

## ORIGINAL ARTICLE

# Forensic Analysis of Physical Disruption of Human Hair after being Treated with Henna and Dyes

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## Abstract :

Nowadays, people are going through a lot of cosmetic treatments. The purpose of certain chemical treatments, such as henna and dye applications, is to improve the colour, texture, and other characteristics of hair. When conducting forensic investigations, hair analysis under a microscope and scanning electron microscopy can provide unique insights into trace evidence (hair) that may be retrieved from crime scenes. The non-permanent hair treatments that are applied are henna and dyes. Henna alters the colour and texture of hair by covering the cuticle when applied to individual hair strands. On the other hand, the dye not only penetrates the hair's cortex but also coats the cuticle, changing the structure and colour of the hair. The motive of this particular study was to examine the effects of henna and dyes on hairs within a 20-day period by microscopic inspection of the hair that has been treated with both. Under a phase-contrast microscope, hair samples treated with various brands of henna (namely, Brand H1, H2, and H3) were examined to show changes in the hair's structure, colour, and scale pattern. Under a microscope and scanning electron microscope, hair samples treated with various hues (black, brown, and burgundy) and brands (Brand D1, D2, and D3) were examined. The results showed changes in the colour, texture, and scale patterns of the individual hair strands. Compared to hair treated with henna, dyed hair had higher damage. Additionally, the degree of damage varies based on the brands and colour used. This study can give an investigator a starting point for reducing the number of suspects who have had their hair dyed or hennaed.

**Keywords:** Brands; Cosmetic-treatment; Dye; Treated hair; Non-treated hair; Hair colour; Texture of hair; Hair scale pattern; Henna; Microscopic comparison; Scanning electron microscopy.

## Introduction :

There are different types of biological samples which are found at a crime scene for example sweat, urine, blood etc. Among all these biological specimens, hairs are considered as the most important trace evidence which is found at a crime scene.<sup>1</sup> Therefore, hair is considered as the key component in criminal investigations. Because human hair is subjective and sheds between 100 and 150 times a day, it is one of the biological specimens that is regarded as the most significant trace evidence.<sup>1-3</sup> These days, styling and colouring hair has become a way of life. Individuals get a variety of hair treatments to improve their hair's colour, texture, and other characteristics. Knowing the origin of the hair samples (animal origin or human origin) obtained from the crime scene is the main goal of forensic science analysis of hair evidence.<sup>4-5</sup> According to Edmond Locard, whenever two things or objects come into contact, transfer happens in such circumstance in forensics.<sup>6</sup>

Various dyes and mehndi treatments are given to the hair strands to change the colour, texture of the hair. Different studies have been done to demonstrate the difference between the treated and non-treated hair. Hair samples obtained from the individuals

(fallen or cut samples) were treated with various commercial brands of henna available in the market. Phase-contrast microscopy was used to examine hair samples that had been coloured with three distinct henna brands: Neha Herbals Herbal Mehndi, Godrej Nupur Henna, and Patanjali Kesh Kanti Herbal Mehndi. Compared to Nupur Henna and Neha Herbals Mehndi, it was discovered that the more long-lasting effect on the texture and colour of the hair as compared to other brands.<sup>7</sup> Historically, transmitted light and polarised light microscopy have been employed by hair analysers to offer details on the physical properties of hair and other fibre evidence. A skilled forensic expert can categorise hair using light microscopy and determine hair modifications, such as if a hair was coloured, burnt, or clipped.<sup>8,9</sup> Scanning electron microscopic technique can be used to see the changes caused to the hair strand by using several cosmetic techniques. A study done by Man Q., Zang L., and Choy., at Gachon University, Korea determines the level of damage caused to the hair by using SEM, characterises the damage in three categories weak, moderate, and high damage.<sup>10</sup>

In this study, we have aimed to study the effects of commercial henna and dyes available in the market, on the hair strand with the interval of time i.e. after 1 day and 15 days. Henna samples of hair treated with three brands (labelled as H1, H2, and H3) and dyes of three brands (labelled as D1, D2, and D3) of colour burgundy are taken and examined under Scanning Electron Microscope. Thus, this study will be very helpful for the forensic examiners which will help them to narrow down their approach in finding the culprit.

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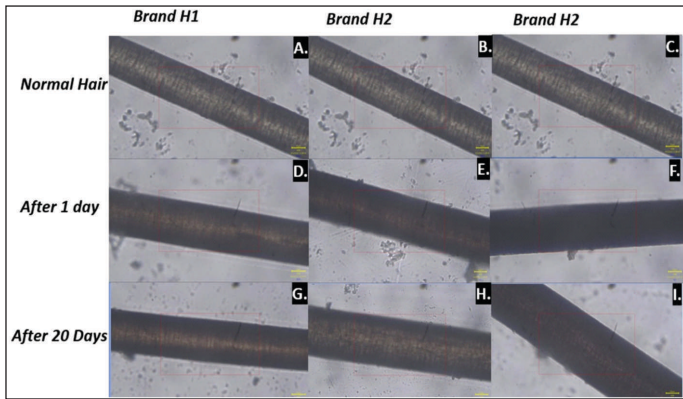


Figure 1. Shows microscopic results of normal hair, hair samples after 1st wash and after after 20 days i.e. after multiple washes. (A,B,C) normal hair i.e. without any treatment; (D,E,F) treated hair after first wash; (G,H,I) treated hair after 20 days i.e. after multiple washes of brand H1, H2 and H3, respectively.

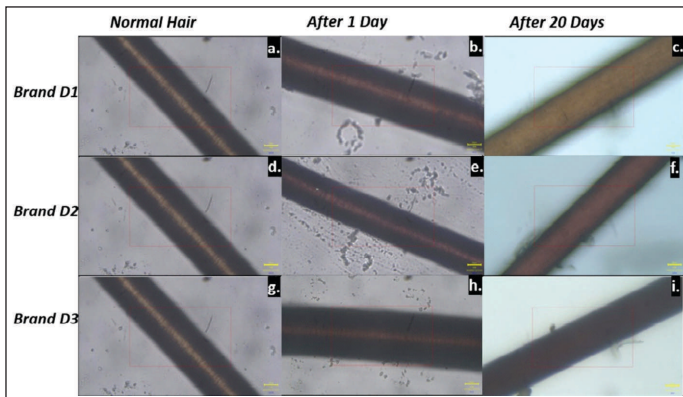


Figure 2. Shows microscopic results of normal hair, hair samples after 1st wash and after after 20 days i.e. after multiple washes. (a, b, c) normal hair i.e. without any treatment; (d, e, f) treated hair after first wash; (g, h, i) treated hair after 20 days i.e. after multiple washes of burgundy colour of three brands of dyes i.e., D1, D2 and D3 respectively.

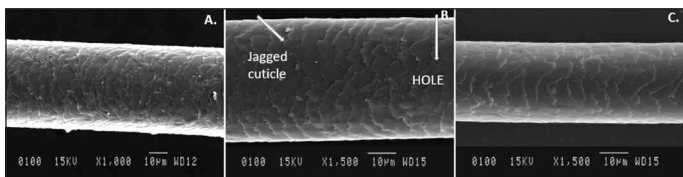


Figure 3. Shows results of hair treated with brand H1 analysed under Scanning Electron Microscope where (A) is Normal hair or non treated hair, (B) is treated hair after first wash and (C) is treated hair after 20 days i.e. multiple washes.

**Methodology:**

Collection of samples: - A total of 45 samples, both male and female, were collected from the five participants in this investigation. The combed treated and non-treated hair samples of individuals were treated with henna of three different brands (labelled as H1, H2, and H3) and were collected in zip lock bags. Similarly, 45 samples of dyed hairs from 5 individuals were collected for three different brands (labelled as D1, D2, and D3) for burgundy colour and compared.

Preparation of samples: - Samples of combed and fallen hair were obtained from people who had dyed and/or applied henna to their hair. Normal hair, that is, hair that has not been treated, was

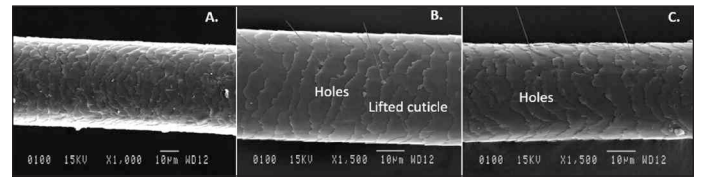


Figure 4. Shows results of hair treated with brand H2 analysed under Scanning Electron Microscope where (A) is Normal hair or non-treated hair, (B) is treated hair after first wash and (C) is treated hair after 20 days i.e. multiple washes.

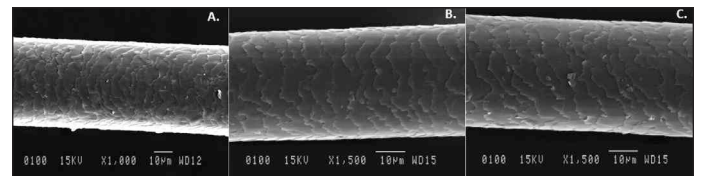


Figure 5. Shows results of hair treated with brand H3 analysed under Scanning Electron Microscope where (A) is Normal hair or non-treated hair, (B) is treated hair after first wash and (C) is treated hair after 20 days i.e., multiple washes.

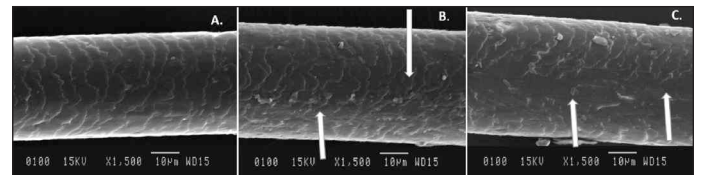


Figure 6. Shows results of hair treated with Burgundy colour of brand D1 analysed under Scanning Electron Microscope where (A) is Normal hair or non-treated hair, (B) is treated hair after first wash and (C) is treated hair after 20 days i.e., multiple washes.

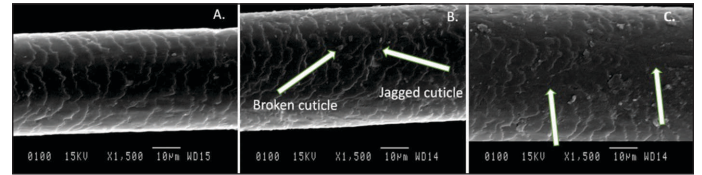


Figure 7. Shows results of hair treated with Burgundy colour of brand D2 analysed under Scanning Electron Microscope where (A) is Normal hair or non-treated hair, (B) is treated hair after first wash and (C) is treated hair after 20 days i.e., multiple washes.

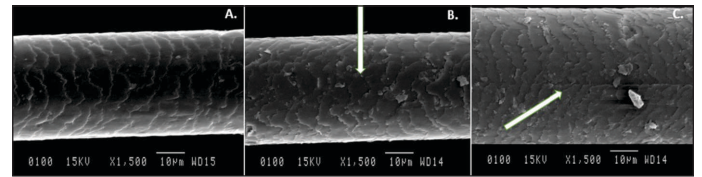


Figure 8. Shows results of hair treated with Burgundy colour of brand D3 analysed under Scanning Electron Microscope where (A) is Normal hair or non-treated hair, (B) is treated hair after first wash and (C) is treated hair after 20 days i.e. multiple washes.

obtained from the subjects who would serve as the control group. Samples were collected both immediately following the treatment, or after one day, and again after 20 days, or after several washes. Separate Ziplock bags, marked properly with all the required details had been taken for the sample collection.

For microscopic analysis, a hair strand had been taken on a clean slide and then covered with a cover slip. Later the sample had been visualised under the microscope. For scanning electron microscopy, double sided carbon tape is pasted on the stubs, 0 number brush had been used to lift the hair sample and placed on

the tape. The stubs are then put in the desiccator, where vacuum is created so that the sample stick to the tape properly and moisture is removed. Then the stubs are placed in coater for 3 minutes in order to coat the samples. The coated hair samples were analysed under scanning electron microscope.

**Analysis of samples:** A physical examination of a person with normal hair reveals that while it has a smooth and shining texture, treatment causes the hair to become rough and discoloured. Next, phase contrast microscopy was used to analyse the hair samples that had been dyed and treated with henna under a microscope.

### Result and Discussion:

As evident from figure 1: Under a microscope, normal hair, or untreated human hair (A, B and C), was observed. Three brands of henna (H1, H2, and H3) were used to treat hair, and following the initial wash, samples were examined under a microscope (D, E, F). After treating normal hair (A) with brand H1, the effects of the first wash were observed (D), indicating that the henna had penetrated the hair's cuticle layer and given the hair a smooth texture. After multiple washes i.e., after 20 days results were noted (G), indicating that the henna's effects had begun to fade and that the hair had scale patterns and a minor roughness. Secondly, normal hair (B) treated with brand H2 analysed under microscope after first day (E) indicates that the cuticle of the hair is covered with henna and slight roughness is also noted. Then after 20 days i.e., after multiple washes (H), scales patterns are visible which shows that henna affects have started to fade and hair has become rough which depicts that the hair has been damaged. Thirdly, (C) normal hair was treated with H3 brand and after first wash (F) results under microscope depicts that hair surface has been covered properly with henna, giving it a smooth and shiny texture- after multiple washes i.e. 20 days later (I) also the henna stays on the hair only slight scale patterns can be seen. According to *Kizawa et al.*, in 2005, colouring hair results in serious harm to the hair. This particular study looked at the impact of bleaching process on hair and found that bleached hair was vulnerable to cuticle fragmentation into tiny peptides, which makes the hair brittle and fragile.<sup>10</sup>

Figure 2. shows the microscopic results of hair treated with burgundy colour of three different brands i.e., D1, D2 and D3. Normal hair (a) which is brown in colour when treated with burgundy colour of brand D1 examined under microscope after 1<sup>st</sup> day (b) indicates that dye has penetrated the hair fibre due to which cuticle as well as scale patterns are totally covered with burgundy colour. Then multiple washes are provided to the hair strand and hair is examined under microscope (c) which shows that the dye is washed away from the hair strand leaving the hair rough and damaged with scales slightly visible again as the normal hair. The normal hair (d) when treated with burgundy colour of brand D2 and examined after 1 day (e) depicts that the burgundy colour dye has penetrated inside the cuticle giving the hair a smooth texture whereas after multiple washes i.e. 20 days (f) the hair fibre has become rough and dye has started to vanish from the hair fibre, leaving it in damaged condition. Normal hair (g) when treated with burgundy colour of brand D3, after 1 day (h) also depicts the penetration of the dye and the cuticle layer is covered properly with the dye. After 20 days (i) it shows that the

dye is washed away and the hair appears damaged. In 2022, Verma P and Sharma A. have done a study where microscopic analysis of hair samples treated with various commercial brands of henna has been done. It has been noted under microscope that the henna penetrates the hair shaft and covers the cuticle. Even the differences in hair treated with different brands can also be noted.<sup>7</sup>

Figure 3 depicts the results of Scanning electron microscopy where (A) is having damage of Grade 0 - Virgin hair with a consistent cuticle layer i.e Normal Hair. The normal hair then treated with H1 and hair examined after 1 day (B) shows damage of Grade 2 - A severe lifting up of the cuticle with holes or fissures, but with little to no cortical exposure; the cuticle is fractured and angular. (C) is the treated hair fibre after 20 days or multiple washes shows damage of Grade 1 - Uneven layering of the cuticle without breaks or holes, indicating that the hair is beginning to return to its natural colour. Figure 4 depicts the results of Scanning electron microscopy where (A) is having damage of Grade 0 - Virgin hair with a consistent cuticle layer i.e Normal Hair. The normal hair then treated with henna of brand H2 and hair examined after 1 day (B) and 20 days (C) both shows damage of Grade 1 - There is an uneven layer of cuticle without any fractures, however there are little holes visible. Cuticle is jagged and less breakage can also be seen. Figure 5 depicts the results of Scanning electron microscopy where (A) is having damage of Grade 0 - Virgin hair with a consistent cuticle layer i.e Normal Hair. The normal hair then treated with henna of brand H3 and hair examined after 1 day (B) and 20 days (C) both shows damage of Grade 1 - Uneven layering of the cuticle without breaks or holes, indicating that the hair is beginning to return to its natural colour. In 2017, Enrico et al. investigated the relationship between hair damage that may result from undergoing excessive cosmetic procedures and cocaine absorption from a wettable solution into the hair matrix (simulating owing to external pollution or contamination).<sup>11</sup>

Figure 6 depicts the results of Scanning electron microscopy where (A) is having damage of Grade 0 - Virgin hair with a consistent cuticle layer i.e Normal Hair. The normal hair then treated with burgundy colour of brand D1 and hair examined after 1 day (B) shows damage of Grade 2 - Severe cuticle lifting that exposes the cortex yet has holes or fissures in it. After 20 days (C) shows damage of Grade 3 - Partially exposed cortex. The cuticle appears rugged and hair seems to be damaged. According to a study done in 2021, Qiaoyue et al. discovered with the use of SEM that hair is effectively damaged. Weak, moderate, and high damage levels were used to categorise the degree of harm. To determine the extent of the damage, a convolutional neural network was employed, and a database was created.<sup>12</sup>

Figure 7 depicts the results of Scanning electron microscopy where (A) is having damage of Grade 0 - Virgin hair with a consistent cuticle layer i.e Normal Hair. The normal hair then treated with burgundy colour of brand D2 and hair examined after 1 day (B) shows damage of Grade 1 - There is an uneven layer of cuticle without any fractures, however there are little holes visible. There is less breakage and a jagged cuticle. After 20 days (C) shows damage of Grade 3 - Partially exposed cortex. The cuticle appears jagged and hair seems to be damaged. Figure 8

depicts the results of Scanning electron microscopy where (A) is having damage of Grade 0 - Virgin hair with a consistent cuticle layer i.e normal hair. The normal hair then treated with burgundy colour of brand D3 and hair examined after 1 day, (B) shows damage of Grade 1 – There is an uneven layer of cuticle without any fractures, however there are little holes visible. There is less breakage and a jagged cuticle. After 20 days (C) shows damage of Grade 1 – Irregular overlay of the cuticle without cracks or holes. Cuticle is lifted and broken. Kaliyadan F et al. in 2016 had done a study where hair shaft of volunteer females had been taken who took cosmetic treatments like bleaching, dyeing and straightening. Samples had been analyzed under scanning electron microscope and damage had been visualized which was categorized as grade 0, grade 1, grade 2 and grade 3.<sup>13</sup>

### Conclusion:

The study of trace evidence is crucial to forensic science. Therefore, if recovered at a crime scene, trace evidence from a hair might be used as corroboration in the inquiry. Since hair varies in size, colour, and texture, forensic analysis of hair evidence utilising a variety of techniques can clarify situations and allow a connection to be formed between a suspect and the scene of the crime. This study has shown that applying henna and dyes to hair results in some modifications to the texture, colour, and other features of the hair. The level of damage has been noted with respect to the brands and colours used on the hair. This study also shows that the dyes create more damage if compared with the damage done by henna. As a result, this study will assist the investigators in focusing their efforts and frequently offer crucial details that can help identify a suspect or victim.

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