J. Soc. Cosmet. Scientists Korea
Vol. 38, No. 2, June 2012, 139-145

생체모사 지질을 이용한 모발 표면에 공유 결합된 지방산 단분자층의 회복

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(2012년 3월 16일 접수, 2012년 5월 20일 수정, 2012년 6월 17일 채택)

Recovery of Covalently Linked Fatty Acid Monolayer on the Hair Surface Using Biomimetic Lipid

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(Received March 16, 2012; Revised May 20, 2012; Accepted June 17, 2012)

요 약: 모발표면에는 독특한 형태의 지방산이 있다. 18-methyleicosanoic acid (18-MEA)는 모발 큐티클의 최외각 층에 공유결합된 특이한 ante-iso 형태의 지방산이다. 18-MEA 층은 CMC의 β-층 위에 위치하면서 모발의 외부 표면에 표면에너지와 마찰저항을 낮춰준다. 18-MEA 분자의 높은 유동성은 계면 전단력을 감소시켜 외부로부터 전달되는 지질들이 쉽게 퍼지도록 한다. 본 연구에서는 C10 - 40 isoalkyl acid의 말단에 관능기로 N-hydroxyl succinimidyl ester를 도입하여 모발 표면의 공유결합 지방산 층을 회복시켜보고자 하였다. 모발 표면의 재 소수화를 확인하기 위하여 접촉각을 측정하였다. 서로 다른 습도 조건(40, 55, 70 %RH)에서 모발의 내부 수분 함유량은 전자 수분 분석기를 이용하여 실시하였다. Hydroxysuccinimidyl C10 - 40 Isoalkyl Acidate (HCIA)로 처리한 모발의 표면은 큐티클을 접착하는 것과 같이 지방산 단분자층이 공유결합을 형성하여 큐티클의 균열을 채워줌으로써 매끄럽고 균일할 것으로 생각되었다. 이러한 접착 효과는 atomic force microscope (AFM) 영상의 라인 프로필(line profile)을 통해서 확인되었다. 따라서 모발 내부 구성 물질과 수분이 쉽게 용출되지 않고, 외기의 습도가 변하는 경우에도 최적의 모발 습도가 항상 유지된다. AFM을 사용한 lateral force microscopy (LFM) 결과에 따르면 HCIA를 처리한 모발 표면의 마찰력이 감소되었고, 이에 대해 15회 샴푸 과정을 수행한 이후에도 동일한 마찰력 값을 나타내었다.

Abstract: There is a unique type of fatty acid in the hair surface. 18-methyleicosanoic acid (18-MEA) is an unusual anteiso fatty acid covalently linked to the outermost surface of hair cuticle. A layer of 18-MEA is located in the upper β-layer of the CMC that is responsible for the low surface energy and low friction resistance of the hair's outer surface. The high mobility of 18-MEA molecule facilitates spreading of extraneous lipid by decreasing interfacial shear strength. In this study, we introduced N-hydroxyl succinimidyl ester functional group to the one end of C10 - 40 isoalkyl acid for regenerating hair surface with covalently bound fatty acid layer. The re-hydrophobicization of hair surface has been investigated by contact angle measurement. The inner moisture content of hair at different levels of humidity (40, 55, 70 %RH) was measured by electric moisture analyzer. Treatment with Hydroxysuccinimidyl C10 - 40 Isoalkyl Acidate (HCIA) was supposed to make hair surface smoother by filling the cracks between cuticles with covalently bound fatty acid monomolecular layer like cuticle glue. This glue effect was also confirmed with line profile of AFM images. Therefore, the moisture and structural components of inner hair were not easily flown out and the optimum moisture content could be kept constantly though the outside humidity level was changed. The lateral force microscopy (LFM) by using atomic force microscope showed that the friction force of hair surface treated with HCIA was decreased. It also showed the constantly sustained friction value even after shampooing repeated 15 times.

Keywords: *hair surface, lipids, biomimetic, 18-MEA, covalent link*

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1. Introduction

When hair got damaged, the chemical and physical transition of hair structure occurred at macroscale and microscale. Therefore, rejuvenation of hair is not the temporarily enhancing sensory feeling. It should be fundamental reinforcement of hair structure changing due to hair damage.

There is the unique type of fatty acid in the hair surface. 18-methyleicosanoic acid (18-MEA) is an unusual anteiso fatty acid covalently linked to the outermost surface of hair cuticle. 18-MEA was naturally selected with unique methyl branch and covalent bond. 18-MEA has lower melting temperature than normal fatty acid due to high local segmental mobility of methyl branch. Therefore it shows liquid like behavior. As a result, 18-MEA monolayer shows low frictional resistance. This high mobility of 18-MEA molecule facilitates spreading of extraneous lipid due to decreasing interfacial shear strength. A layer of 18-MEA is responsible for the low surface energy and low friction resistance of the hair's outer surface.

Covalently linked fatty acid monolayer was removed easily by permanent waving and coloring process. But it didn't wash out via daily shampooing process cause of covalent linkage. It is the important point of our study. Generally, most of hair conditioning agents were removed by one or more times of shampooing.

Hair matrix is composed of protein moiety. So, we searched amino acid chain modification agents to make covalently linked fatty acid monolayer. In the field of protein modification, a common method is called PEGylation. PEGylation is the process of covalent attachment of polyethylene glycol polymer chains to another molecule, normally a drug or therapeutic protein. It can "mask" the agent from the host's immune system (reduced immunogenicity) and increase the hydrodynamic size (size in solution) of the agent which prolongs its circulatory time and provide water solubility to hydrophobic drugs and proteins.

However, we intended to make covalently linked fatty acid monolayer on the hair surface not the PEG. And we combine the fatty acid and coupling chemistry of PEGylation to form functionalized new materials. It is like hair lipidation. It is the covalent attachment of lipid to the amino acid side chain.

In this article, we verified long lasting coating effect for biomimetic functionalized lipid and what the role of fatty acid monolayer on hair surface is. We expected hair regeneration to the original state and additionally, long-lasting effect via covalent linkage.

2. Materials and Methods

2.1. Hair Tress Preparation

In this experiment, 2 g of dark brown oriental hair tresses (DeMeo brothers, New York) were used for instrumental analysis. Tresses were washed rubbing in circular motion for 30 seconds with a solution of sodium laureth sulfate (1.5 % w/w) in deionized water. They were rinsed under warm (40 ℃) running water for enough time. Excess water was squeezed from the tresses by pulling them between two fingers. The tresses were laid on a clean paper towel and allowed to air dry overnight at ambient conditions (25 ± 1 ℃ and 50 ± 5 % relative humidity). To get hair damaged, hair tress was placed in a bleaching solution composed of 4 % of MEA, 0.28 % of ammonia, and 5 % of hydrogen peroxide for 1 h, then rinsed thoroughly with water and dried in air.

In this study, we introduced N-hydroxyl succinimidyl group to the one end of fatty acid for regenerating hair surface with covalently bound fatty acid monolayer. N-hydroxysuccinimidyl (NHS) ester functional group is commonly used to protein modification for drug delivery. It was well known that NHS-ester can be reacted to the amine group existing in lysine and N-terminal of hair keratin. We called this funcionalized lipid 'Hydroxysuccinimidyl C10 - 40 Isoalkyl Acidate (HCIA)'.

2.2. Hair Regeneration Measurement

Various methods have been developed for measuring hair regeneration. We evaluated moisturizing, surface hydrophobicity, friction force. The instrument used in this experiment is shown in Figure 1. First is moisture balance test. Electric moisture balance was employed to

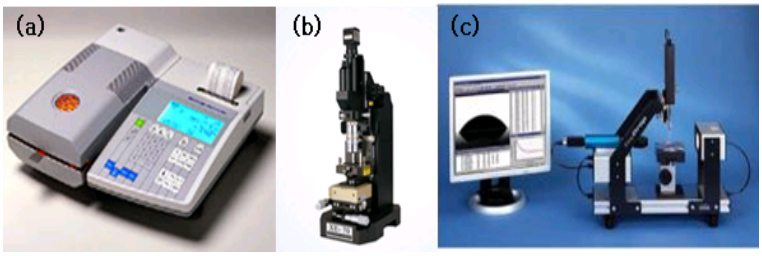

Figure 1. Evaluation instrument : (a) Electric moisture balance, (b) Atomic force microscopy, (c) Contact angle goniometer.

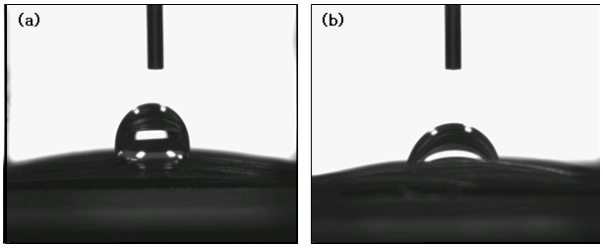

Figure 2. Optical Image of contact angle (a) Normal black hair surface, (b) Damaged hair surface (Bleached).

measure moisture of hair. 1g of hair sample was placed on balance dishes, and weight change with heating was recorded every 1 minutes. The tress was heated for the first 20 min at 65 ℃ to evaporate surface water, and for the next 20 min at 150 ℃ to evaporate bound water contained in hair.

And the second one is contact angle measurement to evaluate surface wetting properties of hair. There are two methods, one is single fiber wilhelmy method and the other is sessile drop method on hair tress. Single fiber wilhelmy method has been applied to measure advancing and receding contact angles of single fiber. For the accurate calculation, both sides of the solid must have the same properties. But, the properties of hair like cuticle scale, surface roughness, ellipticity and diameter was different from each single fiber. We cannot calculate the exact meaning of contact angle of single fiber. We used static sessile drop method. The sessile drop method was measured by a contact angle goniometer using an optical subsystem to capture the profile of a pure liquid on a solid substrate. The contact angle image of normal, untreated and damaged, bleached hair is shown in Figure 2. The angle formed between the liquid/solid interface and the liquid/vapor interface is the contact angle. These instruments em-

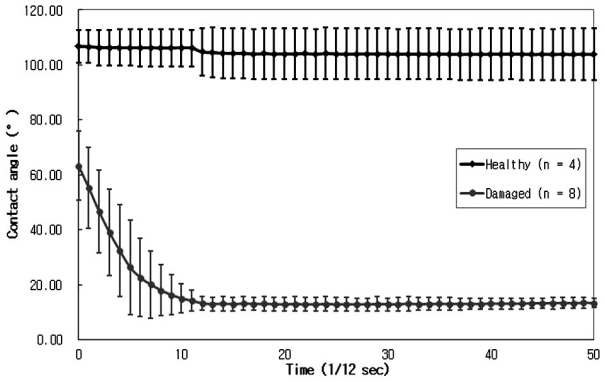

Figure 3. Static contact angle shift of Black, untreated (diamond) and Damaged, bleached (square) hair surface.

ploy high resolutions cameras and software to capture and analyze the contact angle. We customized the zig to make the hair tresses being fixed flatly.

Atomic force microscopy can be used for measurement of hair generation. In contact mode AFM, We can evaluate the RMS roughness of surface and lubricity. It is called lateral force microscopy, LFM. And itis also possible to measure the adhesion force between the tip and the surface using the frequency of tip.

3. Results and Discussion

3.1. Contact Angle Measurement

The result for contact angle for black and damaged hair surface was plotted in Figure 3. This result showed that black, undamaged hair has high, stable contact angle shift due to its fatty acid monolayer. In case of damaged hair, it is rapidly decreasing.

The re-hydrophobicization of hair surface has been investigated by static sessile drop method. For comparison, we treated hair tress with normal conditioner,

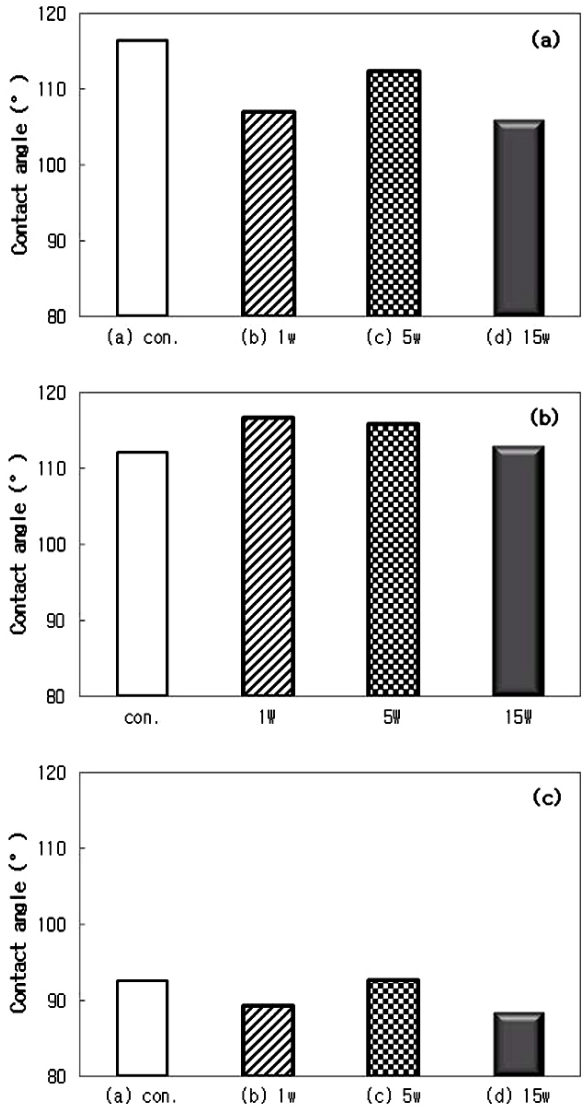

Figure 4. Contact angle change via shampooing process. Con. means no shampooing process after treatment. 1, 5, 15 w means each 1, 5, 15 times shampooing after treatment. (a) Conditioner treated damaged hair, (b) HCIA treated damaged hair, (c) HCIA with surfactant treated damaged hair.

HCIA and HCIA with surfactant. And the experiments were made to develop durability by each 1, 5 and 15 times shampooing after 1 times treatment. As seen in Figure 4, all cases have the durability of hydrophobic oil film. The surface treated with HCIA and surfactant together showed lower contact angle due to surfactant's emulsifying effect. In case of conditioner, silicone ingredient plays an essential role of durable re-hydrophobicization.

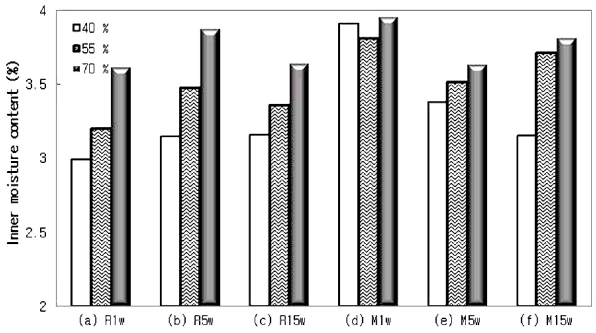

Figure 5. Inner moisture content durability of hair tress treated conditioner vs. HCIA with surfactant with different humidity. First bar of each group is 40 % of relative humidity (dry state), second is 55 % of RH (normal state), last one is 70 % of RH (wet state). R series : (a) R1w, (b) R5w, (c) R15w ; each 1, 5, 15 times of shampooing treated hair tress after conditioner treatment, M series : (d) M1w, (e) M5w, (f) M15w ; each 1, 5, 15 times of shampooing treated hair tress after HCIA with surfactant treatment.

3.2. Moisture Balance Test

Primary transpiration moisture means surface moisture of hair. And secondary moisture means inner, bound moisture of hair. We only use the secondary moisture value. The inner moisture content of hair in different humidity (40, 55, 70 % relative humidity (RH)) was observed by electric moisture analyzer. In Figure 5, there are R series and M series. R series are R1w, R5w and R15w. These term means each 1, 5, 15 times of shampooing treated hair tress after conditioner treatment. M series are M1w, M5w and M15W, which means each 1, 5, 15 times of shampooing treated hair tress after HCIA with surfactant treatment.

In Figure 5, the inner moisture content of hair in R series increased with humidity (40, 55, 70 % RH). But, in M series, it shows similar inner moisture content in different humidity. It is considered that the hair surface treated with functionalized fatty acid has been smooth and uniform because the crack between cuticles filled up with covalently bound fatty acid monomolecular layer like cuticle glue. This glue effect was also con-

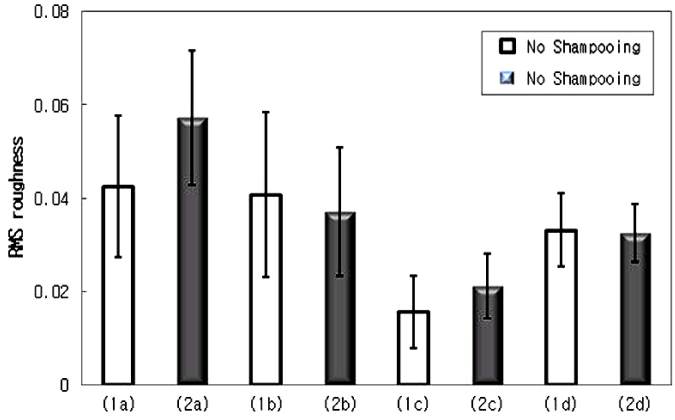

Figure 6. RMS roughness of hair surface by AFM measurement shown in Box-whisker plot, 1 : No shampooing after treatment, 2 : 15 times of shampooing after treatment in each graph, (a) conditioner treated, (b) HCIA treated, (c) HCIA with surfactant treated (d) Hydrosuccinimidyl C10 - 40 alkyl acidate (HCAA) treated, The range of Y axis is different from each plot.

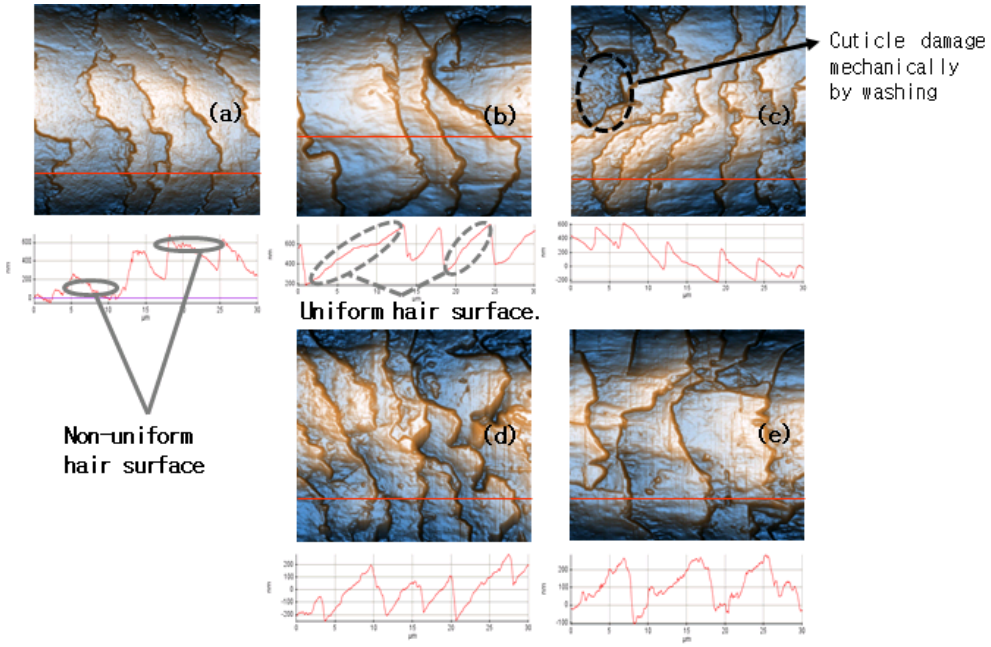

Figure 7. AFM images and Height Profile of (a) Bleached untreated, (b) HCIA treated, (c) 15 times shampooing after HCIA treatment, (d) conditioner treated, (e) 15 times shampoo after conditioner treatment.

firmed with line profile of AFM images. Therefore, the moisture and structural component of inner hair was not easily flown out and the optimum moisture content could be kept constantly though humidity in outside air was changed. The cuticle defect due to mechanical washing have occurred after 15 times of shampooing.

Damaged hair is crumbly in dry state of 40 % RH, and damp in wet state of over 70 % RH. This is due to disappearance of fatty acid monolayer. We assume that this flexible fatty acid monolayer can control inner optimum humidity although humidity in atmosphere was changed with weather condition.

3.3. AFM Examination

Friction measurement using AFM was performed. We called this method lateral force microscopy (LFM) on contact mode AFM measurement. Based on Gaussian probability, we measured the surface roughness with AFM to identify how the covalently linked fatty acid monolayer affects roughness of the hair surface. This surface roughness is related to friction force. To reduce the variation between each hair fibers, we measured the 18 different area of a hair fiber. This result for surface roughness with conditioner treated, HCIA treated, HCIA treated with surfactant, Hydroxysuccinimidyl C10 - 40 Alkyl Acidate (HCAA) treated hair is summarized in "Figure 6". Friction force of hair surface treated with conditioner was significantly increased after 15 times shampooing. In contrast, the friction force of hair surface treated with HCIA was decreased than the hair surface treated with conditioner. It also showed the same friction value even after 15 times shampooing process. Figure 6 also shows the LFM result as surface treated with HCAA. It also shows same friction value even after 15 times shampooing process. In case of treatment with HCIA and surfactant, Friction force was increased a bit after 15 times shampooing, but the difference was very smaller than the gap of friction force for hair surface treated with conditioner.

In Figure 7, AFM image and height profile of damaged non-treated hair, conditioner treated, HCIA with surfactant treated hair was shown. The surface of damaged hair is non-uniform because protein component and CMC (cellular matrix complex) flow out during bleaching process. In Figure 7 (b) and 7 (c), damaged hair treated with HCIA and surfactant was shown. The very uniform and smooth hair surface was shown. It is considered that this surface is coated with fatty acid monolayer. We tested the durability of fatty acid monolayer. It was confirmed that a majority of fatty acid monolayer was remained after even 15 times shampooing treatment. But there is some cuticle defect by mechanical shampooing process.

It is a conditioner treated damaged hair in Figure 7 (d) and 7 (e). It shows smooth hair surface due to conditioning agents like fatty alcohol, silicone etc. We also measured conditioner treated surface after doing 15 times shampooing. We verified that conditioner ingredient was easily removed by shampoo treatment. This is like damaged, non-treated hair surface.

4. Conclusions

In conclusion, the treatment of the hair surface with functionalized fatty acid leaves a covalently linked monolayer that regenerates some properties of the original fiber. The functionalized fatty-acid monolayer described here is covalently linked and substantially remained even after fifteen cycles of shampoo.

Acknowledgment

The authors thank Mr. Yoonsuk Kim for his advice on AFM measurement and Mr. Inkee Yoo for consulting conditioner formulation and test method.

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