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News Release

**Milbon Develops Technology Enabling Shape Memory at
the Molecular Level of the Beautiful Condition of Hair
Immediately after Coloring
-Cross-linking of Hair Proteins Using Plant Components-**

Milbon Co., Ltd. (President and CEO: Ryuji Sato) has developed a new haircare technology that, when applied immediately after hair coloring, can memorize the shape of the hair at the protein molecular level and maintain that condition. This technology uses plant phenolic compounds^{*1} to semi-permanently bind hair protein molecules together.

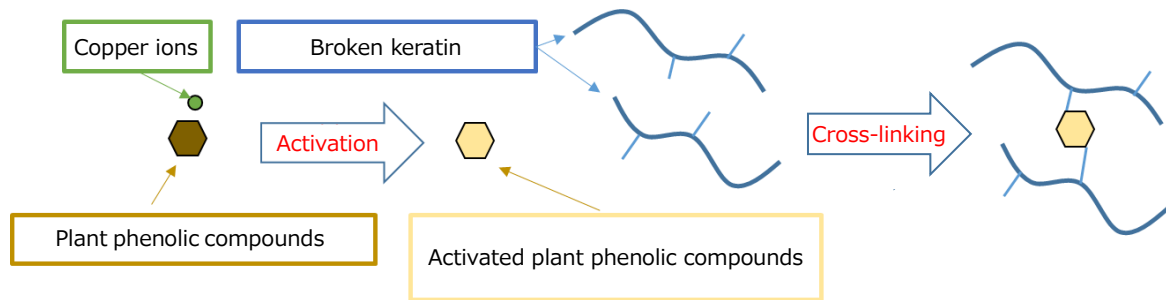
The research was advanced in cooperation with the Osaka Research Institute of Industrial Science and Technology (ORIST)^{*2}, and the results were presented at a Research Presentation Meeting of The Textile Machinery Society of Japan held on November 10, 2020.

[Key Points of the Technology]

Approximately 85% of hair is composed of a particular protein called keratin. When hair coloring is performed at a salon, the hair becomes very glossy and beautifully shaped immediately after the treatment. However, repeated hair washing afterwards gradually distorts the shape of the hair, leading to hair breakage and split ends. This is caused by the keratin damaged by the hair coloring leaching from the hair on repeated hair washing.

As hair is made up of dead cells, it is difficult to repair damaged keratin. Thus conventional haircare method has only provided temporary enhancement in the appearance and texture of the hair by applying repair ingredients. However, this method has failed to fundamentally prevent the leaching of keratin, which had remained a major challenge.

Therefore, Milbon focused on technology for improving the firmness of gelatin protein by binding it with phenolic compounds which had been developed in the food industry, and worked on research to apply it to keratin repair. We examined about 50 types of plant phenolic compounds, such as polyphenols, which have a track record in the cosmetics field and are useful for living organisms due to their antioxidant effects. Our findings revealed that chicoric acid contained in the Echinacea purpurea plant^{*3} had the greatest effect. When chicoric acid is activated by copper ions and applied to the hair immediately after hair coloring, damaged keratin proteins are bound together (cross-linked) resulting in a larger molecular weight. Consequently, it became possible to prevent leaching of the protein during subsequent hair washing, and to maintain the beautiful condition of the hair immediately after hair coloring.



[Future Vision]

Milbon will progressively apply this technology to its haircare products, including hair coloring-compatible products to be launched next spring.

[Supplementary Information 1: Why were the proteins cross-linked by the plant phenolic compounds and copper ions?]

When insects hatch, their soft epidermis hardens. This phenomenon is called quinone hardening and occurs due to proteins binding to each other and cross-linking. Biological research has shown that phenolic compounds and enzymes as their active components are involved in this reaction. This phenomenon has already been applied in the food industry with the development of techniques to improve the firmness of gelatin proteins by using copper ions instead of enzymes. Milbon decided to apply this technology to repair damaged hair.

When proteins bind to each other, their molecular weight increases. Therefore, in a model experiment using an aqueous solution of keratin, we searched for components that increase the molecular weight of the proteins, using the SDS-PAGE technique^{*4}. As a result, it was confirmed that when the keratin solution was treated with chicoric acid and copper chlorophyllin^{*5} or with Echinacea purpurea extract and copper chlorophyllin, the color of the band around about 40kDa^{*6} became lighter (Figure 1).

It was also visually confirmed that, although the aqueous solution of keratins was clear, these treatments caused the solution to become colored (Figure 2).

These results suggest that keratin with a size of about 40kDa was cross-linked to become a higher molecular weight, most of which precipitated and colored the keratin solution.

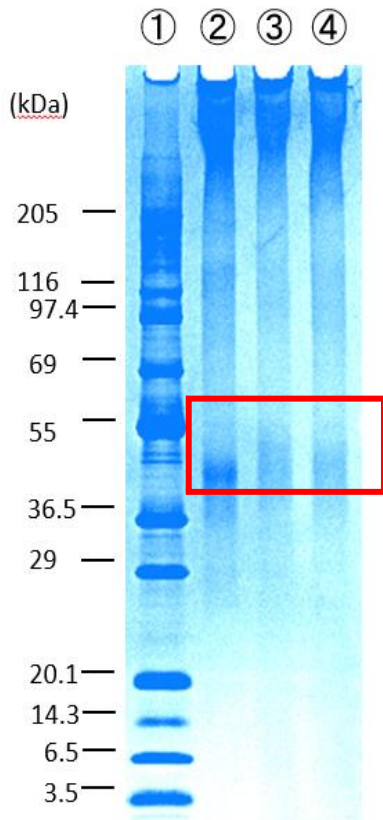


Figure 1. Keratin protein SDS-PAGE results

Color of the band around 40kDa became lighter in ③ and ④ compared to ②.

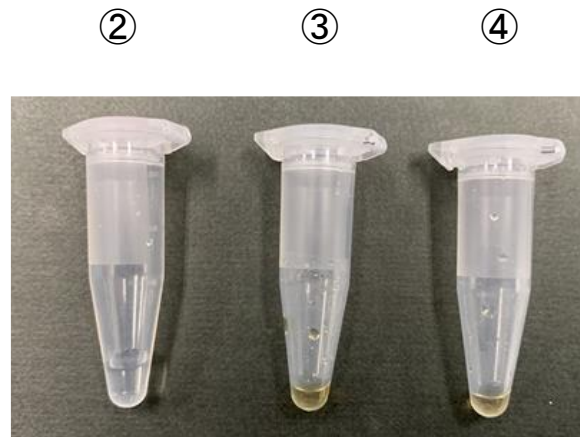


Figure 2. External view of each solution

② is clear, but ③ and ④ are colored

* ①: Molecular weight marker^{*7}

②: Keratin

③: Keratin treated with chicoric acid and copper chlorophyllin

④: Keratin treated with Echinacea purpurea and copper chlorophyllin

[Supplementary Information 2: Verification results with human hair]

1. Increased hair strength

To confirm that the technology is actually effective on human hair, we treated damaged hair with Echinacea purpurea extract and copper chlorophyllin and measured its strength in a tensile test. The results showed that the strength increased significantly together with the concentration of Echinacea purpurea (Figure 3).

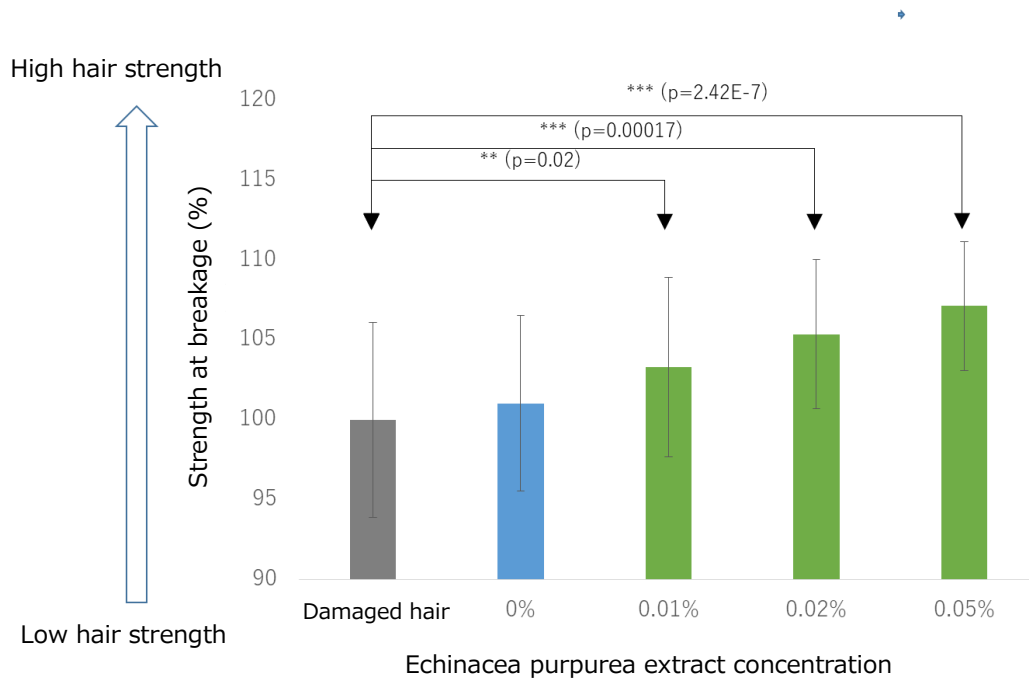
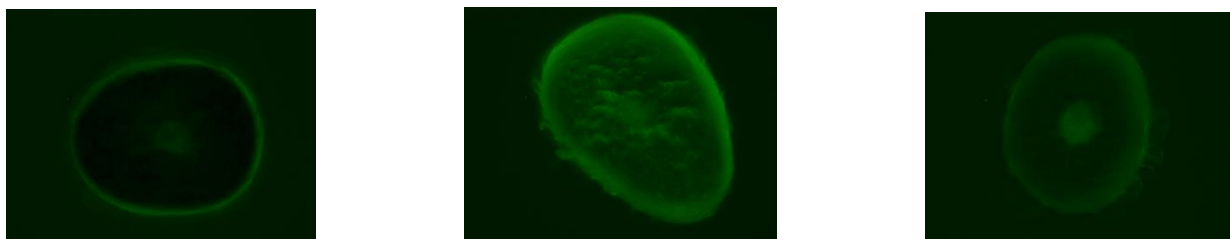


Figure 3. Results of tensile strength testing of hair

2. Confirmation of repair of damaged hair areas

Cross-sectional observation of the hair was conducted by staining with a fluorescent dye that reacts to damaged areas of hair. The cross section of undamaged and untreated hair hardly showed fluorescence. Damaged hair fluoresced strongly, but when it was treated with Echinacea purpurea extract and copper chlorophyllin, the fluorescence intensity weakened, confirming that the damaged areas had been repaired (Figure 4).



Untreated hair

Damaged

Treated hair

Figure 4. Results of fluorescence

[Notes]

***1 Phenolic compounds**

“Phenolic compound” is a general term for substances with a hydroxyl group (-OH) on the benzene ring. Those with multiple hydroxyl groups in a molecule are called polyphenols.

***2 Osaka Research Institute of Industrial Science and Technology (ORIST)**

ORIST is a local incorporated administrative agency that aims to contribute to economic and industrial development and improve the lives of residents, as a center of support for knowledge and technology that supports industrial technology and manufacturing. It conducts testing, research, and other support related to industrial technology while promoting the dissemination and practical application of the results of such research.

***3 Echinacea purpurea**

A perennial herb of the genus *Echinacea purpurea* of the Asteraceae family.



***4 SDS-PAGE technique**

A method of protein analysis that separates proteins by molecular weight.

***5 Copper chlorophyllin**

A compound derived from chlorophyll. Also used as a coloring agent in food and medicines.

***6 Da**

The unit “dalton” refers to the size of molecules and means the molecular mass. “40kDa” refers to 40,000 Da.

***7 Molecular weight marker**

A mixture of proteins of known molecular weight used to evaluate the relative molecular sizes of proteins.

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