The influence of hair bleach on the ultrastructure of human hair 
with special reference to hair damage

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Key Words: Human hair, Ultrastructure, Bleach, Melanin

Summary: The influence of human hair bleaching agents with different bleaching strength on the ultrastructure of human hair was studied using a transmission electron microscope (TEM) and an energy dispersive X-ray spectrometer equipped with TEM (EDS-TEM). Two kinds of bleaching agents were used: a lightener agent with a weak bleaching effect and a powder-bleach with a stronger bleaching effect.

From the comparison of the bleaching properties obtained by the electronic staining of black and white hair samples, it was suggested that the permeability of hair was increased by bleaching, and there was an increase of the stainability of hair subjected to electronic staining. The bleaching action provoked the decomposition of melanin granules and the flow out of granular contents into the intermacrofibrillar matrix. Some metal elements were detected in the melanin granular matrix by EDS-TEM. As a result, the diffusion of metal elements into the intermacrofibrillar matrix promoted further damage to the hair by catalytic action with the hydrogen peroxide in the bleaching agents outside the melanin granules. Further study will lead us to the edge of the development of a new bleaching agent, which reacts only with melanin granules and causes the minimum of damage to outside the melanin granules.

Introduction

A human hair is composed of 5 to 10 sheets of cuticle that surround the cortex, and a sheet of cuticle consists of ultrastructural components such as epicuticle, A-layer, exocuticle, endocuticle, and inner layer as shown in Fig. 1-3). The structure of the cuticle CMC (cell membrane complex), which originates from the cell membrane, has the role of connecting cuticle cells with each other and provides a penetration route for moisture to reach the inner part of the hair9). The CMC consists of two lipophilic β-layers and a single intervening hydrophilic δ-layer. The cortex consists of ultrastructural components such as macrofibrils, intermacrofibrillar matrix, nuclear remnant, and the cortex CMC derived from the cortex cell membrane. Melanin granules are mainly found in the intermacrofibrillar matrix of the cortex, and the color of hair depends on the melanin granules because the substances making up the cuticle and the cortex are colorless.

When applying hair coloring techniques to black hair, it is necessary to first decompose a part of the melanin granules with a bleaching agent before applying hair dyes. Therefore the oxidative hair coloring, which is used widely today, simultaneously dyes and bleaches hair, and consequently allows a one step hair coloring procedure to obtain the desired color, even for black hair.

There are roughly two kinds of hair bleaching agents, one is a lightener which bleaches black hair to brown, the other is a more powerful powder-bleach which bleaches black hair to light brown. The basic mechanism of the lightener is the decomposition of melanin granules by oxidation with hydrogen peroxide under an alkaline condition using ammonia or ethanolamine. Powder-bleach has a stronger bleaching effect than lightener and uses persulfate salt and hydrogen peroxide. Most of the oxidative hair coloring agents contain oxidative dyes, such as p-phenylenediamine, and the same active ingredients as lightener, and thus have the same bleaching effect as a lightener.
On the other hand, hair bleaching also causes some hair damage, and awareness of this fact has been increasing in recent years. Ideally bleaching agents should act only on melanin granules. However since the melanin granules exist in the cortex, it is reasonable to assume that bleaching agents would have a considerable impact on the ultrastructural components on their way to the melanin granules and the inner section of a hair. If so, this would cause damage to the hair. For example, it has been known that some oxidative decomposition of disulfide bonds in keratin molecules is caused by the action of lightener, and more serious decomposition is found in hair treated with powder-bleach5).

So far hair damage caused by bleaching agents has been actively studied ultrastructurally, but there is little research concerning ultrastructural changes of hair and the resultant color in relation to the strength of hair bleaching agents. Therefore in this study, the influence of two kinds of bleaching agent on the ultrastructure was studied using a conventional transmission electron microscope (TEM) and an energy dispersive X-ray spectrometer equipped with TEM (EDS-TEM).

### Materials and Methods

Human black hair and white hair without any chemical treatment were used as samples of untreated normal hair, and were treated with commercially available lightener (Promaster LT, Hoyu Ltd., Nagoya, Japan) and powder-bleach (Powder-Bleach, Hoyu Ltd., Nagoya, Japan) at 30°C for 40 minutes. The amount of bleaching agent used was three times the weight of the hair. The active ingredients and the bleaching effect of these agents are shown in Table 1. Then, both lightener-treated hair and powder-bleach-treated hair were washed twice in a 10% sodium lauryl sulfate solution followed by washing in distilled water for one minute, and the hair samples were allowed to dry naturally. A number of hair samples were also subjected to powder-bleach three times, by which the color changed from black to pale yellow, and this was referred to as 3 times powder-bleach-treated hair.

Samples of all these hairs were cut into about 1 cm lengths. Each hair sample was fixed with a 1% osmium tetroxide solution (OsO₄) for 2 or 4 hours, then subjected to a dehydration process with absolute ethanol and propyl-

<table>
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<th>Hair</th>
<th>Bleaching agents (active ingredients)</th>
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<tr>
<td>Untreated normal hair</td>
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<td>–</td>
<td>Black</td>
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<tr>
<td>Lightener-treated hair</td>
<td>Promaster LT (ammonia, hydrogen peroxide)</td>
<td>30°C, 40 min.</td>
<td>Brown</td>
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<tr>
<td>Powder-bleach-treated hair</td>
<td>Powder-Bleach (potassium persulfate, ammonium persulfate, hydrogen peroxide)</td>
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ene oxide, and then embedded in epoxy resin (TAAB Laboratories Equipment Ltd., UK) according to the Luft’s method. The mixing composition of epoxy resin was as follows; EPON 812 Resin (4.16 g), methyl nadic anhydride (6.02 g), dodecenyl succinic anhydride (1.90 g), and 2,4,6-tri(dimethylaminomethyl)phenol (0.15 g). 70 nm thick ultrathin sections containing the cross section of hair for TEM observation were prepared by using a diamond knife. The ultrathin sections were put on a formvar covered copper grid, and electronic stained with uranium acetate for 20 minutes and lead citrate for 3 minutes (U-Pb), then observed with TEM (HITACHI H-7000, 75 kV , Hitachi Ltd., Tokyo, Japan). Ultrathin sections of hair samples without OsO₄ fixation were also observed without U-Pb staining.

Several ultrathin cross sections of unfixed normal hair samples without any electronic staining were subjected to elemental qualitative analysis by EDS-TEM (JEM-2100, 200 kV equipped with JED-2300T, JEOL Ltd., Tokyo, Japan).

Results

A. Morphological observation of the cuticle and cortex

When the ultrathin sections of untreated normal hair fixed with OsO₄ for 2 hours were stained with U-Pb, as shown in Fig. 2, it was generally observed that the exocuticle was a uniform structure with low electronic density, while the endocuticle was a structure with substantially high electronic density. In addition, the electronic density of the A-layer was a little higher than the main part of the exocuticle, but the texture was similar to the exocuticle and no distinct contrast difference was observed between them. It was not possible to clearly distinguish between the A-layer and the epicuticle, or the endocuticle and the inner layer as shown in Fig. 1. In the cortex, the intermacrofibrillar matrix filled the space between the macrofibrils, and its electronic density in the untreated normal hair was slightly higher than the macrofibrils. Melanin granules were usually found in the intermacrofibrillar matrix in the case of the black hair, and were the highest electron dense structures in the hair. The cuticle CMC and the cortex CMC showed a slightly lower electronic density than the structures around them. In the white hair samples, melanin granules were very few or non existent, but the rest of their structures were basically the same as those of black hair.

The TEM images of the hair treated with lightener (Fig. 3A) or powder-bleach (Fig. 3B), which were fixed with OsO₄ for 2 hours and stained with U-Pb are shown. The electronic density of the A-layers in the cuticle was slightly increased in the hair treated with lightener compared to those in untreated normal hair. In the hair treated with powder-bleach, the electronic density of the endocuticle showed a greater increase than that seen in the lightener-treated hair in addition to the A-layer. However, the electronic density of the circular or amorphous structures in the endocuticle was not as high as that of the matrix around them, so it was thought that those structures in the endocuticle were accentuated as a result.

In addition, the electronic density of the intermacrofibrillar matrix in the cortex was increased in the lightener-treated hair and this made it easier to distinguish each macrofibril. This tendency was more exaggerated in the powder-bleach-treated hair, especially the intermacrofibrillar matrix around melanin granules became wider, and it was thought that some degradation products of melanin granules were included in them. On the other hand in the white hair treated with lightener (Fig. 4A) or powder-bleach (Fig. 4B), the electronic density of the A-layer and endocuticle was increased in the same way as in the black hair. However, because of the absence of melanin granules, the structural degradation or decomposition of melanin granules was not seen so that the structural changes seen in the bleached black hair were not observed in their intermacrofibrillar matrix.

In the lightener-treated hair, the electronic density of melanin granules was extremely high and their matrix was found to be homogeneous, and these characteristics were the same as the melanin granules in the untreated normal
Fig. 3. TEM micrographs of black hair treated with lightener (A) and powder-bleach (B), which have been fixed with OsO₄ and stained by the U-Pb procedure. The electronic density of the A-layer, endocuticle, and intermacrofibrillar matrix are higher, and then heterogeneous structures in the endocuticle are more clearly visible, especially in the hair treated with powder-bleach. In the powder-bleach-treated hair, low electron-dense portions have appeared in the melanin granules and substances from the decomposed melanin granules flow out into the intermacrofibrillar matrix. The intermacrofibrillar matrix around the melanin granules is wider and darker than that of the sections further away. The darker materials seem to be diffused from the decomposed melanin granules. Scale bar = 1 μm.

Fig. 4. TEM micrographs of white hair treated with lightener (A) and powder-bleach (B), which have been fixed with OsO₄ and stained by the U-Pb procedure. There are no melanin granules in the cortex. The intermacrofibrillar matrix is low electron dense and not wide compared with that of the lightener-treated and powder-bleach-treated black hair. Scale bar = 1 μm.
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On the other hand, some spots with low electronic density appeared in the melanin granules of the hair treated with powder-bleach and it would seem to indicate the decomposition of melanin granules. Taken together with the increase of the electronic density of the intermacrofibrillar matrix mentioned above, these results led us to imagine that the matrix substances of the melanin granules decomposed by hydrogen peroxide, an ingredient of the bleaching agent, defused to the intermacrofibrillar matrix around the melanin granules.

However in the untreated normal hair, the electronic density of the A-layer in the cuticle and the intermacrofibrillar matrix in the cortex was increased when OsO₄ fixation was prolonged from 2 to 4 hours, which made it easy to distinguish each macrofibril (Fig. 5). Such changes of the electronic density were similar to those observed in the lightener-treated hair or in the powder-bleach-treated hair, except for the melanin granules. The intermacrofibrillar matrix surrounding melanin granules, however, was not so notably widened.

**B. The morphological changes of melanin granules under different bleaching conditions**

In the morphological analysis for the melanin granules with TEM, the effects of OsO₄ fixation and electronic staining seems to be basically important because their matrix usually show high electronic density. Therefore the author compared the melanin granules of TEM images of several samples: hair fixed with OsO₄, ultrathin sections and stained with U-Pb (Os+Es images), and hair without the fixation and the staining (-Os-Es images) as shown in Fig. 6. The hair groups used for this purpose were untreated normal hair (A), lightener-treated hair (B), powder-bleach-treated hair (C), and 3 times powder-bleach-treated hair (D), respectively.

In the Os+Es images (Fig. 6 upper row), as mentioned previously, the matrix of the melanin granules found in the untreated normal hair and lightener-treated hair was homogeneous and highly electron-dense, while in the powder-bleach-treated hair, the matrix of melanin granules was considerably heterogeneous because of the development of low electron-dense portions. The electronic density of melanin granules in the 3 times powder-bleach-treated hair decreased further, and hole-like structures indicating the collapse of their matrix were seen.

On the other hand in the -Os-Es images (Fig. 6 lower row), the electronic density of melanin granules in the untreated normal hair was notably high compared to the macrofibrils around them, however many clear punctuations or depressions were seen in their slightly low electron-dense granular matrix compared to those seen in the Os+Es images.

In the -Os-Es images obtained from lightener-treated, powder-bleach-treated, and 3 times powder-bleach-treated hairs, the electronic density of the melanin granules was remarkably low, and fine granular and polymorphic structures appeared in their granular matrix. However, in the -Os-Es images of the 3 times powder-bleach-treated hair,
the fine punctations found in the -Os-Es images obtained from the untreated normal hair were not able to be distinguished because the electronic density of their granular matrix was extremely low. Further, the relation of these fine granular or polymorphic structures found in the granular matrix and clear punctations found in those of untreated normal hair was also unclear.

C. Elemental qualitative analysis for melanin granules

In the -Os-Es images, it is considered that the elements with a larger atomic number exist in many of the areas with higher electronic density. The main molecular component of hair is keratin, and its elemental component with the largest atomic number is sulfur. The electronic density of melanin granules in the -Os-Es images was clearly higher than that of the A-layer in the cuticle, which was considered to have extremely high sulfur content, so that the existence of metal-like materials with larger atomic number than sulfur were supposed. Therefore, a qualitative elemental analysis by EDS-TEM was performed on the A-layer, the melanin granules, and the macrofibrils (Fig. 7). In these organelles, the main elemental components of the keratin, such as carbon, oxygen, nitrogen, and sulfur were detected. On the other hand, some metal elements including aluminum, magnesium, potassium, calcium, ferrous, and zinc were detected in the melanin granules in addition to the main elemental components of keratin (Fig. 7B). Thus, it was considered that the high electronic density of melanin granules found in the -Os-Es images would be reflecting the existing metal elements.

Discussion

A. Change of the electronic density caused by bleach relating to changes of the hair permeability and reactivity

The A-layer has a high cystine content and a strong chemically resistant component, and the endocuticle has a low cystine content and a hydrophilic component. The electronic density of the A-layer is increased but that of the endocuticle is decreased with cold permanent wave treatment, which contains thioglycolic acid as a reducing ingredient, and it is thought that OsO₄ reacts with the cleaved disulfide bond of cystine through the action of the reducing agent. On the other hand, in the Os+Es images of this study, the electronic density of both A-layer and endocuticle was increased in the bleached hair (Fig. 3 and 4), so that it is difficult to consider that the electronic density only in the structural components with high contents of cystine will increase. In fact, Rogers et al. report that...
OsO₄ reacts with not only amino side chains containing sulfur but also other amino side chains.

Compared to untreated normal hair, bleached hair absorbs moisture more easily and is also more permeable to dyes⁹. In addition, not only was an increase of cysteic acid caused by the oxidative cleavage of the disulfide bond found, but also the generation of amino acid residues such as tyrosine, threonine, methionine, lysine, and histidine in the bleached hair was observed¹¹,¹². From these facts, the electronic density of the A-layer and the endocuticle in the untreated normal hair was increased by prolongation of the fixation time from 2 to 4 hours (Fig. 4), like those in the hair treated with lightener or powder-bleach agents which were fixed for 2 hours with OsO₄, suggest that the permeability of OsO₄ will increase or the reactivity of amino residues of the hair will increase, or both will occur. As a result, it is considered that the electronic density of the A-layer and the endocuticle in the bleached hair is increased in comparison with those in the untreated normal hair.

In the Os+Es images, the electronic density of the endocuticle parenchyma in the powder-bleach-treated hair was increased, which made it possible to observe more clearly the circular or polymorphic structures in the
endocuticle (Fig. 3). This result corresponds to the report of Kawasoe et al.\textsuperscript{13}. Arguably, those irregular structures in the endocuticle seem to derive from the cell organella including mitochondria, nuclei, and etc., which are susceptible to the powder-bleach agent, and as a result they seem to become more easily stainable.

When comparing lightener-treated or powder-bleach-treated black hairs (Fig. 3) and white hairs treated in the same way (Fig. 4), less changes were found in the white hair than the black hair, because the area of the intermacrofibrillar matrix had not widened like that of the black hair. Therefore, it seems that the spread of the intermacrofibrillar matrix around the melanin granules in the black hair was larger than that seen in the white hair mainly due to those substances that flowed out from the decomposed melanin granules.

On the other hand, it is considered that increasing the permeability and reactivity of the active ingredients such as hydrogen peroxide in the bleaching process resulted in the weakening of hair and thus led to hair damage. Concretely, it is thought that there arise physical changes such as decreasing the tensile strength of hair fibers\textsuperscript{14} and increasing the friction on hair surfaces\textsuperscript{15}, and some chemical changes such as decreasing 18-methyleicosanoic acid\textsuperscript{16} which is a specific hair lipid, and increasing cysteic acid by the oxidative cleavage of the disulfide bond of cystine\textsuperscript{17}.

B. Metal elements in the melanin granules and their relation to bleach

Melanin granules have the property of being able to coordinate metal elements\textsuperscript{17}, and actually metal elements have been detected in the melanin of the iris of a human by EDS-TEM\textsuperscript{18}. It became clear in this work that high concentrations of metal elements existed in the melanin granules of human black hair, but less than the detection limit in other areas such as the cortex (Fig. 7). It was considered that the high electronic density of the melanin granules even in the -Os-Es image of the untreated normal hair was due to the influence of metal elements naturally present (Fig. 6). Wolfram et al.\textsuperscript{19} supposes that the melanin granules react more easily with hydrogen peroxide than the other hair proteins, because the decomposition speed of hydrogen peroxide solution with brown hair is faster than with white hair. When purified \textit{sepia} melanin without metal elements is added to an alkaline hydrogen peroxide solution, the reaction is very mild. However adding \textit{sepia} melanin coordinated transition metal elements to the solution gives a very intense reaction with effervescence and the \textit{sepia} is quickly bleached (unpublished data). Taken together with the results in this study, it is considered that the metal elements in the melanin granules act as a decomposition catalyst for hydrogen peroxide and this is a powerful reason for the suppositions of Wolfram et al.\textsuperscript{19}. These metal elements will be released from the melanin granules when they are decomposed by the action of bleach. This is supported by the fact that, in the -Os-Es images obtained from the powder-bleach-treated hair in this study, the electronic density of melanin granules was extremely low.

According to naked eye observations, the color of hair subjected to lightener treatment changes from black to brown, and from black to light brown with powder-bleach treatment. Under electron microscopic observation however, no remarkable changes in the melanin granules were observed in the lightener-treated hair, or even in the powder-bleach-treated hair, when compared to untreated normal hair. Very little degradation of melanin granules was observed and moreover the electronic density of the granules was still high in the Os+Es images in both the lightener-treated and the powder-bleach-treated hair. This shows that the changes of stainability for the electronic staining agents are unexpectedly small between the untreated normal hair and both types of bleached hair. In this case, the remaining brown color in the hair showed that at least, a part of those structural components determining the hair color still remained in the melanin granules. Further, the electronic density of melanin granules is very high in the Os+Es images, but in the -Os-Es images it had lowered considerably. This shows that the metals coordinating with melanin granules are lost, while the ability to coordinate metals of melanin granules still remains. The melanin granular parenchyma is composed of fine particulate structures\textsuperscript{20}, and these fine particulates still remain after bleaching with hydrogen peroxide solution\textsuperscript{21}. This suggests that the molecular frames of melanin granules are still able to appreciably coordinate metals after bleaching.

On the other hand, in the 3 times powder-bleach-treated hair, the electronic density of melanin granules in the Os+Es images as well as in the -Os-Es images was extremely low (Fig. 6D), and this, being especially so in the Os+Es images, shows that the melanin granules have almost lost their ability to coordinate metals.

Moreover, the fine clear punctations or particulate structures in melanin granules were possible to observe because the electronic density was a little lower in the -Os-Es images than in the Os+Es images. This phenomenon shows that taking advantage of the electronic density due to the metals naturally existing in the melanin granules is favorable for the observation of the structural changes based on their degraded state after bleaching and without OsO\textsubscript{4} fixation and electronic staining.

C. Mechanism of melanin decomposition by bleaching agents and problems in the future

The two phenomena seen to occur in the decomposition of the melanin granules caused by the bleaching agents are the solubilization of the melanin granules and the decomposition of the chromospheres of the melanin molecule\textsuperscript{22}. Consequently, the following processes are considered in relation to the decomposition mechanism of the melanin granules by the bleaching agent.
Step 1: A part of the melanin granule is soluble.
Step 2: Chromosomes of the solubilized melanin molecules are decomposed and the hair color becomes lighter. Most of the metal elements coordinated with the melanin molecules are released, but most of the molecular frames remain and they are able to coordinate with the metal elements during fixation or electronic staining.
Step 3: The molecular frames of melanin granules are decomposed and no longer able to coordinate with metal elements in electronic staining.

Therefore in the conventional use of lightener or powder-bleach agents, Step 1 and Step 2 will account for changes to the hair color from black to brown or light brown, and all steps up to Step 3 must have occurred to change the hair color from black to pale yellow by the repeated use of the powder-bleach agent.

In this context, the electronic density of the melanin granules in the untreated normal black hair is higher than in the bleached hair even in the Os-Es image, and this suggests that some metal elements naturally coordinate to the melanin granules. In the hair treated with bleaching agents, however, these metal elements coordinating to the melanin granules flow out during the bleaching process and become a catalyst for hydrogen peroxide in other areas than the melanin granules, and it is considered to promote a vicious spiral of increasing hair damage and the consequent augmentation of permeability and reactivity of the hair. This phenomenon is supported by the result that the cleavage of disulfide bonds in the cortex of white hair by bleaching with powder-bleach agent is lower compared to black hair. Therefore, further study will lead us to the edge of the development of a new bleaching agent, which reacts only with melanin granules and causes the minimum of damage to outside the melanin granules.

Acknowledgement

The author would like to thank Dr Takashi Nakano and Dr Masaru Kimura (Department of Anatomy, Aichi Medical University School of Medicine) for instruction, advice, thoughtful discussion, and critical reading of the manuscript. The author thanks Kazuo Kato and Masanao Niwa (Hoyu Corporation) for the opportunity to provide this research.

References
1) Swift JA: Morphology and histochemistry of human hair. EXS 1997; 78:149–175.