Int. J. Morphol.,
38(1):96-100, 2020.

Scanning Electron Microscopy Approach for Evaluation of Hair Dyed with *Lawsonia inermis* Powder: *in vitro* Study

Método de Microscopía Electrónica de Barrido para la Evaluación de Cabello Teñido con Extracto de *Lawsonia inermis*: Estudio *in vitro*

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BIANCHI, S.; BERNARDI, S.; CONTINENZA, M. A.; VINCENTI, E.; ANTONOULI, S.; TORGE, D. & MACCHIARELLI, G. Scanning electron microscopy approach for evaluation of hairs dyed with *Lawsonia inermis* powder: *in vitro* study. *Int. J. Morph. 38(1)*:96-100, 2020.

SUMMARY: During aging, usually graying of the hair occurs as a result of oxidative stress. Driven by social acceptance and self-perception of the exterior appearance, both men and women rely on hair dyeing products, in order to mask the graying hair. At the same time, a frequent use of synthetic products and treatment can damage the hair shaft; for this reason, this study aimed to evaluate the morphological effect of the herbal dye derived from *Lawsonia inermis* (henna), on hair. Dyed hairs were evaluated by means of SEM. Subsequently, they were compared, qualitatively and quantitatively, with undyed hairs. Results showed a positive impact on the cuticula pattern and on the diameters of the examined samples, after henna application. Different results, about the degree and the type of morphological changes occurring on pigmented hairs, may depend on the phenotype and on the health condition of hair, before dye treatment.

KEY WORDS: Hair dyes; Hair fibers; Hair cosmetics; SEM; Herbal dye.

INTRODUCTION

Human hair is a precious physical feature of the human body, of strong psychological and social importance, especially for women. Their biological functions consist of protection of the skin and thermic regulation (Buffoli *et al*., 2014). Specifically, scalp hair protects head skin from sunlight rays, avoids the thermic dispersion, owns sensory properties and plays a key role in social communication. For these reasons, scalp hair disorders, such as alopecia, hirsutism or simply hair graying can cause psychological discomfort and affect the quality of life (Buffoli *et al*.).
Like all other tissues, the scalp hair also undergoes an aging process, graying and thinning: a recent study, in fact, reported that 6–23 % of people have 50 % gray hair by 50 years of age (Ji *et al*., 2017) and an early appearance of gray hair causes stress and negatively influence the psychological condition of a person (Trüeb, 2015).

Moreover, different factors may promote the appearance of gray hair: among them, genetic heritage, ethnic variation and extrinsic factors, including cosmetic hair treatments, smoking and ultraviolet light (Ji *et al*.) . In Caucasians usually, gray hair appears between 25 – 43 years, while in Africans and Asians the average age is 43.9 ± 10.3 years (Ji *et al*.). The pattern manifestation of the hair graying differs between men and women: while in the male gender, the graying process begins from the temporal and occipital area; in women, the mechanism starts at the boundaries of the scalp (Ji *et al*.; Kumar *et al*., 2018).

Melanin is the hair pigment, it is produced by melanocytes, according to the cycle of the hair follicles. Indeed, melanogenesis occurs during the anagen phase of the cycle of hair follicle (Buffoli *et al*.).

The external factors influencing the pigmentation process, the gradual accumulation of Reactive oxygen species (ROS) along with the loss of follicular melanocytes activity, result in hair graying (Ji *et al*.).

Color, length, and style of scalp hair play a key role

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in self-perception, and the easy-change of these features resulted in the development of products and technique aimed to improve hair appearance, using color and texture correction (Draelos, 1995). In this context, hair dye treatments were developed to color the hair, by removing the existing pigment and/or adding a new color (Guerra-Tapia & Gonzalez-Guerra, 2014).

Hair dyes comprehend:
· Vegetable or herbal products
· Mineral products
· Synthetic products

In this study, we analyze the effects of an herbal dye, derived from *Lawsonia inermis* (henna), on different hair phenotypes. Specifically, *Lawsonia inermis*' leaves contain 1-1.4 % lawsone, a natural herbal dye, used for years as dyeing treatment for hair, skin, and nails (Lascaratos *et al*., 2004). Naphthoquinone glycoside lawsone determines the key color of this dye and influences henna's ability to split glycosidic bonds when applied on hair/skin with water.

Besides, while in Oriental and Mediterranean cultures hair dyeing is only requested for special events, in western countries it is considered an instrument for social acceptance (Lascaratos *et al*.; Saive *et al*., 2018). Nowadays, both men and women have to rely on hair dyeing, in order to hide the signs of aging (Infante *et al*., 2016), often driven by the desire to be accepted in their society. Social networks, which have an important role in the perception of exterior appearance, also enhance this trend: as reported by Perloff (2014), social media and photo-activity have a negative effect on young women's body self-perception, by favoring the development of body image disorders.

Besides, a frequent use of synthetic products can damage the hair shaft, for this reason, the aim of our study is to evaluate, through Scanning Electron Microscopy (SEM), the morphological effects of the herbal dye, derived from *Lawsonia inermis* (henna), on different phenotypes of hair.

MATERIAL AND METHOD

Samples. The study group included sixty human Caucasian hair samples: twenty hair (samples) were collected from blonde, twenty from brown and twenty from black phenotype. 10 samples for each group were analyzed before dyeing application; the others were studied after the treatment. The history of the subjects who donated the samples did not included the use of previous synthetic dyes, but only the normal procedures of hair hygiene using shampoo, conditioners and hair drying.

Hair dyeing used derived from *Lawsonia inermis* powder (Henna red Iran n.3®, Phitophilos), and was applied according to the manufacturer's instructions.

In particular, the powder derived from the leaf of *Lawsonia inermis* was mixed with warm water until to obtain a batter-consistency with a ratio powder/water of 100 gr/ 220 ml.

The henna has been applied for 10 minutes. The hairs were then washed with water at room temperature.

Scanning Electron Microscopy (SEM) analysis. The samples were prepared for conventional SEM analysis. Briefly, the dried samples were put on aluminum stubs and gold coated (Kaliyadan *et al*., 2016; Palmerini *et al*., 2018; Bernardi *et al*., 2018, 2019). Images of hair samples were captured with the scanning electron microscope (Philips XL30CP, the Netherlands) at 20 kV and at a magnification of 1000x and 2000x.

Qualitative Image Analysis. The following parameters were evaluated. general appearance, presence of cuticle, regularity of overlaying, lifting up, presence of holes and cracks.

Quantitative Image Analysis. By Image J® software we performed the quantitative image analysis. For each experimental group we analyzed at least 20 SEM micrographs. The parameters analyzed were the diameter of the hair and the height of the scale (Ali *et al*., 2015). The diameter was measured on each SEM micrographs. The scale height was measured on an area equal to the square of the diameter per each SEM micrograph. The control and henna groups outcomes were statistically compared, using the T student test, by the SAS University Edition software.

RESULTS

The different scalp hair phenotypes were observed with SEM, and the obtained microphotographs were analyzed and compared either qualitatively and quantitatively.

Control samples- Qualitatively analysis. From a general point of view, in the examined samples, splitting was not revealed; cuticles were always present, with no exposure of hair cortex (Fig. 1). Overall, the hair fibers present a rough surface (Fig. 1) revealing an irregular overlaying of the cuticles. The surface of the brown group hair fibers appears smoother than the others (Fig. 1c-inset). Indeed, the cuticle cells, derived from brown hair fibers, form sequenced and continuous cuticle pattern, consisting of a few lifted up cuticles. Black and blonde hairs shafts present a more severe lifting up if compared to the brown phenotype, with loss of tightly overlapping cuticular scales (Fig. 1a-inset). In addition, the blonde fibers presented also holes and cracks (Fig 1b).

Henna-dyed samples. Qualitatively analysis. Observing SEM images of henna-dyed samples, absence of splitting was detected, and cuticles were always present, with no exposure of hair cortex (Fig. 2a-c).

Black and brown groups presented an evident lifting up of the cuticles, with a surface rougher than the blonde ones (Fig. 2a-c). Indeed, the hair shaft of the former do not present a regular overlaying along the surface. Blonde samples showed a well-ordered cuticle pattern, with a decrease in the cuticular lifting up (Fig. 2b). Noticeable is the absence of holes and cracks in all groups.

Quantitatively and Statistical Analysis. In control group, black phenotype represents the thickest fiber hair, followed by blonde and brown fibers. In addition, the black one presents the highest mean scale height (Table I).

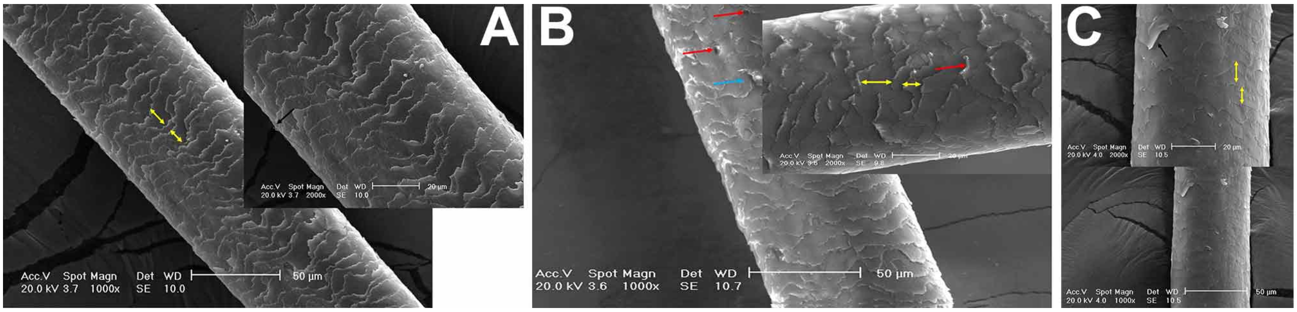

Fig. 1. Representative SEM micrographs of control hairs (A) black phenotype, (B) blonde phenotype and (C) brown phenotype. Black arrows indicate the lifting up of the cuticles. Yellow arrows indicated the overlay of the cuticles that appear very irregular in (A) and (B) insets. Red arrows point the holes and the blue arrows the cracks in (B) inset.

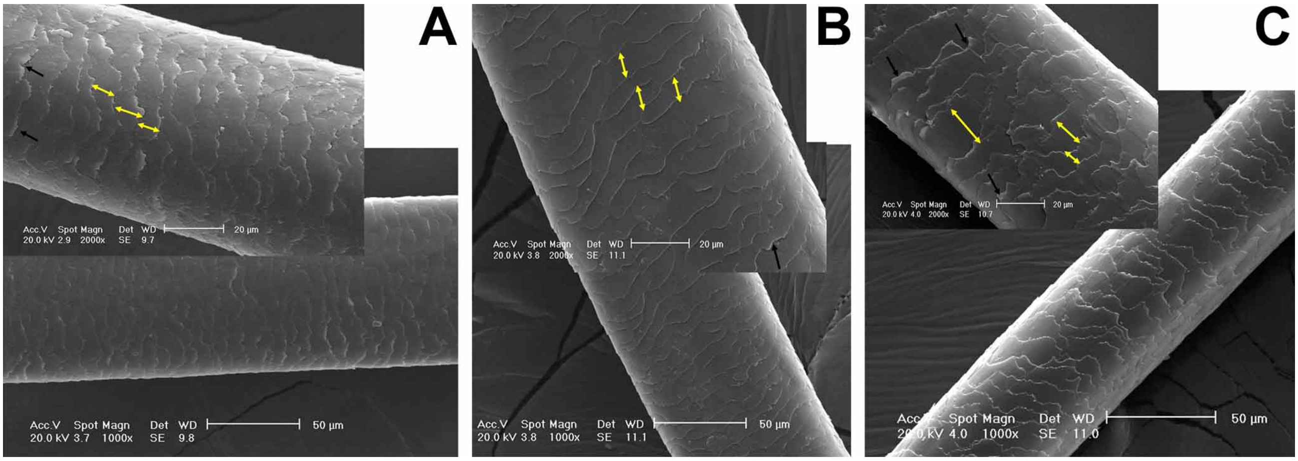

Fig. 2. Representative SEM micrographs of hair dyed (A) black phenotype, (B) blonde phenotype and (C) brown phenotype. Black arrows indicate the lifting up of the cuticles. Yellow arrows indicated the overlay of the cuticles that appear very irregular in (A) and (C) insets but more regular in (B) insets.

Table I. Statistical comparison of parameters.

Sample	Diameters µm (mean ± SD)	T Test (P value)	Mean Scale Height µm (mean ± SD)	T Test (P value)
Black phenotype Control	72.4 ± 5.4	<0.05	8.2 ± 3.2	0.8
Black phenotype Henna dye	83.2 ± 11.2		8.1 ± 2.0	
Blonde phenotype Control	72.1 ± 5.9	<0.05	7.9 ± 2.8	0.7
Blonde Phenotype Henna dye	87.5 ± 0.6		7.7 ± 2.6	
Brown Phenotype Control	55.0 ± 5.6	<0.05	7.3 ± 2.2	<0.05
Brown Phenotype Henna dye	68.8 ± 12.6		9.9 ± 2.7	

The diameter of all hair phenotypes increased significantly after henna application ($p < 0.05$). The mean scale height did not increase significantly in the blonde and black phenotypes. A significant variation occurred in the brown phenotypes ($p < 0.05$).

The statistical comparison, of the two parameters, showed that the henna dye application can significantly influence the diameter and the mean scale height (Table I).

DISCUSSION

This study investigated the morphological changes in the ultrastructure of different phenotypes of hair, after dyeing with a natural herbal colorant, *Lawsonia inermis* powder. Henna's effects on hair were evaluated through Scanning Electron Microscopy (SEM), which provides magnified micrographs of hair samples surface and is useful to reveal possible morphological defects (Kim *et al*., 2010).

Hair dyes are divided into two categories: synthetic (most used category, with a longer life) and natural (such as those derived from the leaves of *Lawsonia inermis*) (Guerra-Tapia & Gonzalez-Guerra). A recent study demonstrated that 64 % of French women rely on the use of scalp hair colors and among them only the 2.64 % uses dyes, based on henna powder (Bernard *et al*., 2016). However, the synthetic dyes affect the hair structure significantly enhancing the oxidation of melanin and any dyes previously applied; this process creates a gap on the hair cortex, allowing the deep penetration of the chromophores (Draelos; Guerra-Tapia & Gonzalez-Guerra, 2014). It has been reported that the frequent use of chemical agents is the principal source of damage to the hair shaft, with significant changes visible by electron microscopy (Kim *et al*.; Lee *et al*., 2014).

Through its inner dyeing mechanism, a synthetic dye induces morphological changes in the hair, leading to a long-term damage of the hair shaft (Lee *et al*.). Moreover, due to the risk cancer of the exposed hairdresser, the chemicals ingredients contained by these products are constantly controlled (Harling *et al*., 2010). The herbal dyes, instead, react with the keratin protein, present on the surface of hairs and skin. The lawsome binds to the keratin protein, leading to a wider diameter of the hair and a superficial covering of the hair surface (Zumrutdal & Ozaslan, 2012; Singh *et al*., 2015).

Lawsonia inermis is a natural henna product free of chemicals and does not cause severe damage to the hair shaft, compared to synthetic dyes (Ali *et al*.). In fact, our morphological evaluation in different phenotypes of hair fibers (brown, blonde and black hair fibers) shows a significant enhancement in cuticle pattern, which regularity improves after herbal dye application. Instead, SEM micrographs derived from hair fibers not yet subjected to dye treatment, appear fragile, with lack of homogeneity, rough surface and with presence of cuticular lifting up. The latter, in controls, could be due to a frequent use of shampoos, or to the high temperature during drying or straightener, which can damage hair fibers (Zhang *et al*., 2015). The two phenotypes that most showed a benefit from the herbal dye application were the brown and blonde phenotypes. The improvement might be due to the reduction of the cuticular detachment and to the use of a free-chemical product which does not penetrate the hair, but reduces friction among hair shafts and reduces cuticle scales by increasing their adhesion to the shaft (Bolduc & Shapiro, 2001). Our SEM qualitative analysis shows that *Lawsonia inermis* powder might be capable of recovering the cuticle damage, binding keratin proteins, and provides an overall profile of more smooth moisture-rich cuticles and strengthen hair fibers.

The quantitative analysis of hair fiber phenotypes showed that the mean diameters increased after the henna application, as reported also by Ali *et al*. Regarding the other measured parameter, the mean scale height was significantly higher on the brown phenotype hair. Indeed, the study of Ali *et al*. reported a general increase of the mean scale height in the hairs dyed with commercial henna, speculating that chemicals added into the product may damage the hair, uplifting the cuticles. However, the product used in in our study was free from chemicals. Therefore, the difference of the mean scale height results in blonde and brown could be due to the interaction between *Lawsonia inermis* and keratin proteins of the different hair phenotypes considered in this study.

The use of *Lawsonia inermis*, on different hair phenotypes, produces morphological changes on cuticles and diameters of the hair fiber. Specifically, after henna application, cuticles lifting up decreases, with a general healthy appearance of the hair. Moreover, henna dye preserves hair shaft structure, inducing an increase in the diameter of all hair phenotypes. The degree and type of morphological changes may depend on phenotype and health condition of the hair. This aspect can be useful to provide new information, for the impact of natural dye products on hair health.

BIANCHI, S.; BERNARDI, S.; CONTINENZA, M. A.; VINCENTI, E.; ANTONOULI, S.; TORGE, D. & MACCHIARELLI, G. Método de microscopía electrónica de barrido para la evaluación de cabello teñido con extracto de *Lawsonia inermis*: Estudio *in vitro*. *Int. J. Morphol., 38(1)*:96-100, 2020.

RESUMEN: Durante el envejecimiento, generalmente se produce el envejecimiento del cabello como resultado del estrés oxidativo. Motivados por la aceptación social y la autopercepción de la apariencia, tanto hombres como mujeres confían en productos para teñir el cabello para enmascarar las canas. Al mismo tiempo, el uso frecuente de productos y tratamientos sintéticos puede dañar el tallo del cabello. Por esta razón, este estudio tuvo como objetivo evaluar el efecto morfológico del tinte derivado de *Lawsonia inermis* (henna) en el cabello. Los cabellos teñidos se evaluaron mediante SEM. Posteriormente, se compararon, cualitativa y cuantitativamente, con cabellos sin teñir. Los resultados mostraron un impacto positivo en el patrón de la cutícula y en los diámetros de las muestras examinadas, después de la aplicación de henna. Los diferentes resultados, sobre el grado y el tipo de cambios morfológicos que ocurren en los cabellos pigmentados, pueden depender del fenotipo y del estado de salud del cabello, antes del tratamiento con tinte.

PALABRAS CLAVE: Tintes para el cabello; Fibras capilares; Cosméticos para el cabello; SEM; Tintes herbales.

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Received: 13-09-2019
Accepted: 25-07-2019