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Internal structure changes of eyelash induced by eye makeup

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Synopsis

To investigate how eye makeup affects eyelash structure, internal structure of eyelashes were observed with a scanning X-ray microscopic tomography system using synchrotron radiation light source. Eyelash samples were obtained from 36 Japanese women aged 20–70 years and whose use of eye makeup differed. Reconstructed cross-sectional images showed that the structure of the eyelash closely resembled that of scalp hair. The eyelash structure is changed by use of eye makeup. There was a positive correlation between the frequency of mascara use and the degree of cracking in cuticle. The positive correlation was also found between the frequency of mascara use and the porosity of the cortex. By contrast, the use of eyelash curler did not affect the eyelash structure with statistical significance.

INTRODUCTION

Whether eye makeup induces characteristic changes in the structure of eyelashes is of interest. The growth cycle of the eyelash is approximately 90 days (1), which is shorter than that of scalp hair of two to six years (2). Therefore, there would appear to be less opportunity for eyelashes to be damaged than scalp hair. It has been reported that characteristics of eyelashes are not influenced by eye makeup use (3). However, the makeup treatments that are applied to the eyelash differ from those that are applied to the scalp hairs, e.g., mascara, eyelash curler, false eyelashes, and eyelash extensions. Eyelashes might be damaged by these treatments. In this study, we investigated the effects of mascara and eyelash curler use on eyelash internal structure.

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The internal structures of the eyelashes were observed using a differential phase-contrast scanning X-ray microscopic computed tomography (CT) system (4). This system enables to observe density distribution of intact samples by measuring the phase gradient of transmitted X-ray probe.

MATERIALS AND METHODS

EYELASH SAMPLES

Eyelash samples were obtained from 36 Japanese women whose use of eye makeup differed. A questionnaire was used to determine their age and whether the women used mascara and/or an eyelash curler, and if so, with what frequency in last two months. The number of women in each age category is shown in Table I, and the frequencies of two eye makeups are shown in Table II.

OBSERVATION OF EYELASH STRUCTURE

The measurement was performed at the undulator beamline 20XU of SPring-8 (Hyougo, Japan). We employed a differential phase-contrast scanning X-ray microscopic CT system (4), which has been developed to observe the internal structure of human hair sample (5).

The differential phase-contrast image representing the variation of refractive index of sample is obtained by measuring the angle of deflection of the transmitted X-ray beam. The decrement of refractive index from unity is approximately proportional to the electron density. The electron density is generally proportional to mass density. The samples were set in the air. So, the phase-contrast image reconstruction by the CT system corresponds to the mass density map of eyelash samples.

The sample setup of the scanning X-ray microscopy system is shown in Figure 1. The eyelash sample was positioned such that the eyelash bulb was uppermost, and regions of the eyelash shaft located 2 mm from the eyelash bulb were scanned. The eyelash samples were observed in the atmosphere. More details of the experimental setup are described elsewhere (4).

A focused 8 keV X-ray beam with a diameter of 100 nm was used as a scan probe. The transversal scan pitch was 100 nm. For a CT measurement, transversal scan was repeated

Table I
Age Distribution of Volunteers

Age (years)	Number of volunteers
20–30	7
30–40	8
40–50	8
50–60	8
60–70	5

Table II
Frequency of Eye Makeup Use

Frequency	Mascara use (n)	Eyelash curler use (n)
More than 4 times/week	15	16
2 or 3 times/week	3	4
1 time/week	2	3
Almost never	8	4
Never	8	9

600 times by changing the rotation angle by 0.3°—step to cover 180°. In the case of the typical measurement condition with the diameter of the hair sample of 100 μm, more than 1000 points are required for each transversal scan with 100 nm pitch. Therefore, totally more than 600,000 scan points (1000 transversal scan × 600 rotational scan) are required for one CT measurement. In such a measurement condition, it takes approximately 30 min for a CT scan.

STRUCTURE ANALYSIS OF RECONSTRUCTED EYELASH IMAGE

Shape and size of eyelash. The reconstructed cross-sectional image of eyelash was assumed to be an ellipse. The longest diameter of the cross-sectional image was deemed to be the major axis of the fiber. The minor axis of the fiber was assumed the longest diameter perpendicular to the line of major axis. From the length of major axis and minor axis, the area of the fiber was calculated. The medulla was assumed to be ellipse- and the length of major axis and that of minor axis were measured by the same method of eyelash fiber measurement. Thickness of cuticle was estimated by defining the average of four locations of cuticle space, two of them were contained in the major axis in the fiber and the other two were contained in the minor axis of the fiber.

Ranking of the degree of crack formation in cuticle. Cracks were observed in the cuticles of eyelashes. The degree of crack formation was ranked into one of five categories: (i) almost

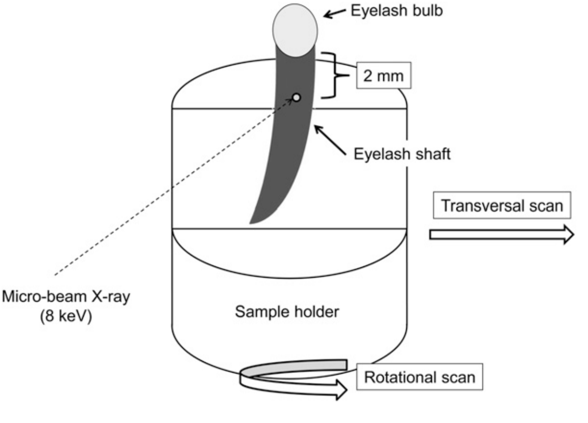

Figure 1. Set up of the scanning X-ray microscopy system with an eyelash sample.

absent, (ii) one small crack, (iii) several small cracks, (iv) a large crack in a narrow part of the cuticle, and (v) a large crack in a wide part of the cuticle. At first, five typical cross-sectional images agreed with these categories were selected from images of all 36 eyelash samples. Second, other 31 samples were ranked with resemblance to the typical images.

Porosity of cortex. The electron density mapping of the reconstructed cross-sectional image of eyelash was binarized. The threshold value of binarized image was determined sensibly to fit the pore space of each cross-sectional image. The porosity of cortex region was calculated using Image J (http://rsbweb.nih.gov/ij/).

STATISTICAL ANALYSIS

Statistical analyses were performed using SPSS 12.0J for Windows (IBM-SPSS Japan, Tokyo, Japan). The correlations among the categories of age distribution, frequency of mascara and/or eyelash curler, and degree of cracking in cuticle were analyzed by Spearman's method using the five ranks of each category. The correlation analyses, thickness of cuticle and porosity of cortex, were performed using Pearson's method. The correlation analyses between the each category and the each character mentioned above were performed using Spearman's method.

RESULTS AND DISCUSSION

RECONSTRUCTED CROSS-SECTIONAL IMAGES OF EYELASHES

Thirty-six eyelash samples were observed using a differential phase-contrast scanning X-ray microscopic CT system. Typical CT image of an eyelash measured with this system is shown in Figure 2. Three components (cuticle, cortex, and medulla) were observed in the structure of the eyelash. Melanin granules were observed as small white dots in the cortex, indicating that they are relatively dense. Small pores were observed as small black dots in the cortex. These characteristics are very similar to those of scalp hair observed in the previous study

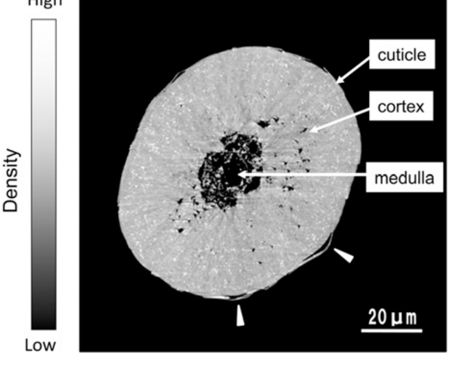

Figure 2. Cross-sectional image of an eyelash. The arrow head in the eyelash image indicates a crack in the cuticle.

(5). The resemblance between eyelash and scalp hair is in agreement with the previous reports that observed eyelashes using transmission electron microscopy (TEM) (4,6). The difference of internal structure between eyelash and scalp hair is not clear at present. As shown below, the shapes and sizes of eyelashes were varied with samples from different-people. We have observed scalp hair samples from only one person (5), and the structural characteristics of scalp hair were in the range of variations of eyelash samples.

Using the reconstructed cross-sectional images, the sizes of specific regions of the eyelashes were measured (Table III). In the reconstructed cross-sectional images, cross section of eyelashes had an elliptical shape, the mean length of the major axis was 77.8 μm, and the mean length of the minor axis was 63.2 μm. They are consistent with a previous study in which eyelash diameter was measured from digital photograms (4). By contrast, the mean thickness of the cuticle layers was 2.0 μm in this study, whereas it was measured to be 5.3 μm from TEM images in the previous study (4). In the TEM observation, the eyelash samples were pretreated by many steps of procedures containing fixation steps using glutaraldehyde and osmium tetroxide. In this study, the eyelash samples did not undergo prior processing. The pretreatments prior to TEM imaging might have induced morphological changes in the cuticle.

The relationships among these measurements were investigated (Table IV). The area of the fiber correlated positively with the length of the major axis of the fiber and the length of the minor axis of the fiber. The ratio of minor axis of length versus major axis length did not correlate with area of fiber. These correlations indicate that eyelashes of different sizes tend to have a similar shape. In contrast, the area of the fiber correlated positively with the ratio of fiber area versus medulla area. This means that the percentage of medulla space in total fiber space tends to be wider in larger eyelashes. Similar correlation has been found in scalp hair (7). The thickness of the cuticle correlated positively with the major axis length, but not with the minor axis of the length. This characteristic seems to reflect the negative correlation between the ellipticity of fiber, the ratio of minor axis of length versus major axis length, and the thickness of cuticle.

EFFECT OF EYE MAKEUP ON EYELASH STRUCTURE

Cracks were observed in the cuticles of eyelashes (indicated by arrows in Figure 2), and the extent of the cracks varied among the samples. Factors underlying the formation of

Table III
Shape and Size of the Eyelashes

	Mean	Standard deviation
Length of the major axis of the fiber (μm)	77.8	13.3
Length of the minor axis of the fiber (μm)	63.9	10.5
Ratio of minor axis length vs. major axis length	0.826	0.073
Area of the fiber (×100 μm^2)	39.9	12.6
Area of the medulla (×10 μm^2)	31.1	20.0
Ratio of fiber area vs. medulla area	0.076	0.034
Thickness of the cuticle (μm)	2.05	0.64

Table IV
Correlations between the Shape and Size Characteristics of the Eyelashes

	Length of the major axis of the fiber	Length of the minor axis of the fiber	Ratio of minor axis length vs. major axis length	Area of the fiber	Area of the medulla	Ratio of fiber area vs. medulla area	Thickness of the cuticle
Length of the major axis of the fiber	1						
Length of the minor axis of the fiber	0.860 (**)	1					
Ratio of minor axis length vs. major axis length	−0.335 (*)	0.184	1				
Area of the fiber	0.961 (**)	0.958 (**)	−0.077	1			
Area of the medulla	0.610 (**)	0.703 (**)	0.1	0.677 (**)	1		
Ratio of fiber area vs. medulla area	0.438 (*)	0.533 (**)	0.067	0.480 (**)	0.841 (**)	1	
Thickness of the cuticle	0.459 (*)	0.25	−0.436 (**)	0.361 (*)	0.068	-0.088	1

Correlations were analyzed using Pearson's method. Correlation coefficients are shown. $*p < 0.05$, $**p < 0.01$.

Table V
Correlations between the Characteristics of the Volunteers and Eyelash Structure

	Age of volunteer	Eyelash curler use	Mascara use	Cracking of the cuticle	Porosity of the cortex
Age of volunteer	1				
Eyelash curler use	0.15	1			
Mascara use	−0.133	0.528 (**)	1		
Cracking of the cuticle	−0.051	0.051	0.348 (*)	1	
Porosity of the cortex	0.057	0.025	0.389 (*)	0.156	1

Correlations were analyzed using Spearman's method. Correlation coefficients are shown. $^*p < 0.05$, $^{**}p < 0.01$.

these cracks were investigated by correlation analysis among the characteristics of the volunteers (Table V). Interestingly, the rank of crack formation showed positive correlations with the frequency of mascara use, whereas it was not significantly correlated with eyelash curler use. There was a relatively strong positive correlation between the frequency of mascara use and the frequency of eyelash curler use. Under these conditions, the difference between mascara and eyelash curler use was found. Similar correlation was also found between porosity of cortex and the frequency of mascara use. These results indicate that mascara induces structural changes to eyelashes, namely crack formation in the cuticle and increased porosity of the cortex.

The relationships between the characteristics of shape and size of eyelash shown in Table III and the characteristics of volunteers were investigated as shown in Table VI. There were negative correlations between volunteer age and the area of the fiber, the length of the minor axis of the fiber, and the area of the medulla. These results indicate that older women tend to have thinner eyelashes. Similar results had been reported in the case of scalp hair (8). There was a positive correlation between the frequency of mascara use and the thickness of the cuticle. This correlation indicates that mascara induces structural

Table VI
Correlations between the Properties Shown in Table V and the Eyelash Shape and Size Characteristics

	Age of volunteer	Eyelash curler use	Mascara use	Cracking of the cuticle	Porosity of the cortex
Area of the fiber	−0.335 (*)	−0.105	0.05	−0.054	0.061
Length of the major axis of the fiber	−0.309	−0.092	0.075	−0.021	0.134
Length of the minor axis of the fiber	−0.348 (*)	−0.094	−0.008	−0.142	0
Area of the medulla	−0.402 (*)	0.173	0.031	0.008	−0.021
Ratio of minor axis length vs. major axis length	0.097	−0.13	−0.223	−0.172	−0.274
Ratio of fiber area vs. medulla area	−0.368 (*)	0.283	−0.029	−0.068	0.111
Thickness of the cuticle	−0.179	−0.069	0.361 (*)	0.282	0.263

Correlations were analyzed using Spearman's method, with the exception of correlations with porosity of the cortex, which were analyzed using Pearson's method. Correlation coefficients are shown. $^*p < 0.05$.

change of cuticle. As shown in Table V, mascara induces cracking of cuticle. The crack formation might be hypothesized to affect measurement of cuticle thickness; however, crack formation did not significantly correlate with the thickness of the cuticle (Table VI). These correlations indicate that mascara use induces other structural changes in cuticle, for example, swelling of the cuticular cell scale.

CONCLUSIONS

To investigate how eye makeup affects eyelash structure, internal structure of eyelashes were observed using a scanning X-ray microscopic tomography system. We found positive correlation between the frequency of mascara use and the rank of cracking in cuticle, the thickness of cuticle, or the porosity in cortex. These correlations indicate that mascara treatment induces crack formation or swelling in cuticle structure and increases the porosity of cortex. Eye makeup using mascara contains two steps of actions, application and removing. In general, it is recognized that mascara, especially the waterproof variety, can be extremely hard to get off. There are many comments from consumers from the internet concerning eyelash damage during removing. The structural changes of eyelash found in this study provide scientific evidence to the eyelash damage induced by mascara use.

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