Novel hair coloring system using eumelanin precursor, DHI (5,6-Dihydroxyindole) based on Hair Science

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Abstract

The hair coloring agents using oxidative dyes have high dyeability and wide coloring variation, however, there are problems with hair damage and allergic symptoms because of using in combination with hydrogen peroxide and alkaline agents. Therefore, we propose a novel hair dye using DHI (5,6-dihydroxyindole), a precursor of eumelanin, which is the origin of black hair, to ensure high safety. Firstly, we found that treating hair with a cationic polymer under alkaline conditions (pH 10), which are optimal for DHI coloration, increased dyeing efficiency by raising the zeta potential on the hair surface. Additionally, by staining the hair with a cationic fluorescent dye and using confocal fluorescence microscopy to calculate the integrated brightness value of the hair surface, we confirmed that the brightness significantly decreased after cationic polymer treatment, indicating that the polymer adsorbed onto the hair surface electrically. Next, we successfully achieved a range of colors from black to light brown by combining DHI with basic dyes/HC dyes. Therefore, we developed and report a novel hair coloring system with high safety, excellent dyeing properties, and color variation by washing with shampoo containing specific cationic polymers and then using a hair dye containing DHI and basic/HC dyes.

Keywords: DHI (5,6-Dihydroxyindole); dyeability; color variation; zeta potential; hair coloring system

Introduction.

People tend to think, "I want to stay young and healthy forever" and "I want to be seen as younger than my actual age." Hair dye is one of the means to maintain a youthful appearance, and it is used by many people regardless of gender. Oxidative hair dye is divided into two agents: Agent I (oxidative dye intermediate and alkaline agent) and Agent II (hydrogen peroxide). By mixing Agent I and Agent II and applying the mixture to the hair, it is possible to both bleach the melanin pigment in the hair and color it with the coloring compounds produced by the oxidative polymerization reaction. Hair color using oxidative hair dye has high dyeability, long-lasting color, and the ability to create a wide range of bright colors. On the other hand, the use of hydrogen peroxide and alkaline agents can damage the scalp and hair, induce the death of melanocyte stem cells, and sometimes cause allergic reactions [1, 2]. Against this background, we focused on melanin, which constitutes the color of human hair, and explored a new hair coloring technique that involves dyeing gray hair with 5, 6-dihydroxyindole, a precursor of eumelanin. There are two types of melanin: black-brown eumelanin and yellow-red pheomelanin. Eumelanin is biosynthesized from tyrosine via dopa and dopaquinone to produce DHI (5,6-dihydroxyindole), which undergoes auto-oxidation [3]. On the other hand, cysteinyl dopa is produced when cysteine reacts with dopaquinone, leading to the biosynthesis of pheomelanin through auto-oxidation. It is known that the color of human hair is determined by the balance of these eumelanin and pheomelanin [4, 5]. Although DHI can now be supplied stably, it has low dyeability and can only dye black, posing a challenge for achieving a variety of colors. Additionally, the stable supply of cysteinyl dopa remains an issue. Therefore, this study aims to establish a new hair coloring system with high dyeability in the hair color range of eumelanin, from black to light brown, using DHI (5,6-dihydroxyindole) as the precursor of eumelanin. Given that DHI develops color under alkaline conditions around pH 10, it is assumed that the negative charge on the hair surface and DHI will create an electrostatic repulsion, making it difficult for DHI to penetrate the hair. To address this, we pretreated the hair with a cationic polymer to change the surface charge of the hair to positive before dyeing it with DHI, aiming to improve dyeability. Additionally, we dyed the hair with ionic fluorescent dyes and used fluorescence microscopy to microscopically evaluate the charge state of the hair surface. We then combined DHI with various dyes to target a range of colors from black to light brown. By applying hair colorant combinations of DHI and various dyes to hair pretreated with cationic polymers, we evaluated the dyeability and variety of colors. Furthermore, we evaluated dyeability and variety by applying hair colorant after washing the hair with shampoo containing cationic polymers, aiming to develop a new hair coloring system.

Materials and Methods.

Materials.

As a hair color dye, 5,6-Dihydroxyindole (DHI, CAS#: 3131-52-0) was purchased from Shanghai PI chemicals (China) (Figure 1). As cationic polymers, Poly-L-Lysine (*α*-PL) was purchased from FUJIFILM Wako Pure Chemical Corporation (Japan) and ε-Poly-L-Lysine (*ε*-PL) was purchased from JNC Corporation (Japan), and Poly [oxyethylene (dimethylimino) propyl (dimethylimino) ethylene] (PA-1) was provided from NICCA CHEMICAL CO., LTD. (Japan) (Figure 2) [6]. Standard material: N/400 potassium polyvinyl sulfate (PVSK) and indicator: toluidine blue (TB) for colloid titration were purchased from FUJIFILM Wako Pure Chemical Corporation (Japan). As a cationic fluorescent reagent, rhodamine 6G was purchased from FUJIFILM Wako Pure Chemical Corporation (Japan) (Figure 3). Tresses of gray hair were commercially purchased from Beaulax Co., Ltd. (Japan). Gardenia red extract, gardenia yellow extract, and paprika pigment were purchased from Glico Co., Ltd. (Japan).

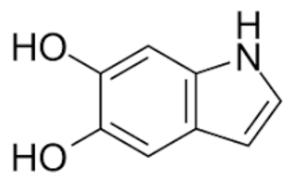

Figure 1. Structural formula of 5,6-Dihydroxyindole

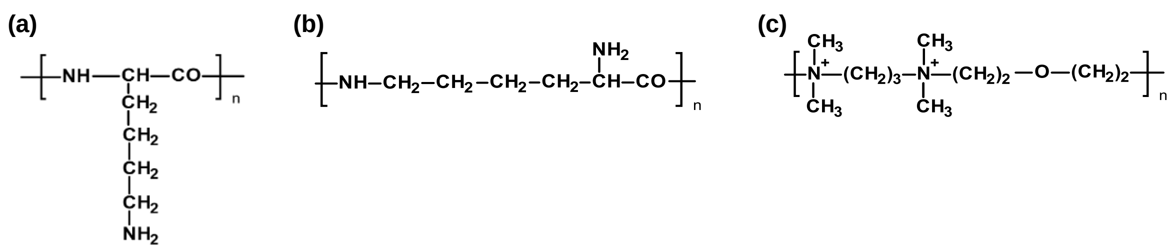

Figure 2. Structural formula of cationic polymers; (a) α-PL, (b) ε-PL, (c) PA-1

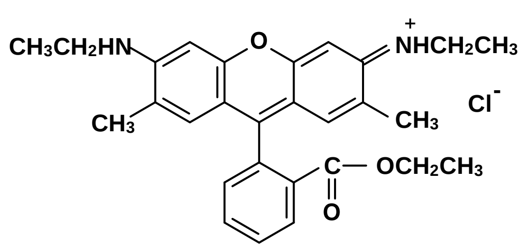

Figure 3. Structural formula of cationic fluorescent reagent; rhodamine 6G

Preliminary test: dyeing experiment with DHI-containing hair colorant

After preparing 0.5 wt%, pH 7 aqueous solutions of α-PL, ε-PL, and PA-1, gray hair tresses were immersed in each solution for 5 minutes and then allowed to air dry. Separately, a model formulation of hair dye containing DHI at pH 10 was prepared in an anaerobic chamber. The prepared base formulation (approximately 4 g) was applied evenly to both sides of a single tress (approximately 2 g) over 1 minute. The treated tresses were then placed in a constant temperature and humidity chamber set at 30°C and 55% humidity for 2.5 minutes, turned over, and left for another 2.5 minutes. After this, the tresses were rinsed with tap water for 1 minute and dried with a hair dryer. This dyeing process was repeated three times. After dyeing, the reflection spectra of each tress were measured using an ultraviolet-visible spectrophotometer (JASCO Corporation, V-650) to evaluate the dyeability.

Measurement of the Degree of Cationization of Cationic Polymers

After preparing 50 ppm aqueous solutions of α-PL, ε-PL, and PA-1, each solution was adjusted to pH 3, pH 7, and pH 10 using ethanolamine and lactic acid. Each solution (10 mL) was then mixed with 100 µL of TB. Using a burette, PVSK was titrated into the solution, and the color change from blue to reddish-purple was observed visually. The degree of cationization of each cationic polymer was calculated based on the volume of PVSK added.

Measurement of Zeta potential on hair surface

After preparing 0.5 wt%, pH 7 aqueous solutions of α-PL, ε-PL, and PA-1, hair bundles were immersed in each solution for 5 minutes and then allowed to air dry. After drying, the surface potential of the hair was measured using the SurPass 3 from Anton Paar GmbH. A 1.0 mmol/L KCl aqueous solution was used as the measurement electrolyte, and pH adjustments were made using 0.1 mol/L sodium hydroxide solution or 0.05 mol/L hydrochloric acid.

Observation of Hair Surface Charge State

A 10 µM rhodamine 6G aqueous solution was prepared and adjusted to pH 10 using monoethanolamine. After preparing 0.5 wt%, pH 7 aqueous solutions of α-PL, ε-PL, and PA-1, gray hair tresses were immersed in each solution for 5 minutes and then allowed to air dry. After drying, the tresses were immersed in the 10 µM rhodamine 6G aqueous solution for 10 seconds, rinsed with distilled water for 10 seconds, and then towel-dried. Subsequently, the hair surface was observed using a fluorescence microscope (Olympus Corporation, IX71) with a confocal unit (Hamamatsu Photonics, MAICO MEMS Confocal Unit C15890 series). Furthermore, the brightness values of these observation images were calculated using the image analysis software Fiji.

Observation of Dye Penetration Pathways Using Fluorescent Dye

The hair surface was observed using a confocal laser scanning microscope (Olympus Corporation, FV1000 IX81). Subsequently, several drops of a 10 µM rhodamine 6G aqueous solution were applied to the observed hair samples, covered with a cover glass. The hair surface state was observed 10 seconds, 5 minutes, 10 minutes, and 20 minutes after the dye application. Additionally, the integrated brightness values of these observation images were calculated using the image analysis software Fiji.

Examination of Color Variations with DHI-Containing Hair Colorant

To achieve a range of colors from black to light brown, hair colorants containing DHI were prepared by adding water-soluble gardenia red and gardenia yellow extracts, oil-soluble paprika pigment, and mixtures of basic dyes/HC dyes (black, dark brown, light brown). Each prepared formulation (approximately 4 g) was applied evenly to both sides of a single hair tress (approximately 2 g) over 1 minute. The treated tresses were then placed in a constant temperature and humidity chamber set at 30°C and 55% humidity for 2.5 minutes, turned over, and left for another 2.5 minutes. After this, the tresses were rinsed with tap water for 1 minute and dried with a hair dryer. This dyeing process was repeated three times.

Next, a model formulation of shampoo containing 0.5 wt% PA-1 was prepared and adjusted to pH 7. After washing with this shampoo formulation, the hair tresses were dyed once with the hair colorant containing the mixture of DHI and basic dyes/HC dyes (black, dark brown, light brown).

Statistical analyses

Student's paired t-test was used to compare the significance between data points. P-values less than 0.05 were considered statistically significant. The significance levels are indicated as follows: n.s. (not significant), *p<0.05, **p<0.01, ***p<0.001.

Results.

Preliminary Test: Evaluation of Dyeability with Cationic Polymer Treatment and DHI-Containing Hair Colorant

Images of gray hair tresses after being treated with α-PL solution, ε-PL solution, and PA-1 solution, followed by dyeing with a model formulation of hair color containing DHI, and the results of the color difference (ΔE*ab) are shown (Figure 4). Compared to the untreated tresses, the tresses treated with ε-PL solution and PA-1 solution clearly showed improved dyeability both visually and in terms of color difference, with significantly higher ΔE*ab values.

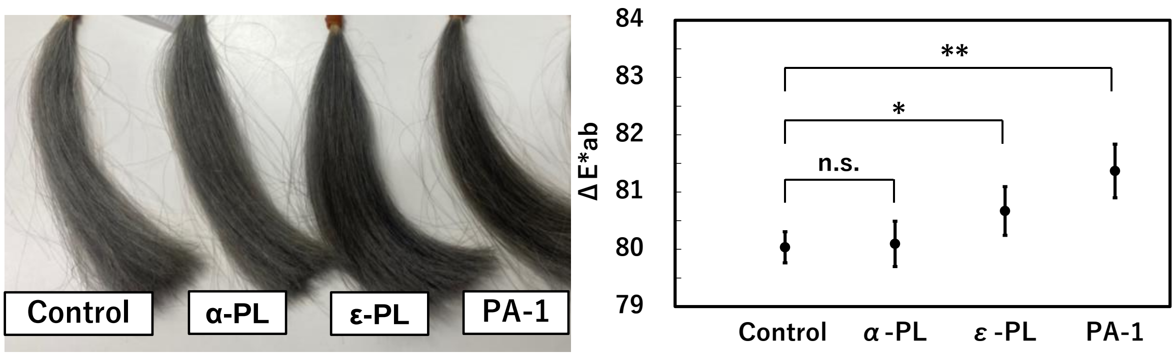

Figure 4. Images of gray hair tresses after being untreated, treated with α-PL, ε-PL, and PA-1, and dyed with a model formulation of hair color containing DHI, along with the results of ΔE*ab measured by a colorimeter.

Degree of Cationization of Cationic Polymers and Hair Surface Potential After Treatment

The results of the pH and degree of cationization of each cationic polymer calculated by colloid titration are shown (Table 1). While α-PL and ε-PL had a degree of cationization of 0 meq/g at pH 10, PA-1 had a degree of cationization of 7.0 meq/g. Additionally, PA-1 showed a constant degree of cationization of 7.0 meq/g at pH 3, 5, and 10. At pH 7, α-PL had a degree of cationization of 0 meq/g, whereas ε-PL had a degree of cationization of 6.6 meq/g.

Next, the results of the zeta potential measurements of gray hair tresses at pH 2 to 10 and the zeta potential measurements of gray hair tresses treated with various cationic polymers at pH 10 are shown (Figure 5). The zeta potential of gray hair tresses was 0 mV around pH 3 and remained constant around -10 mV beyond pH 4. When gray hair tresses were treated with cationic polymers, the zeta potential increased to 2.7 mV with PA-1, while it decreased with α-PL and ε-PL (Figure 5).

Table 1. The pH and degree of cationization of each cationic polymer solution.

	pH 3	pH 7	pH 10
α-PL	2.4 meq/g	0.0 meq/g	0.0 meq/g
ε-PL	8.7 meq/g	6.6 meq/g	0.0 meq/g
PA-1	7.0 meq/g	7.0 meq/g	7.0 meq/g

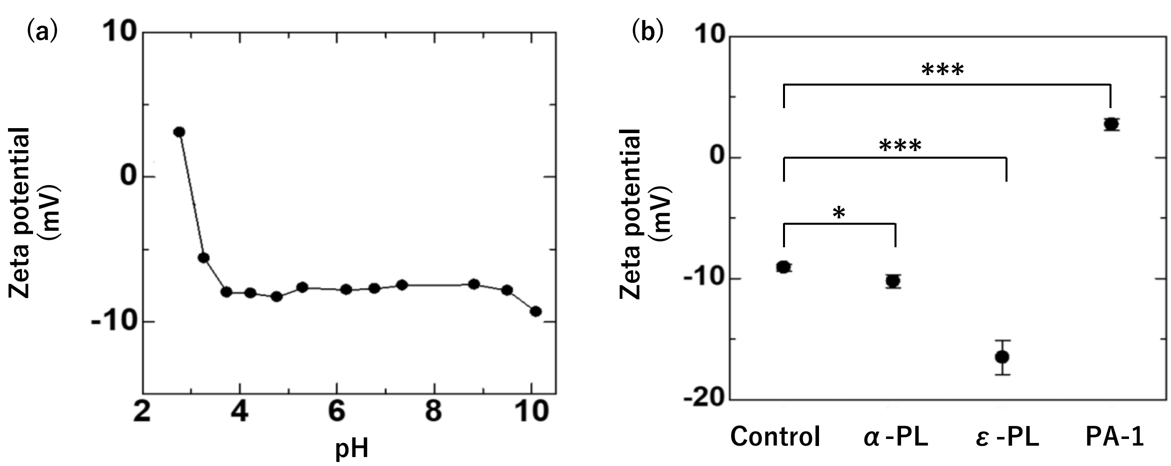

Figure 5. (a) Zeta potential of gray hair tresses at pH 2 to 10 and (b) zeta potential of gray hair tresses treated with various cationic polymers at pH 10.

Visualization of Hair Surface Charge State

The observation results of gray hair tresses treated with various cationic polymers and stained with the cationic fluorescent dye rhodamine 6G using a confocal fluorescence microscope, along with the average brightness values of these observation images (Figure 6). Fluorescence was observed at the cuticle edges of all gray hair tresses. The calculated brightness values of these images showed a significant decrease for ε-PL and PA-1 compared to the untreated samples, while there was no change for α-PL.

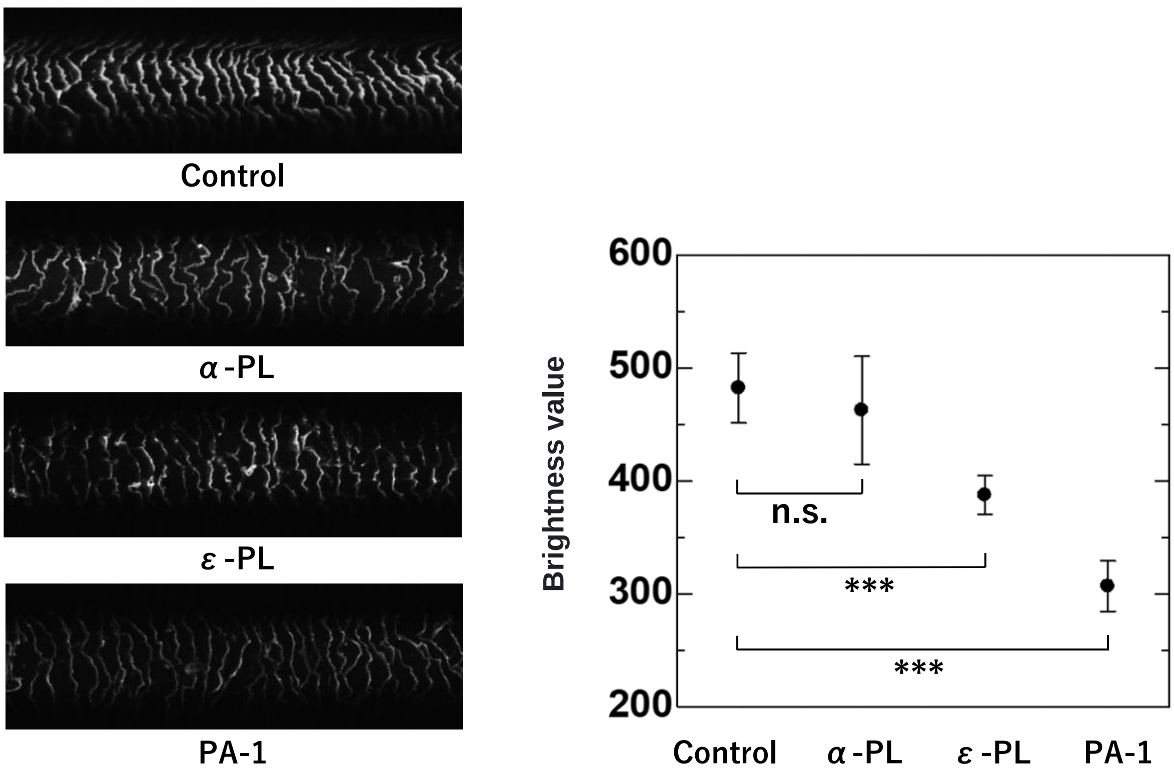

Figure 6. The image of gray hair tresses treated with various cationic polymers and stained with rhodamine 6G using a confocal fluorescence microscope, and the brightness values of these observation images.

Elucidation of Dye Penetration Pathways Using Fluorescent Dye

The time-lapse observation results of gray hair tresses stained with the cationic fluorescent dye rhodamine 6G using a confocal fluorescence microscope, along with the integrated brightness values of these observation images, are shown (Figure 7). It was confirmed that the brightness at the cuticle edges increased over time, reaching a plateau in about 10 minutes.

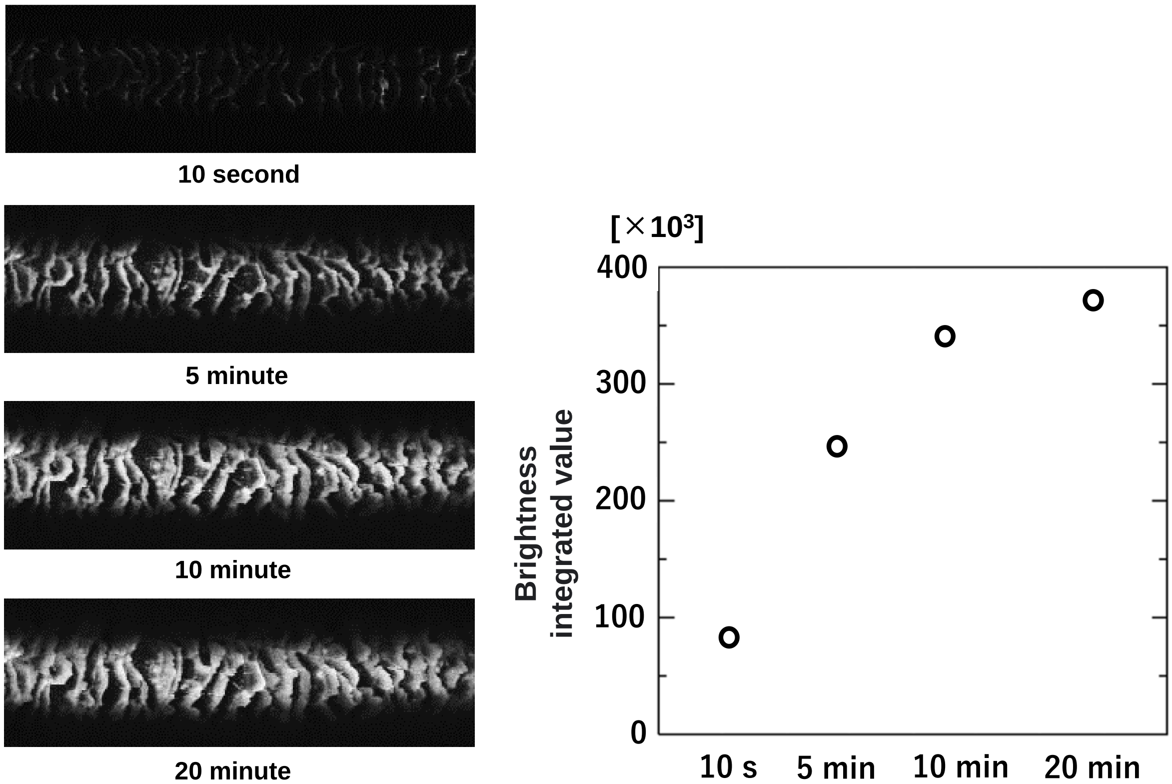

Figure 7. The image of gray hair tresses treated rhodamine 6G using a confocal fluorescence microscope, and the brightness values of these observation images.

Development of Novel Hair Coloring System

The feasibility of achieving dark brown to light brown shades by adding gardenia red, gardenia yellow, and paprika pigment to a DHI-containing hair colorant was investigated. The results showed that brown shades could not be achieved. Next, the dyeability was evaluated by adding basic dyes/HC dyes for black, dark brown, and light brown to the DHI-containing hair colorant. The results indicated that the dyeability was higher for all colors compared to formulations without DHI (Figure 8).

Subsequently, a model shampoo formulation containing 0.5 wt% PA-1 was prepared. After washing gray hair tresses with this shampoo, they were dyed once with a hair colorant containing a mixture of DHI and basic dyes/HC dyes. The results confirmed high dyeability in the black to light brown range (Figure 9).

Figure 8. The images of Hair Tresses Dyed with the Following hair colorant

(a)

1. White hair
2. Black hair
3. DHI
4. Half DHI
5. Half DHI + half amount of gardenia red
6. Half DHI + gardenia red
7. Gardenia red
8. Gardenia yellow
9. DHI + gardenia yellow

(b)

1. White hair
2. Black hair
3. DHI
4. DHI + paprika pigment
5. Paprika pigment
6. Brown hair

(c)

1. Black hair
2. White hair
3. DHI + basic dye/HC dye (black)
4. Basic dye/HC dye (black)
5. DHI + basic dye/HC dye (dark brown)
6. Basic dye/HC dye (dark brown)
7. DHI + basic dye/HC dye (light brown)
8. Basic dye/HC dye (light brown)

Figure 9. The images of Hair Tresses Dyed with the Following hair colorant

1. White hair
2. Basic dye/HC dye (black)
3. DHI + basic dye/HC dye (black)
4. Basic dye/HC dye (dark brown)
5. DHI + basic dye/HC dye (dark brown)
6. Basic dye/HC dye (light brown)
7. DHI + basic dye/HC dye (light brown)

Discussion.

DHI adsorbs onto the hair surface and forms 7-9-mer eumelanin through auto-oxidation. The optimal dyeing condition for DHI is around pH 10. Given that the pKa of DHI is 9.81 and the pKa of hair keratin is around 3.5, both are negatively charged under these conditions. This negative charge repulsion was expected to hinder DHI's approach to the hair surface, thereby reducing dyeability. Therefore, this study investigated whether the zeta potential of the hair surface could be positively charged using cationic polymers with different degrees of cationization.

Previous research has reported on the adsorption and desorption behavior and potential changes of hair and cationic polymers, showing that the adsorption behavior varies depending on the molecular weight and degree of cationization of the cationic polymers [7]. We confirmed through zeta potential measurements that coating the hair surface with PA-1, a cationic polymer with two quaternized amine groups in its molecule, resulted in a positive charge on the hair surface. Furthermore, using a confocal fluorescence microscope, we calculated the integrated brightness values of the hair surface stained with the cationic fluorescent dye rhodamine 6G. The significant decrease in brightness after treatment with the cationic polymer indicated that the polymer was electrostatically adsorbed onto the hair surface.

When the hair was treated with PA-1 and then dyed with a DHI-containing hair colorant, we observed an increase in dyeability compared to untreated hair. Additionally, the cationic fluorescent dye was found to adsorb only to the hydrophilic edges of the hair surface and penetrate over time. This suggests that the dyeing mechanism of DHI involves the concentration of water-soluble DHI at the hydrophilic edges of the hair surface, creating a concentration gradient that enhances DHI's penetration into the hair. Subsequently, DHI polymerizes into hydrophobic eumelanin, which fixes itself to the hydrophobic parts of the hair.

Moreover, dyeing hair with a hair colorant containing a mixture of DHI and basic dyes/HC dyes (black, dark brown, light brown) resulted in higher dyeability than existing hair color treatments (basic dyes/HC dyes in black, dark brown, light brown). Furthermore, by washing the hair with a shampoo containing PA-1 and then dyeing it with a DHI and basic dyes/HC dyes hair colorant, we developed a high-dyeability hair color system in the range from black to light brown.

Conclusion.

In this study, we developed a novel hair color system focusing on 5,6-dihydroxyindole (DHI), a safe precursor of eumelanin, which is the origin of black hair. By washing hair with a shampoo containing a cationic polymer that maintains its cationic properties under alkaline conditions (the optimal coloring condition for DHI), and then using a hair colorant containing DHI and basic dyes/HC dyes, we were able to develop a hair color system that is equivalent to oxidative hair dyes available on the market in terms of safety, dyeability, and color variety.

Furthermore, it was suggested that the dyeing mechanism of DHI involves its concentration at the hydrophilic edges of the hair surface, followed by penetration into the hair interior where it polymerizes into eumelanin and becomes hydrophobic, thus fixing itself to the hydrophobic parts of the hair surface. The results of this study offer a new alternative for individuals who experience hair damage or allergic reactions from using oxidative hair dyes, contributing to a means of maintaining a youthful appearance for people around the world.

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Conflict of Interest Statement.

NONE.

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