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Review

Hair keratin film as a substitute device for human hair

- Application in hair care science -

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Keratin film prepared from human hair samples can be considered a model which represents an average human hair. In this study, the investigations were focused on whether the keratin film can withstand physical and chemical stimulations such as ultraviolet (UV), bleach, perm, and thermal treatments or not. Obtained data indicated that keratin film responded to such hair damage factors in the similar manner as hair, and responses of keratin film were significantly higher than those of hair. Keratin film can be used as a substitute device for human hair, to accurately evaluate hair damage for the development of hair care related products.

Keywords: Hair damage, keratin film, UV, bleach, perm, heat

Introduction

Human hair is a physiological tissue, which is a specialized part of the skin. This tissue helps to maintain bodily temperature in heat or cold, and it also protects our body from outside harm. Hair that adorns our head continues to be one of the objects of interest for many people, because it is a part of their fashion or anti-aging tool.

The water content of human body should be more than 50%, whereas that of

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hair tissue is less than 20% and 70 to 80% of it consists of proteins. This configuration is made of 3 layers: from out to in, cuticles, cortex, and medulla (Fig. 1). The major components of proteins are keratin (40 to 65 kDa) and keratin associated proteins (KAPs; 6 to 30 kDa). Keratin, which possesses the similar fibrous proteins to collagen and microtubules, forms intermediate filaments that are 10 nm in diameter. They gather systematically on the line of long axis and construct macrofibril and cortex. KAPs function as glue like substance which fills the spaces among microfibrils. The strength and flexibility of hair depends on the multi-layered structure of keratin based fibers (1).

In the development of hair care products, accurate evaluation of hair damage is essential for examining the benefits of pharmaceuticals or devices used. However, I and collaborators think that the research and

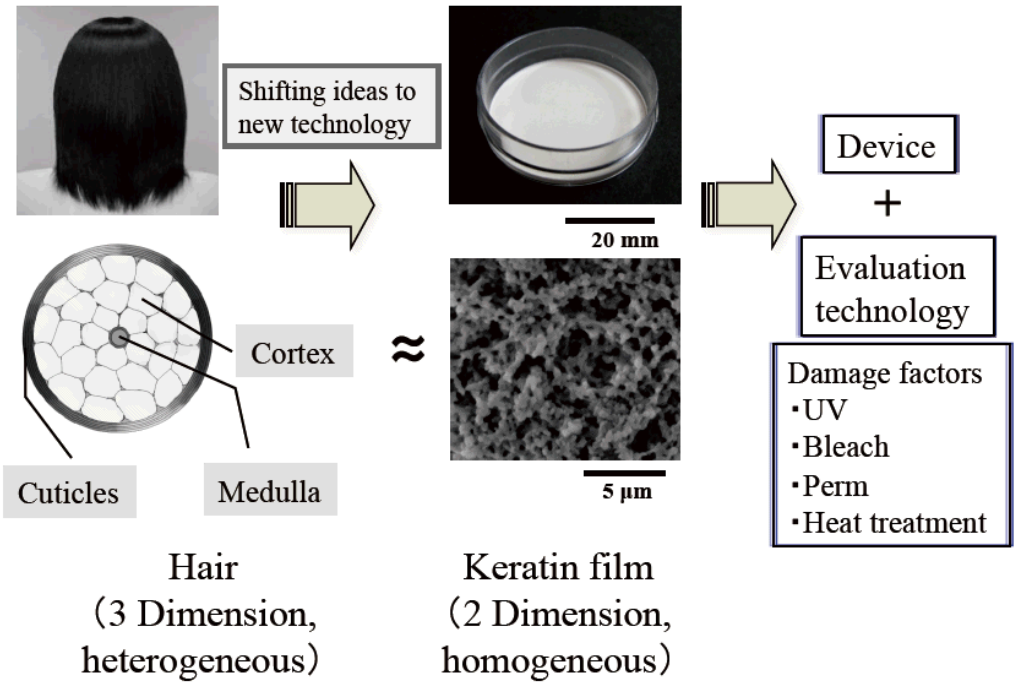

Fig. 1 Fine structures of hair and keratin film and new hair damage evaluation

development have been difficult due to the following problems.

1. Since the experimental material is hair itself, not only the individual differences, but also varying conditions even between roots and end tips within a piece of hair causes samples to be heterogeneous. Therefore, a lot of experiments are needed in order to evaluate hair damages accurately.
2. As shown in Table 1, hair damages are evaluated from various approaches. At present, most of evaluation methods are based on qualitative ways such as the sense of sight and morphological observations. Thus, they might not be suitable for attaining objective viewpoints or quantitative data.
3. As shown in Fig. 1, the analysis of cortex which determines the nature of hair is rather difficult, because it is covered by hard cuticles.
4. The changes in mechanical strength happen at last stage of hair damage and the reproducibility of data is poor.

Table 1 Problems in hair damage evaluation

Methods of damage evaluation
・Senses (Visual or tactile perception)
・Observation of hair configuration
・ Mechanical characteristics (Tensile strength)
・Change in protein conformation
・Carbonylation
・Cysteic acid
・Protein elution

To solve these problems, a novel method has been invoked to estimate accurate hair damage. If the three dimensional and heterogeneous hair samples could be turned into two dimensional and homogeneous film samples, this could be used as a new substitute device for hair, in the field of hair science including hair damage analysis (Fig. 1). Keratin film has been developed and proposed as such new device.

In this review, effects of major hair damage factors such as UV, bleach, perm, and heat on the keratin films are introduced, in comparison to those on hair samples.

Solubilization of proteins from hair and formation of keratin film

We have developed protein extraction method from hair called “Shindai Method” (2, 3). This method is effective on tissues which include hard keratin, such as hair, nails, and wool. Obtaining keratin (hair proteins) solution with little protein degradation is possible in a short time. By using hair without any past history of chemical treatments such as hair coloring or perms, proteins were solubilized by Shindai method.

Hair keratin is a major component of cytoskeletal proteins, which belong to intermediate filaments with a diameter of around 10 nm (4, 5). Intermediate proteins containing keratins retain self-assembly ability even after the depolymerization using denaturant. By changing conditions of the solution, it is possible to induce repolymerization and remold original filaments. Keratin solution, with different denaturant combinations or with reduced pH, would induce aggregate and mold it

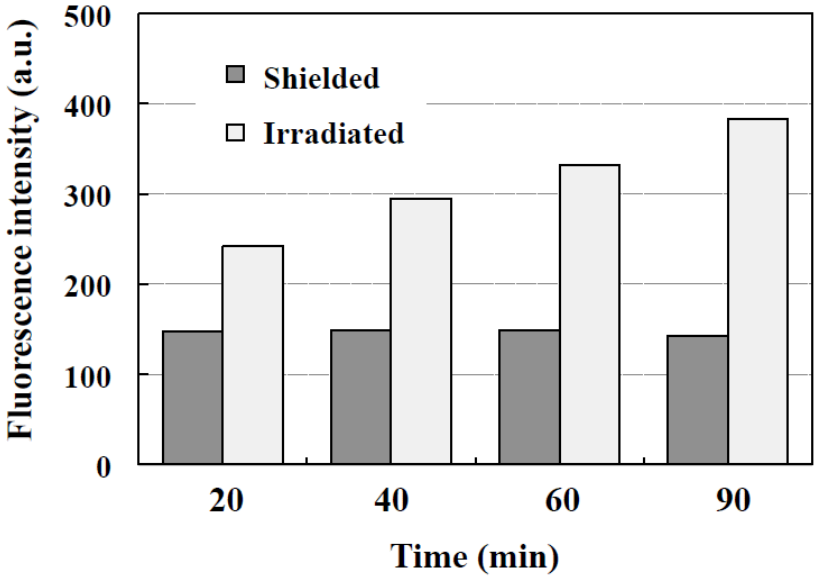

Fig. 2 Fluorescence intensity of keratin film after artificial sunlight irradiation

into a form of film. These methods according to the varying combinations and conditions are named Pre-cast method, Post-cast method, and Soft-post-cast method (6, 7). When solubilizing these films again by Shindai method, the films were found to be consisted mainly of keratin, low molecular weight keratin associated proteins (KAPs), and high molecular weight proteins.

In this study, keratin films prepared in the petri dishes by Pre-cast method were used. These films had smooth surface and white to light beige color in appearance. According to the SEM observation of the keratin film, particles less than 1 μm in diameter in a form of filaments were observed, which constructed irregular and reticular structure around 5 μm in diameter by gathering together (Fig. 1). It has become evident that the keratin films can be used for different types of experiments related with hair damage.

UV

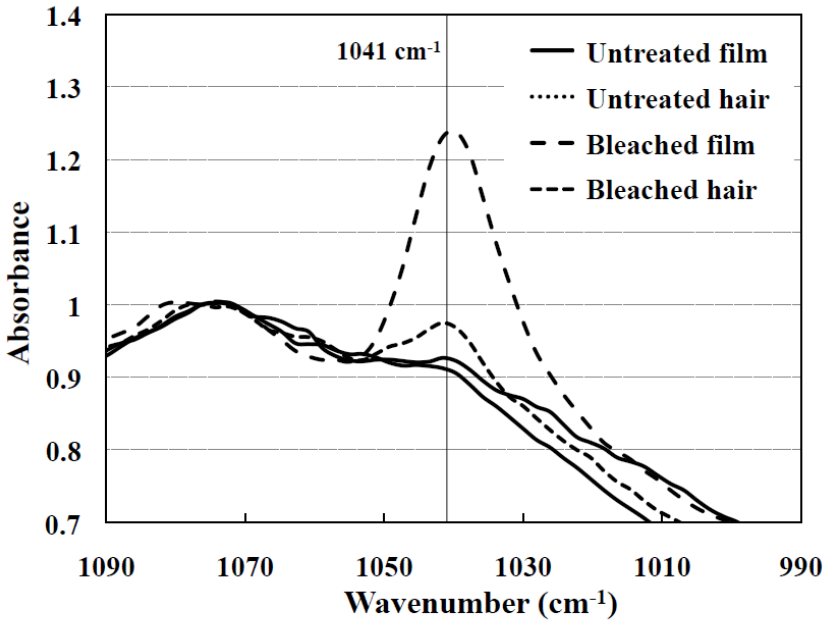

Fig. 3 FT-IR spectra of keratin film and hair after bleach treatment

Carbonyl groups which are generated by UV irradiation in the skin are measured by fluorescent reagent (fluorescein-5-thiosemicarbazide), which bonds itself to carbonyl groups specifically (8). When this method was applied to the keratin films, the fluorescence intensity increased in proportion to irradiation time (Fig. 2). Thus, in keratin films, the formation of oxidized proteins depended on the amount of UV irradiation.

When hair samples were irradiated and observed by fluorescence microscopy, the fluorescence intensity also increased proportionally to UV irradiation time (9, 10). When comparing films with hair samples, it was demonstrated, that the films showed 5-10 times more of sensitivity than hair samples. By applying this advantage, we succeeded in detecting carbonylated proteins which were generated by light irradiation which was almost equal to 30 min of mid-summer sun exposure. Compared to

hair samples, keratin films were more effective in reproducibility of data and more convenient in handling during experiments.

4. Bleach

It is known that when human hair is treated by bleaching agent, disulfide bond is oxidized and forms cysteic acid (11, 12). Thus, the formation of cysteic acid was investigated using FT-IR (Fourier transform infrared spectroscopy) (Fig. 3). Cysteic acid was not detected in untreated hair or keratin film however, when treating hair and film by hydrogen peroxide, which was commonly used in the bleaching agent. The peak appeared around 1041 cm^{-1}, indicating the presence of cysteic acid. Keratin film exhibited 5-10 times more of sensitivity in detection, compared to hair sample (13). Spectra of the film and hair sample

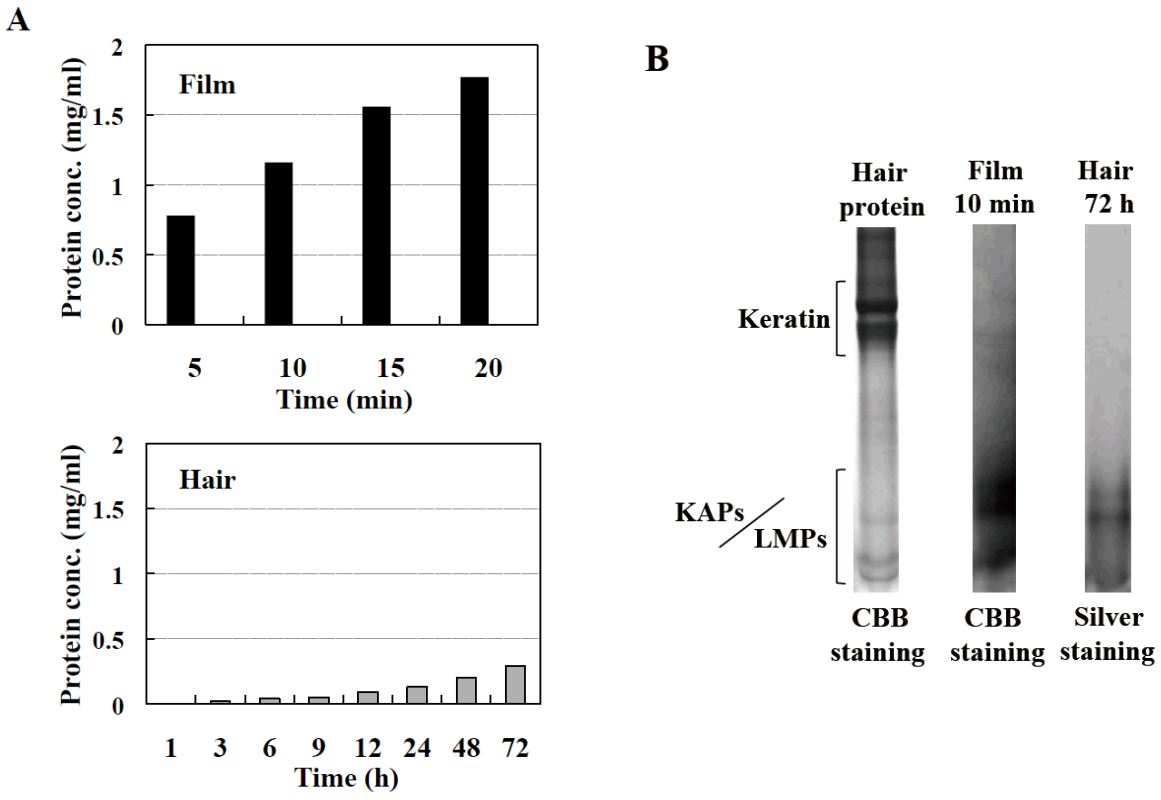

Fig. 4 Protein elution from keratin film and hair after TGA treatment (A) and analysis of Tricine/SDS-PAGE (B)

showed similarity (data not shown), suggesting one of the proofs for keratin film to be a substitute device for hair.

Perm

Permanent wave treatment consists of two steps. The first step cuts plentiful S-S bonds among keratin filaments by reductive treatment and produces -SH group. After setting up a hair style, the second step recombines -SH group by oxidative treatment in order to fix such hair style.

It has been reported that when treating hair with thioglycolic acid (TGA), which is commonly used in reductive permanent reagent, low molecular weight proteins such as ubiquitin and A100 are eluted from hair samples, while keratin is not (14). This is considered to be one of the indicators of hair damage, since irreversible change is generated inside hair (in cortex portion). Interestingly, the process of elution occurred 2,000 times faster in keratin films compared to that of hair samples, thus it only took 5 min for

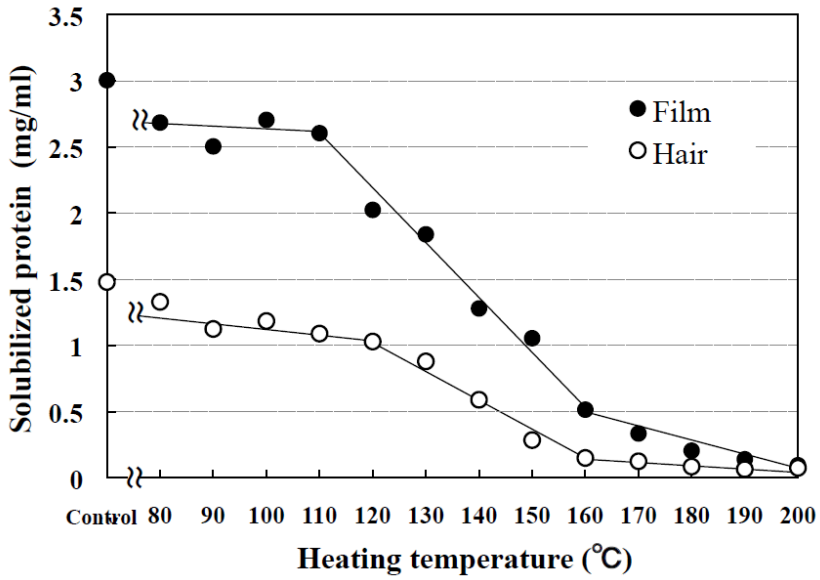

Fig. 5 Effect of heating time on the protein elution from untreated and heat-treated keratin film and hair

keratin films to detect changes which took 2-3 days for hair to detect (Fig. 4A) (15, 16). When using keratin films, the similar elution of low molecular proteins was observed as it was observed in hair samples (Fig. 4B).

Heat

Hair damage due to thermal treatments has been researched, since hair dryers and hair irons are frequently used to dry hair or to set up hair styles. Most thermal damage measurements are done by observing morphology of cuticles, which has become qualitative evaluation method (17).

Keratin films were able to withstand thermal treatment of up to 200°C, and when treated by heat more than 180°C for 10 min, white to light beige colored keratin films turned to light yellow and then to light brown. Such observed color changes were similar to those of Japanese gray hair samples under the same treatment (data not shown).

Mechanical strength of hair is known to increase by thermal treatment; thus cross-linkage among proteins in the cortex portion of hair was presumed to be generated. In order to investigate the

Table 2 Summary of treatment responses between keratin film and hair

Damage Factors	Measured objects	Keratin film	Hair
UV	Carbonylation	◎	○
Bleach (H_2O_2)	Carbonylation	◎	○
	Cysteic acid	◎	○
Perm (TGA)	Transparency	◎	?
	Solubility	◎	○
Temperature (180℃～)	Color difference	◎	○
Temperature (100～180℃)	Solubility	◎	○

○, detection ; ◎, sensitive and/or convenient detection

amount of solubilized proteins after the thermal treatment of 80-200°C for 10 min, solution containing reductive reagent and denaturant was used to incubate keratin films and hair samples at 50°C for 2 h (Fig. 5). The amount of solubilized proteins from keratin films and hair samples both decreased linearly in a temperature-dependent manner. Compared to hair samples, this result was detected twice as high in keratin films.

Summary

Keratin films were able to withstand physical and chemical stimulations such as UV, bleach, perm, and thermal treatments. In order to develop hair care related products, accurate hair damage evaluation method is essential. The effects from different hair damage factors to keratin films and hair samples have been summarized in Table 2 with investigated results. Then, the characteristics of keratin films are compared to hair, from a perspective of keratin film as a device.

1. A number of responses observed in hair samples were also detected in keratin film.
2. Compared to hair samples, responses in keratin film were significantly high; 10 times as high in UV and bleach treatment, 2,000 times as high in perm reductive treatment, and twice as high in thermal treatment.
3. In regard to each hair damage factor, reproducibility of data from homogeneous keratin film was excellent.
4. Keratin film prepared in petri dishes was easier to handle compared to hair samples.
5. Since experiments using keratin film can be carried out in short

amount of time, operational efficiency was improved.

Keratin film prepared from numerous hair samples can be considered a model which represents an average human hair. Since highly sensitive and reproducible experimental system can be constructed, keratin film is considered suitable for the efficient screenings of many chemical and natural substances. Production system and quality management of keratin film have progressed, allowing keratin film as the commercial item and keratin film-related trust examination on contract through Shinshu TLO since 2010 (18). It is our hope that the role we have in the development of hair care products through a number of collaborative relationships will ultimately contribute to the wellness of society.

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References

1. **Robbins, C. R.**: Chemical and physical behavior of human hair, 4th ed., 25-52 (2002).
2. **Nakamura, A., Arimoto, M., Takeuchi, K., and Fujii, T.**: A rapid extraction procedure of human hair proteins and identification of phosphorylated species. Biol. Pharm. Bull., **25** (5), 569-572 (2002).
3. **Kobayashi, S. and Fujii, T.**: Preparation and properties of individual protein products. Current

Topics in Biochemical Research, **9** (1), 29-37 (2007).

4. **Langbein, L., Rogers, M. A., Winter, H., Praetzel, S., Beckhaus, U., Rackwitz, H. R., and Schweizer, J.**: The catalog of human hair keratins. I. Expression of the nine type I members in the hair follicle. J. Biol. Chem., **274**(28), 19874-84 (1999).
5. **Steinert, P. M. and Roop, D. R.**: Molecular and cellular biology of intermediate filaments. Annu. Rev. Biochem., **57**, 593-625 (1988).
6. **Fujii, T., Ogiwara, D., and Arimoto, M.**: Convenient procedures for human hair protein films and properties of alkaline phosphatase incorporated in the film. Biol. Pharm. Bull., **27**(1), 89-93 (2004).
7. **Fujii, T. and Ide, Y.**: Preparation of translucent and flexible human hair protein films and their properties. Biol. Pharm. Bull., **27**(9), 1433-1436 (2004).
8. **Fujita, H., Hirao, T., and Takahashi, M.**: A simple and non-invasive visualization for assessment of carbonylated protein in the stratum corneum. Skin Research and Technology, **13**, 84-90 (2007).
9. **Kawasoe, T., Watanabe, T. and Fujii, T.**: Visualization of modified human hair by artificial sunlight with carbonylated proteins as an indicator of hair damage. J. Jpn. Cosmet. Sci. Soc., **34**(4), 287-291 (2010).
10. **Kawasoe, T. Watanabe, T., and Fujii, T.**: A novel method using a keratin film for quantifying the photo-modification of hair proteins. J. Soc. Cosmet. Chem. Jpn., **45**(2), 100-107 (2011).
11. **Leszek, J. W., Hall, K., and Hui, I.**: The mechanism of hair bleaching. J.

Soc. Cosmet. Chem., **21**, 875-900 (1970).

12. **Signori, V. and Lewis, D. M.**: FTIR investigation of the damage produced on human hair by weathering and bleaching processes: implementation of different sampling techniques and data processing. Int., J. Cosmet. Sci., **19**, 1-13 (1997).
13. **Fujii, T., Ito, Y., Watanabe, T., and Kawasoe, T.**: Effects of oxidative treatments on human hair keratin films. J. Cosmet. Sci., in press.
14. **Inoue, T., Ito, M., and Kizawa, K.**: Labile proteins accumulated in damaged hair upon permanent waving and bleaching treatments. J. Cosmet. Sci., **53**, 337-344 (2002).
15. **Fujii, T.**: Evaluation of damage caused by perm treatment using the hair keratin films. Fragrance J., **7**, 46-52 (2011).
16. **Kawasoe, T., Takayama, S. Ito, Y. and Fujii, T.**: Effects of reductive and/or oxidative treatment during permanent wave procedure on human hair keratin films. J. Jpn. Cosmet. Sci. Soc., **35**(4), 306-311 (2011).
17. **Ruetsch, S. B., and Kamath, Y. K.**: Effects of thermal treatments with a curling iron on hair fiber. J. Cosmet. Sci., **55**, 13-27 (2004).
18. Shinshu TLO homepage address: http://www.shinshu-tlo.co.jp/topics/, SIS., Ltd. homepage address: http://www.sishp.com/.

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