most similar to 18-MEA, which was covalently bound to the hair surface via PCI. 18-MEA was difficult to apply because of cost issues, so it was replaced with PTIS and PCI, which are commercialized raw materials. Both PTIS and PCI are commercially available raw ingredients that are easy to apply to actual shampoo. An evaluation of the hydrophobicity and moisture homeostasis of hair bound with 16-MHA confirmed that it had physical properties similar to the original hair. This study provides an example of the application of biomimetic technology to the outermost layer of hair to recover damaged hair.

## Methods

### Materials

For PCI, a Picassian XL-742 from Stahl (Netherlands) was used. Pentaerythritol tetraisosterate (PTIS) was obtained from Croda (US). *t*-butoxide, *t*-butanol, hydrochloric acid solution, ammonium hydroxide, and isopropyl alcohol (IPA) were purchased from Daejung Chemicals (Siheung, Korea). Sodium laureth sulfate (SLES) was obtained from LG Household and Healthcare (Seoul, Korea). Myristic acid, palmitic acid, stearic acid, and *o*-terphenyl were purchased from Sigma-Aldrich (US). Oleic acid was obtained from Nippon Oil&Fats (Japan).

### Remove of 18-MEA

Hair swatches were purchased from Happy Call (Seoul, Korea). The swatches were washed with SLES for 1 min and rinsed with water for 2 min. After washing, 10 g of hair was weighed and divided into two tresses of 5 g each. To induce 18-MEA damage, one of the samples was soaked in 50 mL of 0.1 M potassium *t*-butoxide/*t*-butanol solution at room temperature. After sufficient exposure to ethanol, the sample was washed with SLES for 2 min at a flow rate of 35 mL/s and then dried with a hair dryer.

### Surface analysis with XPS and AFM

X-ray photoelectron spectroscopy (XPS, Thermo VG Scientific, UK) was used to examine the 18-MEA at the KAIST Analysis Center for Research Advancement (KARA).

The topography of the hair and the friction at the cuticle were evaluated via atomic force microscopy (AFM, XE-100, Park Systems, Suwon, Republic of Korea) via an LFM cantilever (Nanosensors, Neuchatel, Switzerland) with a typical spring constant of  $0.2 \text{ N}\cdot\text{m}^{-1}$  and a resonant frequency of 23 kHz. A lateral force microscope (LFM) was utilized when the AFM was scanned in contact mode. No flattening was performed. XEI software (ver. 5.2.4) was used for the analysis.

### Lipid extraction and analysis

A hair swatch was cut to a length of less than 1 mm with a mass of 250 mg, and the hair residue was transferred to a 50 mL vial filled with extraction solvents of chloroform, methanol, and water, for 30 h. For gas chromatography/mass spectrometry (GC/MS), samples were analyzed on an Agilent (7890 A GC System, US) gas chromatograph coupled to an Agilent detector (5975 C MSD System, US). Separation was performed on an HP-5ms UI column ( $30 \text{ m} \times 0.25 \text{ mm} \times 0.25 \mu\text{m}$ ) using helium as the carrier gas. It was programmed to maintain an initial column

temperature of 80 °C for 1 min, to change to 320 °C at a rate of 6 °C min<sup>-1</sup> and then to hold for 20 min at this temperature. See the SI Materials and Methods for further details.

### PTIS hydrolysis and analysis

To obtain 16-MHA 1% PTIS was added to IPA and suspended at 80 °C for 1 h. *tert*-butanol reaches its melting point at room temperature, making it difficult to handle, so butanol, which has a low melting point, was used.

16-MHA was confirmed through GC/MS at Yonsei University. The samples were analyzed on an Agilent (8890 A GC System, US) gas chromatograph coupled to an Agilent detector (5977B MSD System, US). The injector temperature was maintained at 230 °C during the analysis, while the ion source temperature and interface temperature were 80 and 270 °C, respectively. Separation was performed on an HP-5MS UI column (30 m × 250 µm × 0.25 µm) using helium as the carrier gas. The mixture was programmed to maintain an initial column temperature of 80 °C for 1 min and then to increase to 270 °C at a rate of 6 °C min<sup>-1</sup>, and this temperature was maintained for 20 min.

### Bioconjugation

For the carbodiimide reaction, hair was coated with the first agent, the 16-MHA solution, for 10 min at room temperature and then transferred to the second liquid agent, an aqueous solution of 0.4% PCI (w/w), at 40 °C for 1 h. Carbodiimide-responsive hair was washed five times with SLES. The unwashed hair was gently patted with a paper towel and left to dry naturally.

### Time-of-flight secondary Ion Mass Spectrometry (TOF-SIMS)

TOF-SIMS measurements were obtained at the Korea Advanced Institute of Science and Technology via a TOFSIMS.5 (IONTOF GmbH, Münster, Germany) instrument operated under high vacuum (10<sup>-9</sup> mbar). The hair samples were analyzed via Cs<sup>+</sup> sputtering and Bi<sup>3+</sup> analysis conditions at 500 eV and 30 keV, respectively.

### Hydrophobicity measurements

Contact angle measurements were recorded and analyzed at room temperature via a Sanyo camera with FTA 32 (Ver 2.0). Five strands of hair were tightly fixed in a custom-made jig and set on a DSA-100 (Krüss, Germany) surface analyzer. An empty space below the hair fibers in the center of the jig prevented the hair from touching any surfaces. Next, 0.3 µL of water was placed on the hair via a syringe, and measurements were taken immediately. When water droplets are dropped on hydrophilic hair, they spread and flow into the hair, making it difficult to measure the contact angle on the hair. Even if the hair is hydrophobic, the surface of the hair is round, so the area where the water droplet comes into contact with the hair is relatively large compared with the diameter of the hair. As time passes, the water droplets become unstable and the contact angle decreases. Therefore, the contact angles were compared based at 15 s, which is the middle time of the experiment.

After absorbing 1.2 times the weight of the hair in the water, the hair tress was suspended in a custom-built device that vibrated along a horizontal axis. The hair samples were stored at a constant temperature and humidity at 25 °C and RH=45% for more than 24 h. After the device equipped with the sample was used, the weight of the hair was measured at regular intervals, and the condition of the hair was observed.

### DSC

To understand the internal structure of hair resulting from the endothermic reaction according to the heat supply, differential scanning calorimetry (DSC, DSC204 F1 Phoenix, Netzsch, Germany) was used. One gram of chopped hair was measured in drying mode at 10 °C per minute. A thermal open fan cover (SML-PE19378) was purchased from Shinko M&T (Korea). To prevent oxidation reactions due to temperature, the experiment was conducted by flowing nitrogen gas through the area in which the sample container was located. All hair used in the experiment was washed with SLES 5 times.

### Moisture analyses

The moisture content was analyzed via a halogen moisture analyzer (HX204, Mettler-Toledo, Greifensee, Switzerland). The hair samples were left overnight in a humid room at constant temperature, 25 °C, and 50% relative humidity. Individual tresses were cut into small pieces, and 0.5 g of hair was placed on the balance of the device. Heating was performed for 60 min, and fresh samples were used for each measurement.

### Human patch test

A human skin irritation test was conducted in accordance with Article 16 of the standard guidelines from the LG Household & Health Care Bioethics Board (IRB) and was conducted to a level where there was no reason for disqualification. Twenty adult male and female test subjects were assessed with an IQ Ultimate™, in which a 26 µL sample was applied to the arm of the tester. After the patch was applied for 36 h, it was removed, and the degree of irritation was estimated 36 h later according to the ICDRG (International Contact Dermatitis Research Group) and CTFA (The Cosmetic, Toiletry and Fragrance Association) guidelines. The shampoo formulation is detailed elsewhere<sup>34</sup>.

### Data availability

Data Availability: The data that support the finding of this study are available on request from the corresponding author.

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### Author contributions

S.-H.S. wrote the manuscript, and conducted the experiments. P.H.S. participated in GC/MS experiment and carbodiimide reaction. B.T.L. treated hair samples, and measured moisture analysis. K.S.S. participated in the data analysis.

### Declarations

### Competing interests

All authors are employed by LG Household & Health Care, Ltd.

### Additional information

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**Correspondence** and requests for materials should be addressed to S.-H.S.

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