Simultaneous 3D visualization of hair microstructure and damage using FIB-SEM

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Abstract

Background

In order to develop an effective hair care product, it is important to elucidate details of hair damage. Therefore, we tried to make the microstructure and the damaged part in the hair into 3D at the same time to confirm which part in the hair and how much damage occurred. Furthermore, we searched for ingredients that prevent hair damage.

Methods

The microstructure of hair was directly imaged by FIB-SEM. We sputtered the surface of the specimen with gallium ion beam and observed the new exposed surface by backscattered electron imaging with SEM and repeated the process of sputtering. Finally, we reconstructed the 3D structure by stacking the images of serial section series.
As a next step, we examined the protection effect of treatment with cosmetic ingredients.

Results

By using FIB-SEM, we succeeded in simultaneous 3D conversion of microstructures such as melanins and CMC and damaged parts such as voids in bleached hair. As a result, bleached hair had more voids than non-chemical treated hair. Especially it was confirmed that the size of void in CMC was biggest than void of near melanins.
Following screening of ingredients that prevent hair damage, S-Sulfokeratin showed a high prevent effect. The FIB-SEM measurements showed S-Sulfokeratin inhibited the generation of voids.

Conclusion

In this study, we obtained 3D images of microstructure and void in hair at the same time with FIB-SEM. The 3-D information help in interpreting the structures correctly, allowing that how much damage is in which part in hair to be validated.

Keywords: hair, 3D conversion, keratein, SEM,

Introduction

In order to develop an effective hair care product, it is important to elucidate the details of hair damage. It is well known that void formed in hair as a consequence of the chemical processes such as hair color, bleache, and permanent wave treatments. For observing the hair sample cross-sections, electron microscopy such as scanning electron microscopy (SEM) and transmission electron microscope (TEM) has been mainly employed. However, the micrographs are two-dimensional (2-D) only certain parts of hair can be observed.

Recently, the cosmetic community has shown a strong interest in three-dimensional (3-D) images in hair. Research to achieve this objective have already some acquire 3D images in hair. For instance, it has been reported to acquire 3D images of damaged parts in hair using devices such as X-ray CT or FIB-SEM [1-2]. As another example, it has been reported to acquire 3D images of melanins in hair using FIB-SEM [3]. However, these observed only one part of hair such as hair damage or microstructure and are still lacking comprehensiveness.

The focused ion beam and scanning electron microscope (FIB-SEM) are commonly used in material sciences for imaging and analysis of materials. This instrument is SEM with an attached gallium ion column and the 2 beams, electrons and ions are focused on one coincident point. With the ion beam, some nanometres of the surface are removed, and the remaining block-face is imaged with the electron beam in a repetitive manner. Finally, it can reconstruct the 3D structure by stacking the images of serial section series [4].

Recently, FIB-SEM has progressively found use in biological and cosmetic research. As above, FIB-SEM already used for acquiring 3D images of only voids or only microstructure in hair. However, few studies had examined simultaneous 3D visualization of hair microstructure and void using FIB-SEM.

Therefore, in this study, we tried to make the microstructure in the hair and the voids from damage into 3D at the same time and confirmed which part in the hair and how much damage occurred. Furthermore, we searched for ingredients that prevent hair damage by applying this method.

Materials and Methods

Materials

Hair was collected from woman in their 30s who had no history of beauty treatment (bleach, hair color, permanent wave, straightening) with participants providing an informed consent in writing.

Methods

(1) Sample fixation and embedding

Hair samples were fixed in 2% glutaraldehyde in 0.1 M cacodylate buffer and tannic acid for 3h at 4 ℃. After being rinsed in the 0.1 M cacodylate buffer for overnight at 4 ℃, the samples were post-fixed in 2 % osmium tetroxide in the same buffer for 2 h at 4 ℃. The samples were rinsed with the buffer and distilled water, dehydrated in a graded ethanol series (50 - 100 %). Then the samples were embedded into epoxy-resin.

(2) FIB-SEM observations

The microstructure of human hair was directly imaged by FIB-SEM without any staining. We sputtered the surface of the specimen with gallium ion beam and observed the new exposed surface by backscattered electron imaging with SEM and repeated the process of sputtering and observation until obtained 300 slice images.

(3) Three-dimensional reconstruction image and size calculation.

Longitudinal sections and cross-sections of the hair samples were exposed using an ultramicrotome equipped with a diamond knife (EM-ULTRACUT UCT, Leica Microsystems GmbH, Germany). The resin blocks were subsequently trimmed to a cuboid and glued to the specimen stage for FIB-SEM. The hair cross-sections of the segment in the cuboid were placed vertically to the FIB column, and the surface of the hair faced the SEM column. The specimens were coated with a thin layer of evaporated carbon with a carbon coater (CADE-EMT, Meiwafosis, Japan) to prevent electron charging. The serial electron micrographs were obtained with a FIB-SEM (MI-4000L, Hitachi, Japan), in which the SEM and the FIB columns were orthogonally arranged. The fresh surfaces of the specimen were exposed with gallium ion beam using the FIB, and then the block-face images were captured with the SEM. Serial electron micrographs were obtained automatically by repetitive cutting with the FIB and image capturing with the SEM and repeated the process of sputtering and observation until obtained 300 slice images.

The resultant image stacks were processed using the software Fiji [5]. After segmentation

of components, the 3-D model was reconstructed based on the image stacks. The volume of the components were calculated using the plugin 3D Object Counter of the Fiji software.

(4) Bleaching process

The hair tresses were bleached at room temperature for 30 minutes. After wash, the above procedure was repeated 3 times.

(5) Screening of ingredients that prevent hair damage

In the screening of ingredients that prevent hair damage, hair tresses were immersed into each sample at room temperature for 20 minutes. Hydrolyzed keratins, which are used as cosmetic ingredients, was used in this study. Next, each hair tresses were bleached at room temperature for 30 minutes. After wash, the above procedure was repeated 3 times. There were immersed in water at 40℃ for 24hr, and outflow of hair protein was measured using the Bradford method [6].

Then verification of inhibit damage effect of the screened samples on hair was imaged by FIB-SEM.

(6) Verification of the inhibitory effects of the S-Sulfokeratin on Bleaching damage

Among the screening results, the highly effective S-Sulfokeratin A and B were used to verify the inhibit damage effect on hair.

Hair tresses were immersed into S-Sulfokeratin A and B solution (A: 50%, B: 50%) at room temperature for 20 minutes.

Verification of inhibit damage effect of the screened samples on hair was imaged by FIB-SEM.

Results

The hair samples were examined as block-face images using a FIB-SEM. The block-face images had enough contrast without uranyl or lead staining.

Fig.-1 shows a representative SEM image of the cross-section of untreated hair and bleached hair. Variably sized voids were observed in bleached hair.

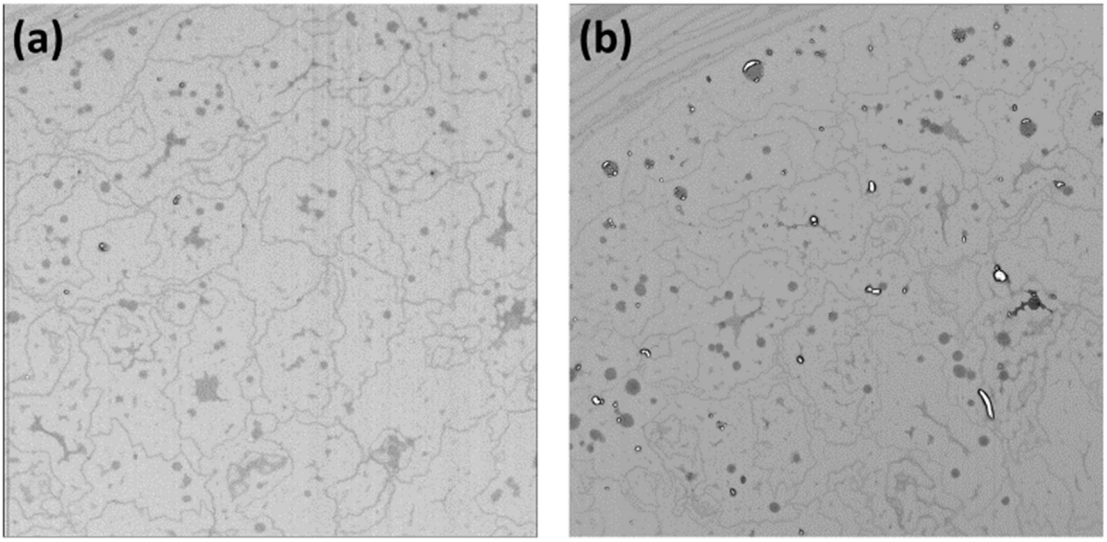

Fig.-1 One image in a sequence of 300 images observed with FIB-SEM

(a) Untreated hair, (b) Bleached hair

In order to compare the sizes of microstructure and damage void in hair, three-dimensional reconstruction was performed using serial SEM images obtained by FIB-SEM observation. Fig.-2 shows 3D reconstructed images of melanins and void of around melanins. It was directly confirmed that the size of void was biggest than that of melanins.

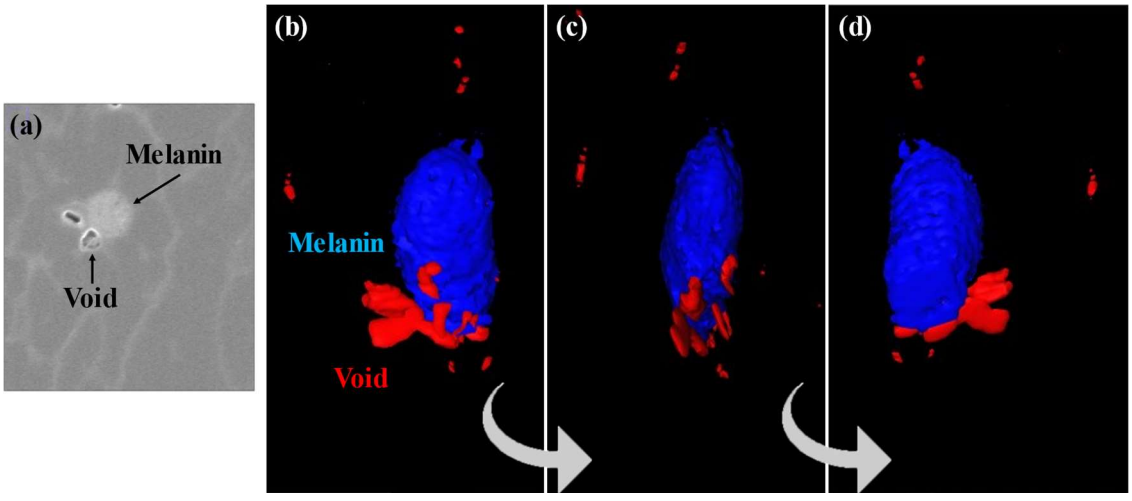

Fig.-2 Image of melanin and void of around melanin.

(a) 2D image, (b) 3D reconstructed image, Blue structure: Melanin, Red structure: Void

(c)・(d) 3D reconstructed image. The view shows the same region however at a different angle from (b).

Fig.-3 shows three-dimensional reconstructed images of melanins and CMC. It was directly confirmed that the size of void in CMC was biggest than that of void near melanins.

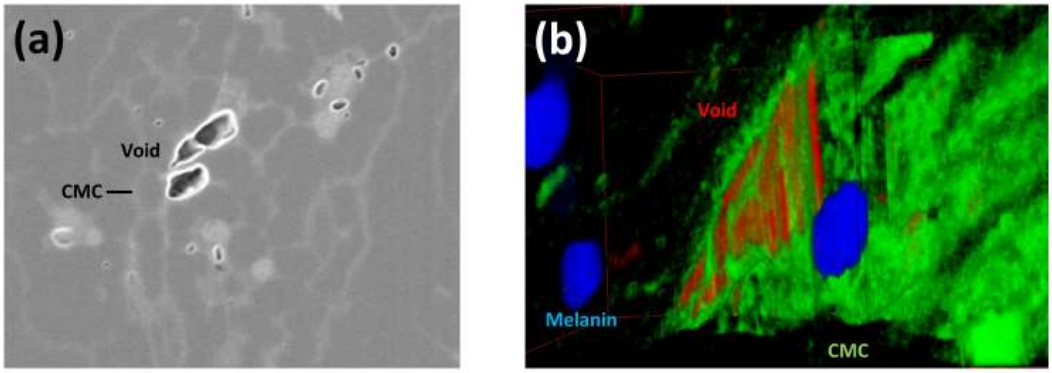

Fig.-3 Image of CMC and void of CMC.
(a) 2D image, CMC is structure like road, (b) 3D reconstructed image, Blue structure: Melanin, Red structure: Void, Green structure: CMC

Table-1 illustrates representative size of microstructure and voids.
The volume of the voids around melanins were smaller than that of melanins. The volume of the biggest void was 15% that of melanin.
On the other hand, the volume of the voids of CMC were bigger than that of melanins. The volume of the biggest void was 2.6 times larger than that of melanin.
This result showed similar trends elsewhere in hair.

Components	Volume (nm^3)
Melanin	51.566
Voids around malenin	0.001 ~ 0.786
Voids of CMC	0.001 ~ 132.865

Table-1 Volume of the components.

Fig.-4 shows verification results for the anti-outflow of hair protein effect of the samples on hair. Hydrolyzed Keratin A and Hydrolyzed Keratin B showed a high inhibitory effect. These two types of hydrolyzed keratin had Bunte salt and called S-Sulfokeratin.

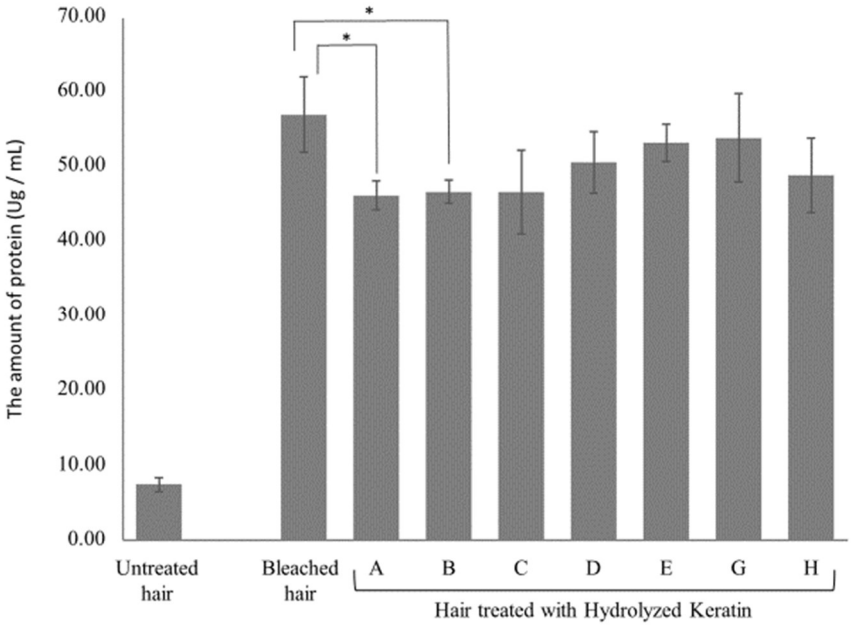

Fig.-4 The amount of protein dissolved from hair.

Fig.-5 shows three-dimensional reconstructed images of voids of bleached hair and S-Sulfokeratin A and B pre-treated bleached hair. It was confirmed that application of S-Sulfokeratin inhibited th generation of voids.

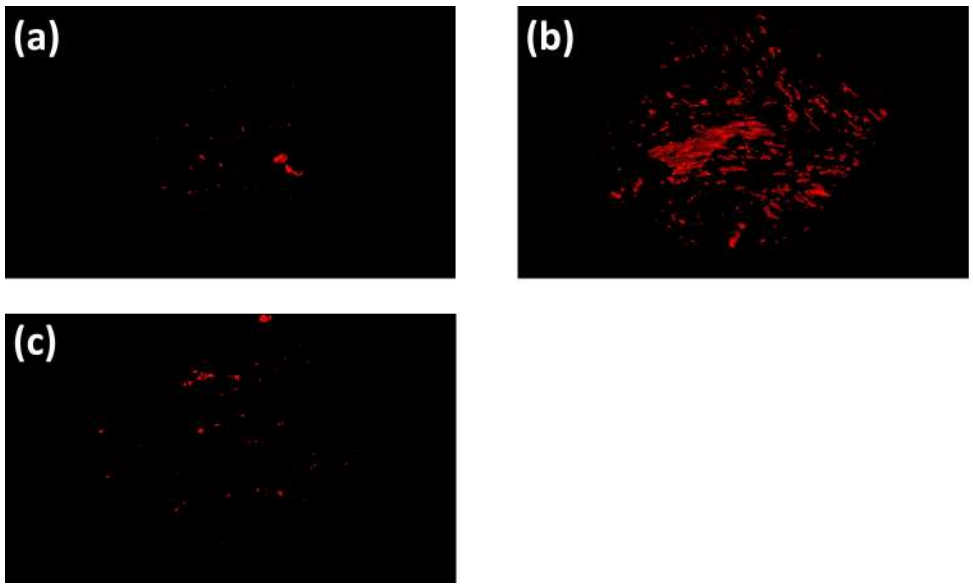

Fig.-5 Representative 3D reconstructed image

(a) Untreated hair, (b) Bleached hair, (c) Hair treated with S-Sulfokerain A and B

Discussion

A hair fiber consists of the medulla, cortex and cuticle from the center to the outside in this order. The major mass of hair fiber is located in the cortical region, which mainly consists of keratin filaments and matrix proteins. On the outside of the cortical region, there is cuticle which has about 5 μm in thickness and consists of sheet like cells. Cell membrane complex (CMC) present in both cuticle and cortical cells is pathways for molecules through the cuticle into the central cortex [7]. Melanins are mainly found in the intermacrofibrillar matrix of the cortex and determine the color of hair [8].

Hair bleach is a global beauty habit that is very popular with people who change their original hair color. When change original hair color, it is necessary to first decompose a part of the melanins with a bleaching agent before applying hair dyes.

Bleach uses persulfate salt and hydrogen peroxide. The basic mechanism of the hair bleach is the decomposition of melanins by oxidation with hydrogen peroxide under an alkaline condition using ammonia or ethanolamine. It has been reported to metal elements coordinating to the melanins flow out during the bleaching process and become a catalyst for hydrogen peroxide in other areas than the melanins [9]. It was confirmed that void generated around melanin by bleach process (Fig.-2). However, since the melanins exist in the cortex, it is considered to active ingredients such as hydrogen peroxide in the bleach agent would have an impact on the microstructural components until their way to the melanins and the inner section of a hair. CMC is route through which active ingredients diffuses into hair, and so we speculate that CMC and around structure could be damaged when it is in contact with bleach agent.

In this study, it was confirmed that the size of void in CMC was biggest than void of near melanins (Fig.-3, Table-1). This result suggest CMC react more easily with hydrogen peroxide than melanins. It is reported that Hydrogen peroxide that has entered the cortex region diffuses throughout CMC in a very short time and the diffusion rate of hydrogen peroxide in the cortex is very slow because there are many SS bonds in the cortex and the crosslink density is high [10]. Therefore, it was presumed to CMC is susceptible to the hydrogen peroxide.

As a next step, we examined the protection effect of treatment with cosmetic ingredients. We first screened hydrolyzed keratin that inhibited outflow of hair protein by bleaching treatments. As a result, it was found that application of S-Sulfokerain before bleaching treatments inhibited outflow of hair protein (Fig.-4).

After that, acquired 3D images hair using FIB-SEM. As a result, it was found that

application of S-Sulfokeratin inhibited the generation of voids (Fig.-5). In particular, it was confirmed that it inhibited the generation of voids in both near melanin and CMC. When evaluated the efficacy of hair care products that prevent bleach damage, it is necessary to distinguish between melanin holes and damage holes. However, this method helped in observing the bleach damage correctly because can simultaneous 3D visualization of hair microstructure and damage.

It has been reported that disulfide bond in hair is oxidatively cleaved to generate cysteic acid by performing bleaching treatments. It is considered that protein elution occurs due to a decrease in disulfide bonds in hair proteins [11]. S-Sulfokeratein has a disulfide bond within the molecule, and it hypothesized that it bonds by an SS/SH interchange reaction with the thiol group of disulfide bond decomposed inside the hair. Based on the above, it seems that S-Sulfokeratin acts as an anti-damaging agent.

This result is only one instance among many. The damage of hair with other chemical process will be further examined in the future.

Conclusion.

In this study, we obtained 3D images of microstructure and void in hair at the same time with the aid of FIB-SEM. The 3D information help in interpreting the structures correctly, allowing that how much damage is in which part in hair to be validated. This method is undoubtedly a promising tool for elucidate the details of hair damage and develop an effective hair care product.

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Conflict of Interest Statement

NONE.

References

1. Takehara K, Inoue T,Uesugi K , et al (2010) Imaging of hair damage structure using X-Ray micro-tomography. J Soc Cosmet Chem Jpn. 44:292- 297
2. Yoshikawa N, Ikuta K, Inaba K, (2013) Cross- sectional Observation and 3-D Imaging of the Human Hair by Cryo FIB- SEM. The 73th SCCJ Research Symposium Abstract. 6-7
3. Kojima T, Yamada H, Imai T, et al (2020) In situ observation of three-dimensional distribution and morphology of melanin granules in human hair. The IFSCC Congress 2020 Yokohama Congress Abstract. 1599-1605
4. Knott G, Marchman H, Wall D, et al (2008) Serial section scanning electron microscopy of adult brain tissue using focused ion beam milling. J Neurosci. 28: 2959–2964.
5. Schindelin J, Arganda-Carreras I, Frise E, et al. (2012) Fiji: an open-source platform for biological-image analysis. Nature Methods. 9: 676–682.
6. Bradford, M.M. (1976) Rapid and Sensitive Method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. Anal Biochem. 72: 248-254.
7. Naito S, Takahashi T, Hattori M, (1992) Histochemical observation of the cell membrane complex of hair. Sen-i Gakkaishi. 48:420-426.
8. Arnaud J C, Bore P, (1981) Isolation of melanin pigments from human hair. J Soc Cosmet Chem. 32: 137–152.
9. Imai T, (2011) The influence of hair bleach on the ultrastructure of human hair with special reference to hair damage.Okajimas Folia Anat. Jpn. 88: 1-9

10. Suzuta K, (2016) Evaluation of Cysteic Acid in Bleached Hair Using Infrared Spectroscopy.J Fiber Sci Technol. 72: 1-8

11. Oku M, Nishimura H, Kanehisa H, (1987) Dissolution of proteins from Hair (1) The analysis of dissolved proteins from bleached hair. J Soc Cosmet Chem Jpn. 21: 198-203