

Morphological degradation of human hair cuticle due to simulated sunlight irradiation and washing

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ARTICLE INFO

Article history:

Received 15 January 2016

Received in revised form 1 June 2016

Accepted 2 June 2016

Available online 04 June 2016

Keywords:

Human hair

Cuticle

Morphology

Photodamage

ABSTRACT

Morphological changes in hair surface are undesirable, since they cause shine loss, roughness increase and split ends. These effects occur more frequently in the cuticle, which is the outermost layer of the hair strand, and thus the most exposed to the environmental damages. Sunlight irradiation contributes significantly to these morphological alterations, which motivates the investigation of this effect on hair degradation. In this work, the influence of irradiation and hand-washing steps on the morphology of pigmented and non-pigmented hair cuticle was investigated using field emission scanning electron microscopy (FESEM) and transmission electron microscopy (TEM). To simulate daily conditions, where hair is hand-washed and light exposed, samples of dark brown and gray hair underwent three different conditions: 1) irradiation with a mercury lamp for up to 600 h; 2) irradiation with the mercury lamp combined with washes with a sodium lauryl sulphate solution; and 3) only washing. A new preparation procedure was applied for TEM samples to minimize natural variations among different hair strands: a single hair strand was cut into two neighbouring halves and only one of them underwent irradiation and washing. The non-exposed half was used as a control, so that the real effects caused by the controlled irradiation and washing procedures could be highlighted in samples that had very similar morphologies initially. More than 25 images/sample were analysed using FESEM (total of 300 images) and ca. 150 images/sample were obtained with TEM (total of 900 images). The results presented herein show that the endocuticle and the cell membrane complex (CMC) are the cuticle structures more degraded by irradiation. Photodegradation alone results in fracturing, cavities ($\varnothing \approx 20\text{--}200\text{ nm}$) and cuticle cell lifting, while the washing steps were able to remove cuticle cells ($\approx 1\text{--}2$ cells removed after 60 washes). Finally, the combined action of irradiation and washing caused the most severe damages, resulting in a more pronounced cuticle extraction ($\approx 1\text{--}4$ cuticle cells after a 600 h irradiation and a 60 times washing). This irradiation dose corresponds to ca. 2 months of sunlight exposure (considering 5 h/day) in Campinas-SP, Brazil, during the day period of maximum irradiation intensity. The combined action of irradiation and washing can be explained by the creation of fragile photodegraded spots in the endocuticle and in the CMC, where the mechanical stress associated to the washing steps are more prone to induce rupture.

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1. Introduction

Lipids, pigments and proteins, which are major constituents of human hair, are modified by solar radiation [1–3], resulting in drier, rougher, more brittle, and opaque hair strands, in addition to the loss of their mechanical strength, color changes and the formation of split ends [4]. These effects are highly undesirable, because hair good appearance is related to self-esteem and health, and they are also cumulative, since damaged hair has no ability to regenerate.

Cuticle is the most external part of the hair strand, and the one most exposed to environmental damages, which increases the interest in evaluating morphological changes in this region. Cuticle cells are

formed by amorphous material and each one is divided into four sub-units that have a distinct chemical composition [5,6]. The epicuticle (layer 1), which is the outermost layer of the cuticle cell, is thin ($\approx 2.5\text{ nm}$), hydrophobic and cystine-rich ($\approx 12\text{ w/w}$) [7]. The A-layer (layer 2) and the exocuticle (layer 3) are both more reticulated and hydrophobic, due to the higher cystine content ($\approx 30\%$ and $\approx 15\text{ w/w}$, respectively). Finally, the endocuticle (E) (layer 4), which is the most internal layer, is made up of non-keratinous material ($\approx 3\text{ w/w}$ cysteine) and is hydrophilic.

The cell membrane complex (CMC) is also part of the cuticle structure and has an adhesive function of binding the cuticle cells together [8]. CMC consists of a hydrophilic central δ -layer ($\approx 15\text{ nm}$), formed mainly by proteins and polysaccharides, sandwiched by two lipid layers ($\approx 5\text{ nm}$ each), called β -layers. β -Layers are composed by monolayer lipids (hydrophobic) that are attached by covalent bonds to the keratin

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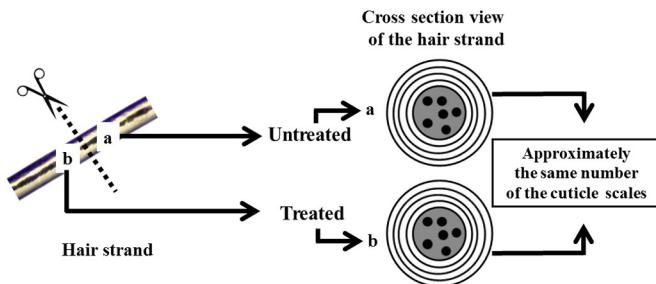


Fig. 1. Scheme of the sample preparation for TEM, aiming the identification of pre-existing morphological features in hair strands and the isolation of washing and irradiation effects.

in the epicuticle and by van der Waals attractive forces to the δ -layer on the hydrophobic end of the fatty acids. There are two β -layers: 1) the inner β -layer, which faces the inner part of an individual cuticle cell, and is formed mainly by palmitic, stearic and oleic fatty acids; and 2) the outer β -layer, facing the outer surface of the cuticle (closer to the adjacent cuticle) and that contains 18-methyl eicosanoic acid (18-MEA), as the main component [9]. The study of the radiation effects on the different layers of the cuticle is remarkably important, due to its protective role for the hair internal structures.

Chemical and physical modifications under irradiation are described in the hair science literature, showing that solar radiation causes severe damages to the strand structure. The modification in hair color is the main reported consequence of photodegradation and represent a physical effect that occurs in both, pigmented and non-pigmented hair [10–15]. The influence of different wavelength radiation ranges (UV, VIS and IR) to hair photodamaging and color changes was previously reported

by this research group [10–13]. The final color after hair irradiation also showed an important dependence on the hair initial color. While hair strands that were initially pale yellow, became more yellow after irradiation, hair strands that were initially more yellow, underwent photobleaching [13].

There are few published papers about the morphology and the ultrastructure of photodegraded hair [16–20]. Weigmann et al. [16,17] used field emission scanning electron microscopy (FESEM) to analyze the effect of UV radiation and humidification cycles in the hair surface. These authors observed that the cuticle was the region most affected by photodegradation, due to its high cystine concentration, and also that the interchange between cycles under different humidification (RH = 95% and RH = 10%), intercalated with UV irradiation periods, intensified the damages to hair morphology, thinning the cuticle cells. According to the authors, the thinning process is highly dependent on the sample hydration state and was observed only with UV exposure under high relative humidity (RH = 95%), and not under RH = 10% [16]. The morphological changes in irradiated hair strands from three different ethnic groups (European, African and Asian) were also analyzed by Won-Soo Lee et al. [20] using transmission electron microscopy (TEM) and scanning electron microscopy (SEM). SEM results revealed damages such as cell lift, loss of cuticle edges and exposure of the cortex cells, which were more severe in African hair than in other hair types, while TEM results showed the formation of holes of variable sizes, rupture along the endocuticle and cell lifting.

Two important points that were not considered in the previously described morphological studies are the number of hair strands considered and the specification of the regions that were analyzed in each strand. Furthermore, there are many intrinsic differences between hair strands from different heads (genetic variability), which is the case in

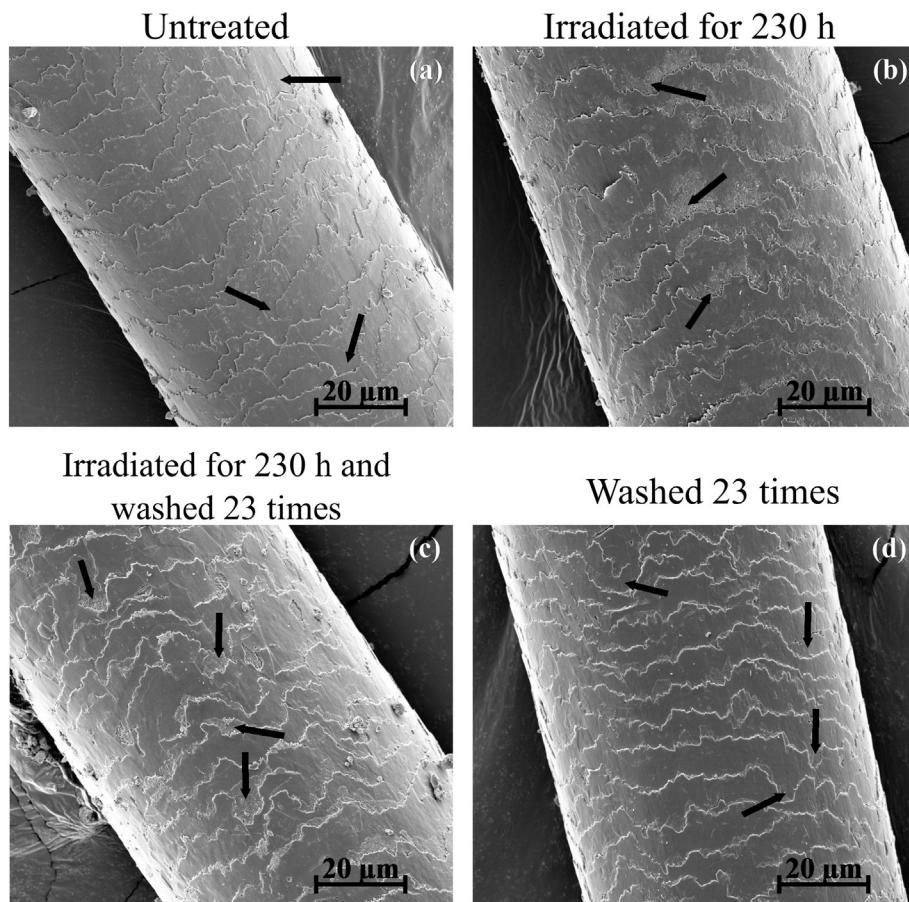


Fig. 2. Images of the dark brown hair surface obtained by FESEM: (a) untreated hair strand; (b) hair irradiated for 230 h; (c) hair irradiated for 230 h and washed after each 10 h irradiation cycle; (d) hair only washed for 23 times. Arrows indicate that some cuticle pieces were broken in (a) and (d) and indicate exposed endocuticles in the hair strand surface in (b) and (c).

blended hair, or between strands with distinct degradation levels from the same head or even between the root and the tip in a single hair strand [21,22]. It is thus a great challenge to distinguish between previous morphological and chemical variations in strands and the effects resulting exclusively from photodamaging. To overcome these intrinsic morphological variations in hair surface, different approaches were used in the present work: 1) A large number of images were obtained in each sample (ca. 25 images/sample in FESEM and 150 images/sample in TEM); 2) Samples from the same donor were compared in each case, instead of blended hair; 3) the central region was analyzed in different strands to minimize the variations from root to tip; and 4) TEM images were prepared by a new methodology, where a single hair strand is divided in two neighboring halves and only one of them is subjected to irradiation and washing. The other half is used as a control, thus allowing the treated and the control sample to have a very similar initial morphology.

Besides this, irradiation steps were combined to hair washing steps in order to simulate everyday conditions, where hair strands are irradiated for a certain period of time during the day and also undergo mechanical stress, though in low level, due to simple daily care procedures, such as washing.

2. Material and Methods

2.1. Samples

Dark brown hair and gray hair were collected from volunteers with no history of chemical treatments. Gray hair is a mixture of white and black strands, which were manually separated by colors. White, black

or dark brown hair were grouped into tresses weighing 0.5 g each. Prior to the experiments, the hair samples were washed as described in reference [13].

2.2. Sample Irradiation and Washing Procedure

A mercury vapor lamp (OSRAM HQL 125 W, São Paulo, Brazil) was used as a radiation source. The lamp has an emission spectrum with UV (367 nm) and visible light (406, 438, 548 and 580 nm) intense lines, in addition to emitting very low infrared (IR) radiation [13]. The overall procedure for irradiation with a mercury vapor lamp is described elsewhere [10,13]. Measurements of light intensity from the source were carried out with a radiometer (PMA 2100, Solar Light Co, USA), considering the incident dose on the samples. The distances from the source to the sample and from the source to the radiometer were the same.

Hair samples were daily irradiated for 10 h, using a mercury lamp full-spectrum (UV, VIS and IR), which was followed by a period in the dark (>14 h), after which the sequence was repeated cyclically, until completing a total irradiation of 230 or 600 h. Two washing conditions were combined to the irradiation steps: 1. hair strands washed after each 10 h irradiation cycle (totalizing 23 or 60 washings in samples irradiated for 230 and 600 h, respectively), and 2. hair strands that were not washed, only irradiated. The effect of washing alone was evaluated in samples that were not irradiated, but only washed for 23 or 60 times. Irradiation was performed inside a fume hood, as described elsewhere [10,13]. The temperature and the relative humidity inside the fume hood were monitored daily and kept under $29.3 \pm 0.9^\circ\text{C}$ and $44 \pm 4\%$ average values, respectively. The radiation intensity obtained for the

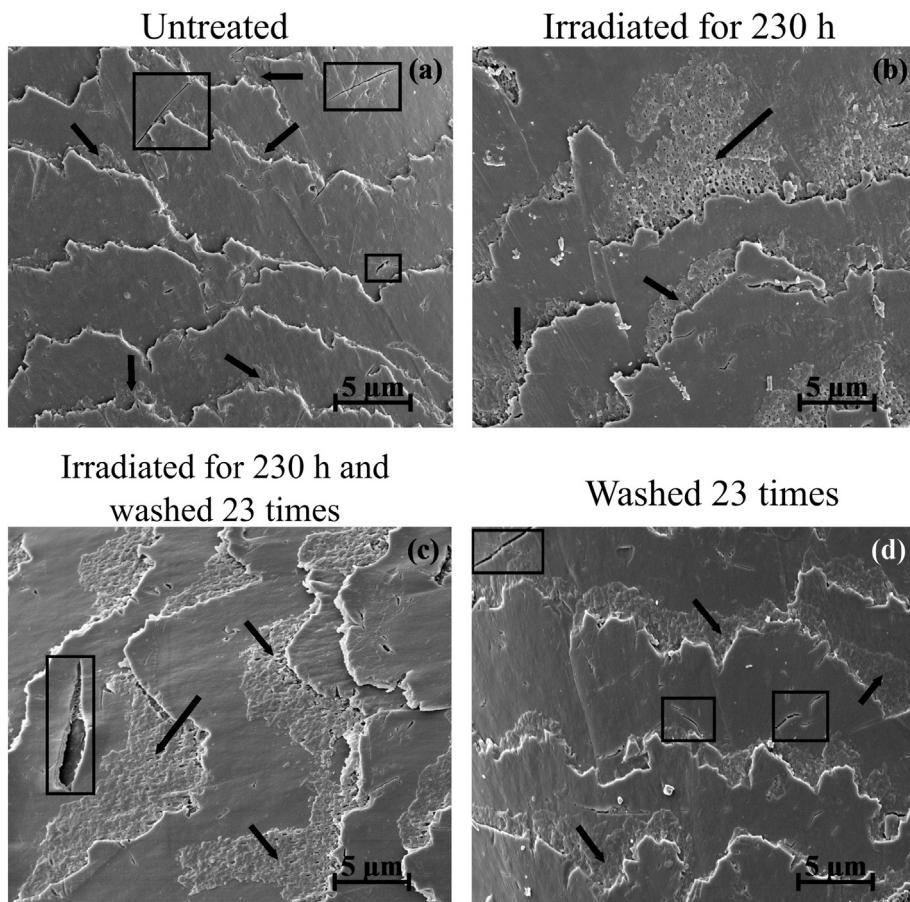


Fig. 3. Images of the dark brown hair surface obtained by FESEM: (a) untreated hair strand; (b) hair irradiated for 230 h; (c) hair irradiated for 230 h and washed after each 10 h of irradiation; (d) hair only washed for 23 times. Exposed endocuticle layers are indicated by arrows and cracks on the cuticle surface are outlined by rectangles.

mercury vapor lamp were as follows: $0.5 \pm 0.1 \text{ W m}^{-2}$ (UVB), $16 \pm 5 \text{ W m}^{-2}$ (UVA), $27 \pm 1 \text{ W m}^{-2}$ (VIS) and $31 \pm 3 \text{ W m}^{-2}$ (IR).

The exposure times were calculated such that the daily doses of UV radiation from the lamp and the sun were comparable. The intensity of sunlight was measured at 12 h, summer period, in Campinas-SP, Brazil ($22^{\circ}53' \text{ S}$; $47^{\circ}04' \text{ W}$). The values of UV radiation intensity obtained for sunlight were $2.3 \pm 0.3 \text{ W m}^{-2}$ (UVB) and $31 \pm 8 \text{ W m}^{-2}$ (UVA). This corresponds to a dose of $\text{UV} \approx 60 \times 10^4 \text{ J m}^{-2}$, the dose is equal to the intensity (W m^{-2}) multiplied by the exposure time (s). Therefore, 5 h of sun exposure (at 12 h, summer period) is equivalent to ≈ 10 h of UV mercury lamp radiation ($\approx 59 \times 10^4 \text{ J m}^{-2}$).

2.3. Field Emission Scanning Electron Microscopy (FESEM)

Secondary electron images (SEI) were obtained in hair strands using a high resolution environmental scanning electron microscope, equipped with a field emission gun (FEI, Quanta 650, USA). Three hair strands were randomly chosen from the samples and a knot was carefully hand made in the central region of each strand (≈ 10 cm from the root end). Then the strands were fixed to the sample holder stub by the length containing the knot, and were cut with a stylet above and below the knot to have a 1 cm final length. Individual hair strand pieces (3 strands/sample) were coated with gold (≈ 16 nm) in a SCD 050 sputter coater (Oerlikon-Balzers, Balzers, Lichtenstein). Both the coater and the microscope were available at the National Laboratory of Nanotechnology (LNNano/CNPEM), in Campinas-SP, Brazil. Images were obtained under vacuum, using a 4 kV accelerating voltage. At least 25 images were obtained on different areas of each sample.

2.4. Transmission Electron Microscopy (TEM)

TEM micrographs were obtained using a Zeiss Libra 120 microscope (UNICAMP - Campinas-SP, Brazil) operating at 120 kV. A specific sample preparation was carried out aiming to discriminate the pre-existing morphological variability in hair from the washing and the photodegradation damages. Thus, three hair strands were selected per treatment and each hair strand was cut into two neighboring halves, as indicated in Fig. 1, resulting in very similar morphological surfaces (a and b) in each half. One of the halves (a) was kept as a control sample and was not exposed to irradiation or washing steps (called untreated). The other half of the hair strand (b) underwent one of the following processes: 1. Irradiation only (strands 1, 2 and 3), or 2. irradiation combined with washing cycles (strands 4, 5 and 6), or 3. only washing (strands 7, 8 and 9). The comparison between the control and its adjacent treated pair in each case facilitates the identification of morphological features caused by the photodegradation and the washing procedure. Both the untreated and the treated parts of the hair strand were fixed, dehydrated, embedded in resin and finally stained together.

Hair strands were fixed in the dark with a 2% (v/v) solution of OsO₄ (Sigma) in a 0.1 mol L⁻¹ sodium cacodilate buffer (pH = 7) for 4 h. Then, they were dehydrated with ethanol solutions of increasing concentration (from 50 to 100% v/v) and propylene oxide. Dehydrated samples were embedded in Spurr resin (formulated with 0.2 g of catalyst) for 5 days and then transferred to moulds and cured at 70 °C for 24 h [23]. The samples were cut using ultra-thin sections and stained with a 2% (w/v) uranyl acetate solution for 1 h and then with 1% (w/v) lead citrate solution for 15 min [24]. At least 300 images were obtained in each hair strand, 150 images in the untreated half and 150 images in the treated one.

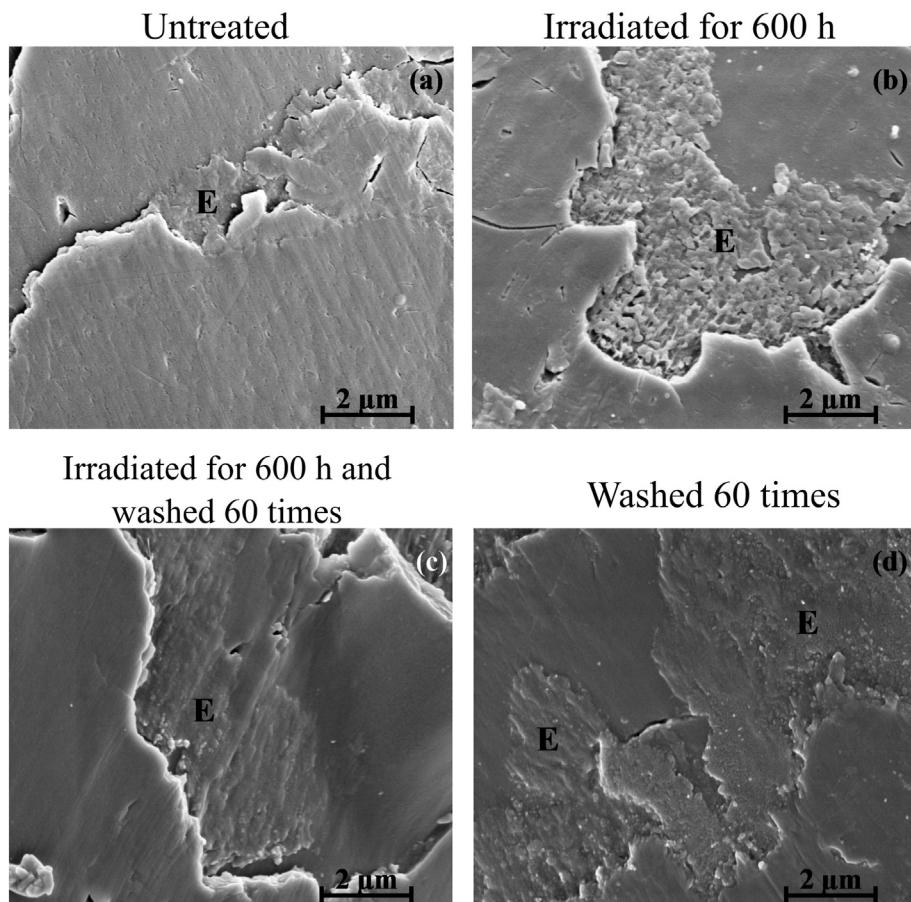


Fig. 4. Images of the dark brown hair surface obtained by FESEM: (a) non-irradiated hair strand; (b) hair irradiated for 600 h; (c) hair irradiated for 600 h and washed after each 10 h of irradiation; (d) hair only washed for 60 times. The index E indicates the exposed endocuticle.

3. Results and Discussion

Hair is daily exposed to solar radiation and washed with shampoo. To simulate these everyday procedures and to evaluate their effects on the cuticle of dark brown and gray hair strands, cycles of irradiation and hand-washing steps were carried out in this work. The morphology of the cuticle, which is the most external layer of hair strands and thus the most exposed to environmental damages, was analyzed using FESEM and TEM. These results were obtained using a systematic methodology, where hair strands were irradiated and washed the same number of times for FESEM and TEM analyses. Washing times were also kept the same in samples that were only washed (1 step) or irradiated and washed (2 steps).

3.1. Field Emission Scanning Electron Microscopy (FESEM)

Figs. 2 and 3 show representative FESEM images with different magnifications obtained on the surface of dark brown hair samples before (a), after 230 h of irradiation (b), after 230 h of irradiation, combined to washing steps after each 10 h irradiation cycle (c) and only washed for 23 times (d). Fig. 2(a) shows that the cuticle cell surface in untreated hair exhibits entire cuticles, in a good general condition, although it is possible to notice some border areas from where broken pieces were removed, as indicated by the arrows in Fig. 2(a). These images were obtained in the central area of the hair strand (10 cm from the root end) and the observed hair morphology is consistent with the previous damages caused by daily care procedures.

In samples of dark brown hair irradiated for 230 h (Fig. 2(b)) and samples where this irradiation period was combined to washing steps

(Fig. 2(c)), it was possible to notice clearer areas (indicated by arrows in (b) and (c)), which corresponds to endocuticles exposed on the surface of the hair strand. Fig. 2(d) shows the surface of a dark brown hair that was only washed for 23 times, not irradiated. The surface of the washed hair strand is very similar to the surface of the untreated hair and some sparse broken border areas can be found, as indicated by arrows in Fig. 2(d).

Fig. 3 shows higher magnification images obtained in the dark brown hair strands of Fig. 2, where it is possible to observe more clearly the morphology of the exposed endocuticle. Before irradiation or washing procedures, the areas of exposed endocuticle in Fig. 3(a), present a smooth surface, as indicated by the arrows. After 230 h of irradiation (Fig. 3(b)) and 230 h of irradiation followed by washing (Fig. 3(c)), wider exposed endocuticle layers are visible, and they are not flat anymore, but uneven and full of cavities, as indicated by the arrows in Fig. 3(b) and (c). These two treatments thus result in very similar morphologies, while hair samples that were only washed (23 times), present a flat endocuticle morphology very similar to the one observed in untreated hair (areas indicated by arrows in Fig. 3(d)).

In Fig. 3(a), it is also possible to verify the presence of previous damages on the hair surface, such as the little cracks, indicated by rectangles in Fig. 3(a). These features are intrinsic to the sample morphology and result from the hair previous history and not from the irradiation or washing steps applied in this work. When hair samples are washed and irradiated, however, more severe damages can be observed, such as the deep fractures, highlighted by the rectangle in Fig. 3(c). In samples that were only washed (23 times), the surface cracks are similar to the ones observed in the untreated sample.

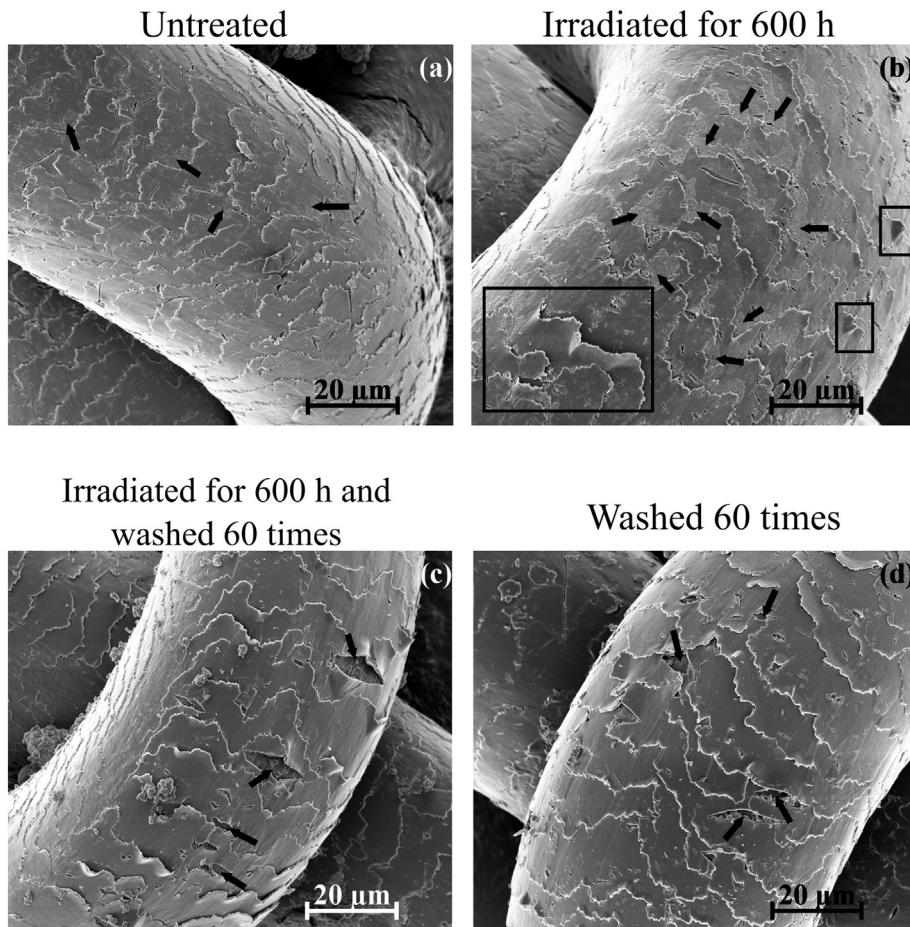


Fig. 5. Images of the knot region in dark brown hair obtained by FESEM: (a) non-irradiated hair strand; (b) hair irradiated for 600 h; (c) hair irradiated for 600 h and washed after each 10 h of irradiation; (d) hair only washed for 60 times.

Fig. 4 shows images of the dark brown hair irradiated for longer periods (600 h) and washed more times (60 times) in comparison to an untreated sample (control) presented in **Fig. 4(a)**. **Fig. 4(b)** shows a representative image of a hair strand irradiated for 600 h, where it is possible to notice an uneven endocuticle (E), full of cavities, just as observed in samples irradiated for 230 h, as previously shown in **Fig. 3(b)**. The effect of a 600 h irradiation combined to washing or the individual washing effect (60 times) can be observed in **Fig. 4(c)** and (d), respectively. The endocuticle morphology (E) in these samples appears partially removed or scratched, probably due to the mechanical stress caused by the washing procedure. It is important to emphasize that the hair strands were gently hand-washed, but this minimum mechanical pressure was enough to promote the endocuticle partial removal.

Therefore, the effect of longer irradiation times (600 h) combined to washing, or washing alone (60 times) differ significantly from the results obtained in their equivalent samples irradiated for 230 h or washed 23 times (**Fig. 3(c)** and (d), respectively). After 230 h of irradiation followed by washing, the endocuticle morphology looks more like the one presented by the hair that was only irradiated for 230 h. On the other hand, the sample that underwent irradiation for 600 h combined to washing is more similar to the hair samples that were only washed (60 times). So, it seems that the main irradiation effect is the creation of cavities in the endocuticle, while washing has a more scratching action. The two effects act synergistically in samples that were irradiated and washed. Irradiation effects are more visible when samples are less washed (23 times), while the washing effects prevail in samples that were longer irradiated and washed more times.

It is also important to notice that the endocuticle morphology after washing the sample 60 times (**Fig. 4(d)**) is not as smooth as the one in

the untreated sample (**Fig. 4(a)**), though it does not contain the visible cavities of a 600 h irradiated sample (**Fig. 4(b)**).

A knot was carefully made in the middle of each hair strand during FESEM sample preparation to emphasize possible damages to the cuticle cells, for instance, detachment and lifting. Images of the knot region in samples irradiated for 600 h and washed 60 times are presented in **Fig. 5**. Lifted cuticle scales can be noticed in hair samples irradiated for 600 h (as in the outlined areas in **Fig. 5(b)**), in samples irradiated for 600 h and washed 60 times (**Fig. 5(c)**) and also in samples only washed for 60 times (**Fig. 5(d)**). In untreated hair (**Fig. 5(a)**), the cuticle cells appear to be closed and in good general conditions. These results indicate that the degradation of the inner layers of the cuticle due to irradiation or mechanical stress during washing can be responsible for the partial detachment and lifting of the cuticle cells, which in turn, can be responsible for macroscopically noticeable optical effects, such as shine loss in hair strands, due to light scattering by irregular cuticle cells.

Cuticle cell lifting was also observed in a previous work of this research group, using atomic force microscopy (AFM) [25], which is a technique that allows the very same area to be scanned on the sample surface before and after irradiation. Line height profiles were measured in different areas of three hair strands for each sample, comparing the same line before and after irradiation, and showing that the height of the steps formed between the edges of two neighboring cuticle scales increased about 100 nm, as a consequence of sample irradiation with a mercury lamp for 500 h. While the average step height for non-irradiated hair strands was 203 ± 23 nm, the average height for the same strands after photodegradation was 338 ± 34 nm (average obtained from 18 cuticle step heights measured).

Also, when hair strands were irradiated and washed or only washed, large cracks appeared on the cuticle surface, as indicated by arrows in

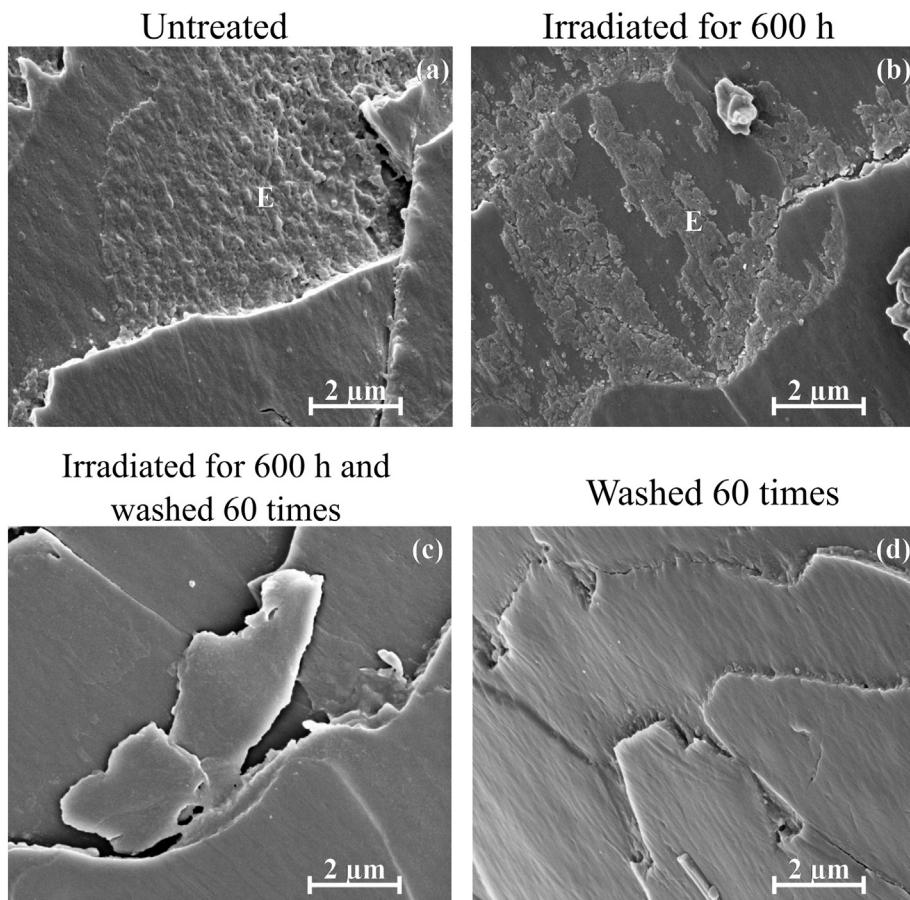


Fig. 6. Images of the gray hair surface obtained by FESEM: (a) non-irradiated hair strand; (b) hair irradiated for 600 h; (c) hair irradiated for 600 h and washed after each 10 h of irradiation; (d) hair only washed for 60 times. The index E indicates the exposed endocuticle.

Fig. 5(c) and (d). This shows a clear effect of the cuticle fragility under repetitive irradiation and washing steps. Some of these fractures are so deep that the cortex fibers underneath are exposed, indicating a complete rupture of the whole cuticle cell layer. The possibility that these cracks were formed due to the little mechanical stress applied on the strand during the knot formation and not during washing cannot be neglected. However, the large cracks appear only in samples that were washed, indicating that the washings have an important effect of weakening the cuticle cells, thus making them more fragile and susceptible to break.

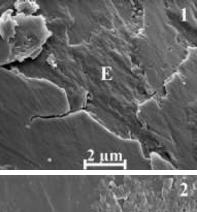
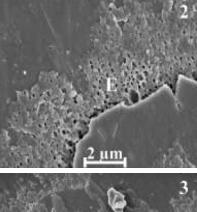
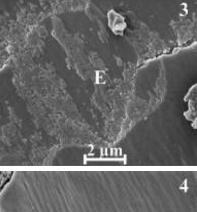
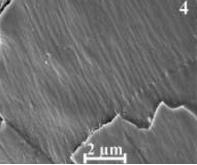
The results in Figs. 2–5 indicate that the mechanical effects, such as material removal and cuticle cracking are caused by washings, while the appearance of the cavities at the endocuticle layers and cuticle lifting are probably effects of irradiation, since it does not appear in any hair sample that has just been washed.

The effect of irradiation on gray hair cuticle was also evaluated. Gray hair consists of a mixture of white and black strands, which were separated in two groups, according to their color and analyzed separately. The results obtained by FESEM on both color groups from gray hair were very similar, so that specific features assigned to melanin presence were not identified. Fig. 6 shows representative FESEM images acquired in gray hair. Untreated strands (Fig. 6(a)) show large areas of exposed endocuticle (E), which indicates the occurrence of previous damages to the hair morphology. Besides this, the endocuticle areas exposed in brown hair (previously shown in Fig. 4(a)) were smooth, while the endocuticle in gray hair is rough and presents small cavities even before irradiation. It seems that the gray hair starts the controlled photodegradation process from an advanced stage, when compared to the brown hair, which can be assigned to the previous irradiation doses received by the samples before they were collected.

After a 600 h irradiation with a mercury lamp, the degradation of gray hair advances and the endocuticle appears partially removed, with small fragments being left on the surface, as shown in Fig. 6(b).

Table 1

Endocuticle (E) classification according to the morphologies observed in different hair types (brown and gray hair) before and after irradiation. The four endocuticle types represent gradual stages of photodegradation.

Classification and degradation stages	Morphology	Types of hair
1 Endocuticle Smooth		Brown hair • Untreated • Washed 23×
2 Endocuticle Rough and with cavities		Brown hair • Irradiated for 230 h • Irradiated for 230 h and washed 23× • Irradiated for 600 h Gray hair • Untreated
3 Endocuticle Partially removed		Brown hair • Irradiated for 600 h and washed 60× • Washed 60× Gray hair • Irradiated for 600 h
4 Endocuticle Totally removed		Gray hair • Irradiated for 600 h and washed 60× • Washed 60×

When gray hair samples were only washed (60 times) or irradiated and washed, the surface of the cuticles became clean, as if cuticle material and endocuticle layers have been removed by washing, as shown in Fig. 6(d) and (c), respectively.

Figs. 2–6 show four different morphologies of the exposed endocuticle, which can be correlated to gradual degradation stages: 1) endocuticle with a smooth surface (observed in brown hair untreated or only washed 23×); 2) rough endocuticle with cavities (observed in brown hair irradiated for 230 or 600 h, irradiated and washed for 230 h and also in untreated gray hair); 3) partially removed endocuticle (observed in brown hair irradiated and washed for 600 h, washed 60× and also in gray hair irradiated for 600 h); and 4) endocuticle completely removed (observed in gray hair irradiated and washed for 600 h or only washed 60×). These four endocuticle morphologies, together with the corresponding hair types are presented in Table 1.

Adopting the classification of the endocuticle morphologies suggested in Table 1, untreated brown hair was initially in stage 1, having a smooth endocuticle. After irradiation and washing processes, brown hair reached stages 2 and 3. On the other hand, gray hair before irradiation already started in stage 2, characterized by a rough endocuticle, full of cavities. This morphology resulted from previous degradation of the gray hair before the irradiation and the washing steps applied in this work. After irradiation and washing processes, gray hair reached stages 3 and 4.

3.2. Transmission Electron Microscopy (TEM)

TEM images provided information on the cross sections of the hair strands in the cuticle region. These images complement then the investigation of the cuticle surface obtained with the FESEM analysis. To isolate the photodegradation effects and to eliminate the intrinsic variability of the hair samples, untreated and treated areas were compared in two neighboring surfaces of the same hair strand. Approximately 150

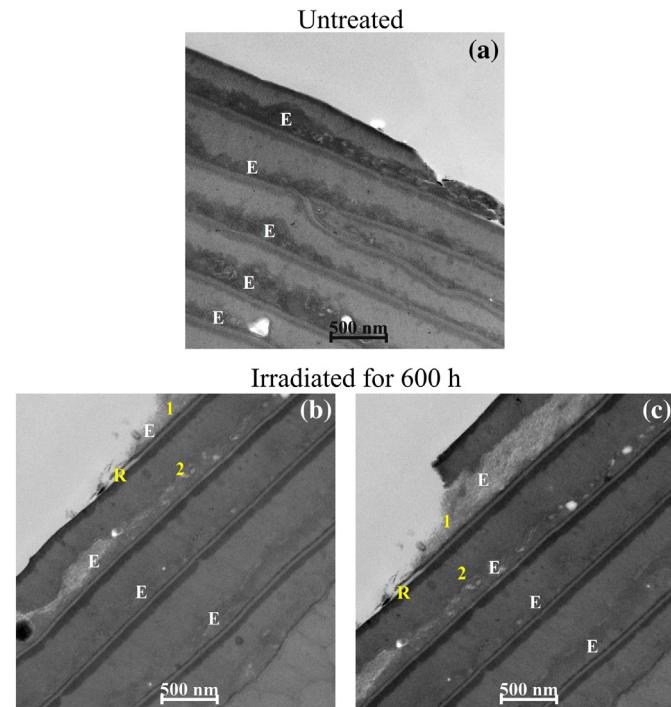


Fig. 7. Images of ultra-thin sections of the cuticle cells in dark brown hair obtained by TEM: (a) untreated hair; (b) and (c) hair irradiated for 600 h with a mercury lamp, showing cuticle cells of the same hair strand. Highlight to the morphology of the endocuticle (E). Number 1 indicates the outermost cuticle cell and number 2 indicates the cuticle just beneath cuticle 1.

images were obtained in three hair strands per sample, totalizing 900 images.

Fig. 7 shows ultra-thin sections of the brown hair strand irradiated for 600 h in comparison to the non-irradiated neighboring half of the strand. Before irradiation, this hair strand had 4 to 8 cuticle cells, as shown in **Fig. 7(a)**, and the endocuticles appear continuous (darker areas indicated by E in each cuticle cell) in **Fig. 7(a)**. After 600 h of irradiation (**Fig. 7(b)** and (c)), the number of cuticle cells remained the same (4 to 8 cells), but the endocuticles of the outermost cuticle cells became brighter, as can be observed in **Fig. 7(b)** and (c). Brighter regions in TEM images may result from less dense material or from a change in the local hydrophilicity, since the stain (uranyl acetate) used for sample preparation acts preferably on hydrophilic areas. This is in agreement to FESEM results for this sample (**Fig. 4(b)**), which showed a degraded endocuticle uneven and full of cavities ($\varnothing \approx 20\text{--}200\text{ nm}$), suggesting a sample with less dense regions and a possible loss of integrity, just as observed by TEM.

These images also show very clearly the preferential degradation of the most external cuticle layers. The images presented in **Fig. 7(b)** and (c) were obtained in neighboring regions of the same hair strand, and the point marked by "R" can be used as a reference that appears in both images. In **Fig. 7(c)** the outermost cuticle cell (1) presents the most degraded (brighter) endocuticle, when compared to the inner cuticle cells. In the penultimate cuticle cell (2) there were small regions with brighter spots in the endocuticle layer. In the adjacent region, where part of cuticle cell 1 was removed (**Fig. 7(b)**) and cuticle cell 2 became the most exposed to irradiation, further endocuticle damages can

be observed. These results show very clearly that the irradiation damages at the endocuticle have a limited effect on the first or second outer cuticle layers and that the inner cells will be damaged only when the top cuticle layers are removed.

Inoue et al. [26,27] suggested that UV radiation breaks the disulfide bonds from S100A3 protein, which is a cysteine-rich calcium-binding protein, located mainly in the endocuticle layer. S100A3 is cross-linked to hair keratin via disulfide bridges, thus providing structural integrity to the hair strand. The authors considered the endocuticle as the weakest element of the cuticle and presented results showing that S100A3 was degraded by UV irradiation for 100 h ($10\text{ J cm}^{-2}\text{ h}^{-1}$). These results were obtained through the elution of S100A3 and by immunoblot analyses. The photodegradation of this protein may be responsible for the endocuticle degradation observed here by TEM (appearance of brighter areas and cavities in **Fig. 7**), and by FESEM (formation of roughness and cavities in the endocuticle, **Figs. 2–5**).

Other result observed in TEM images was the degradation of the outer β -layer, located in the cell membrane complex (CMC), as indicated in **Fig. 8(c)** and (d). The CMC consist of a central δ -layer ($\approx 15\text{ nm}$) sandwiched by two lipid layers called β -layers ($\approx 5\text{ nm}$ each). The inner β -layer faces the inner part of an individual cuticle cell, while the outer β -layer faces the outer surface of the cuticle (closer to the adjacent cuticle) [8,9]. As pointed out in **Fig. 8(c)** and (d), the outer β -layer begins to separate after 600 h of irradiation and it appears broken in many situations, as shown in **Fig. 8(d)**. The degradation of the outer β -layer due to irradiation complicates the analysis of the cuticle cells by TEM. It was very difficult to find

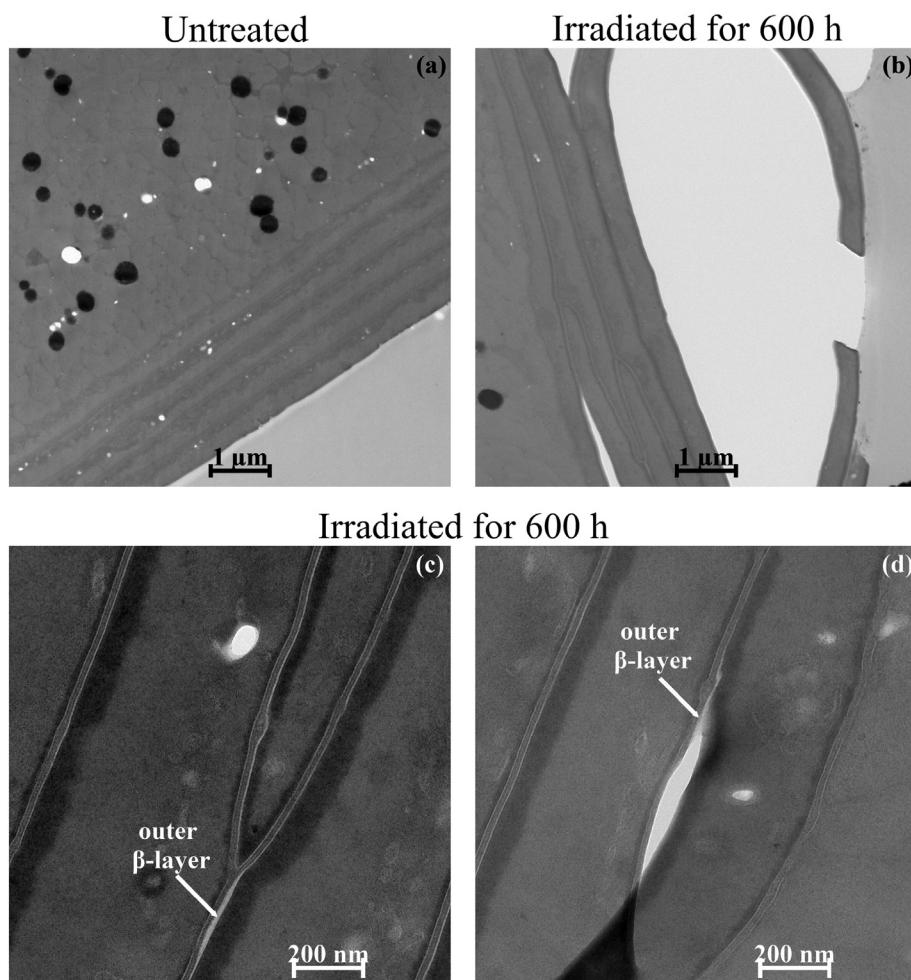


Fig. 8. Images of ultra-thin sections of the cuticle cells in dark brown hair obtained by TEM: (a) untreated hair; (b-d) hair irradiated for 600 h with a mercury lamp. Ruptures in the outer β -layer of the cell membrane complex (CMC) after irradiation are indicated in (c) and (d).

entire sections adhered to the resin and, in most of the irradiated hair sections, the cuticle cells were separated, as shown in Fig. 8(b). This behavior was not observed in any of the untreated hair samples. No resin was observed between the separated cuticle cells in Fig. 8(b) and (d), indicating that the separation of the cuticle cells took place during TEM sample preparation and not during irradiation. However, the cleavage is an indirect consequence of irradiation, since the fragility and the poor adhesion of the cuticle cells after irradiation is a strong indicative of the photodegradation of the β -layer. CMC degradation is also a possible cause to cuticle cell detachment and lifting, as observed in FESEM images (Fig. 5(b)).

TEM images confirm the washing effect on the removal of cuticle cell from the hair surface, which was already observed by FESEM (Fig. 4(d)). Two halves of the hair strand were separated to assure that they had very similar morphologies and cuticle numbers before the experiments. As shown in Fig. 9(a), the untreated half of the dark brown strand had an average of 4.9 ± 1.1 cuticle cells (52 measurements were carried out around the strand diameter), and the structure of endocuticle and of CMC were entire and in good condition. After 60 washes, the other half of the same strand presents an average of 2.7 ± 1.2 cuticle cells (48 measurements), thus indicating the removal of some cuticle cells during washing. The separation of the cuticle cells due to washing occurred generally due to CMC removal (Fig. 9(c)), differently from the irradiated hair samples, in which the separation takes place in the outer β -layer (Fig. 8(c) and (d)). Furthermore, some pieces of the cuticle materials remain on the hair surface, as shown in Fig. 9(b). Thus, both FESEM and TEM images, showed that the washings cause mechanical

damages on the hair strands, removing cuticle cells and leaving some residual cuticle materials on the surface.

The number of cuticle cells was also counted in other strands from dark brown hair before and after washing. Before the treatments, the second strand had an average of 4.1 ± 0.9 ($n = 45$) cuticle cells, while after 60 washes, 3.3 ± 1.0 ($n = 47$) cuticle cells remained. In the case of the third strand, which had an average of 5.7 ± 1.1 ($n = 43$) cuticle cells before the treatment, the cell number became 4.6 ± 0.8 ($n = 41$) after 60 washes. Therefore, about 1 to 2 cuticle cells (considering the three strands) were removed when hair underwent washing.

The new proposed TEM experimental procedure to analyze neighboring slices of the same strand, allowed to count the cuticle cell number in very close regions before and after washings. Therefore, it is possible to affirm that extractable material and cuticle cells were removed when hair washed, and also that the CMC was the structure more prone to rupture and degradation.

Cuticle cell removal is intensified when washings are combined to irradiation. Fig. 10 shows images of the control sample (untreated slice of the strand) and of the corresponding slice in the same strand irradiated for 600 h and washed 60 times. The average number of cuticle cells in the control sample was 6.2 ± 0.6 , as obtained in 54 measurements around the strand diameter, while after 600 h of irradiation, combined to 60 washings, the cuticle cell number decreased to 2.6 ± 1.8 (51 measurements). Also, before irradiation and washings, the endocuticle layers and the CMC were entire and in good condition (Fig. 10(a)), as expected for an untreated hair strand. After irradiation and washings, the CMC was severely damaged and the cuticle cells were being

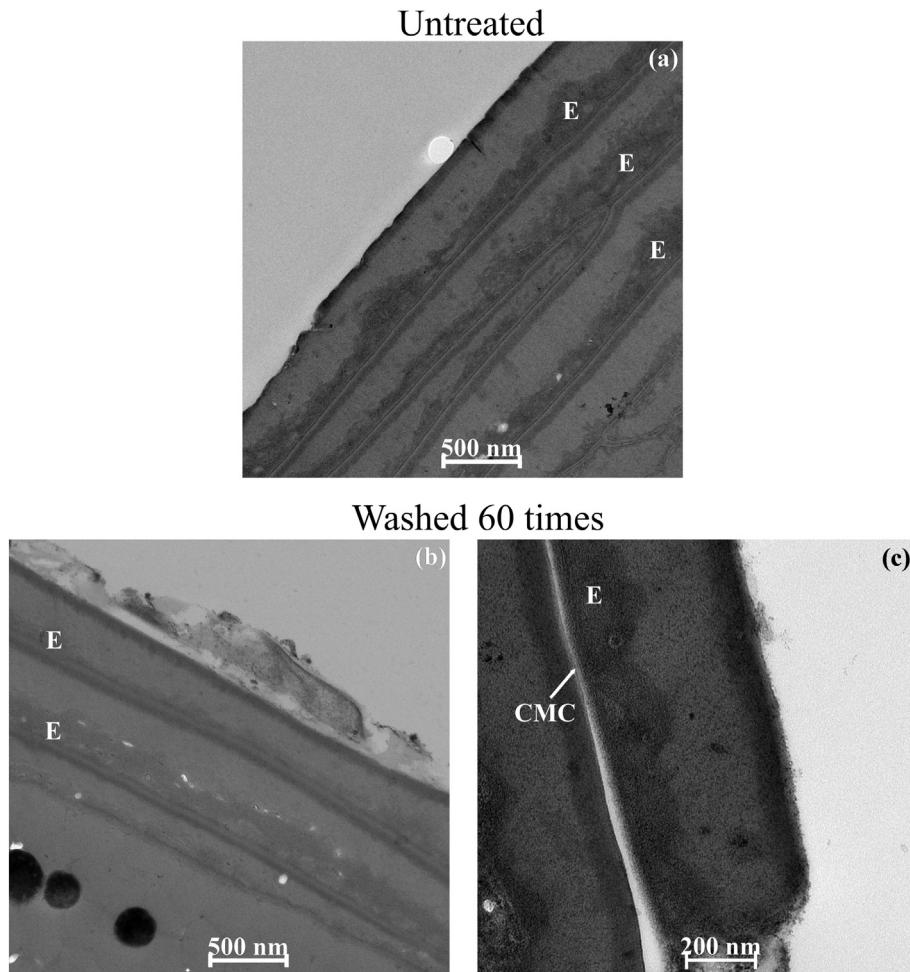


Fig. 9. Images of ultra-thin sections of the cuticle cells in dark brown hair obtained by TEM, showing cuticle cells of the same hair strand: (a) before and (b) and (c) after 60 washing steps. Index E indicates the endocuticle layer and the arrows show the cell membrane complex (CMC).

removed, as indicated by the arrows in Fig. 10(b). Thus, the irradiation and washing combined processes act removing cuticle cells, by cleaving them preferentially at the CMC, as pointed out in Fig. 10(c). Cuticle cells were separated in two ways in hair irradiated for 600 h and washed 60 times: 1) via the rupture of the outer β -layer of the CMC (Fig. 10(c)), similarly to what was observed in hair only irradiated; and 2) via complete removal of the CMC (Fig. 10(b)), the same as observed in hair only washed.

The number of cuticle cells was also counted for other strands used in irradiation and washing treatments. Initially, the second strand had an average of 4.6 ± 1.4 ($n = 50$) cuticle cells, while after irradiation and washings, only 2.5 ± 1.5 ($n = 53$) cuticle cells remained. In the case of the third strand, which presents an average of 1.0 ± 0.7 ($n = 40$) cuticle cells in the control sample, no cuticle cells remained after irradiation and washings. About 1 to 5 cuticle cells were removed (considering the three strands) when hair was submitted to irradiation and washing processes. These results indicate that the combination of irradiation and washing removes a greater number of cuticle cells in comparison to the washing procedure alone. This effect results probably from the combination of photodegradation damages in the layers responsible for adhesion between neighboring cuticle cells, with the mechanical stress caused by washing, which will then remove the more vulnerable cuticle cells. This idea is reinforced by the observation that the endocuticles do not always appear brighter in samples that were irradiated and washed for 600 h (Fig. 10(b)), indicating that the brighter and thus degraded cuticles were already removed. It is important to highlight that a very gentle mechanical stress was applied by the fingers

to these samples during hand-washing. This process is very similar to daily events experienced by hair strands, such as sun exposure, followed by washings, and can be responsible for macroscopically noticeable effects, such as split ends in hair strand, when all cuticle cells are removed and the cortex is exposed.

Images of TEM show that endocuticle and CMC are the regions in the cuticle structure more susceptible to the damages caused by irradiation and washings. Irradiation degrades the outer endocuticle layers and the outer β -layer located in the CMC, which contains 18-methyl eicosanoic acid (18-MEA). Washings remove cuticle cells, generally by cleavage and complete removal of the CMC structure. When hair undergoes irradiation and washings, the damages caused by both processes were simultaneously observed. In this case, the cuticle cells were removed, as occurred in washed hair, and the outer β -layer was degraded, as occurred in irradiated hair. To our knowledge, this is the first study detailing the morphology of a region of untreated hair in comparison to a treated hair strand.

4. Conclusions

The results presented herewith show that both irradiation and hand-washings cause morphological damages to the hair cuticle, which are described in detail in this work. Photodegradation is more harmful to the endocuticle and to the outer β -layer, and its main damages are the formation of cavities in the endocuticle (with diameters of 50–700 nm) and the detachment of the outer β -layer. Washings are responsible for mechanical damages to the hair structure, such as partial

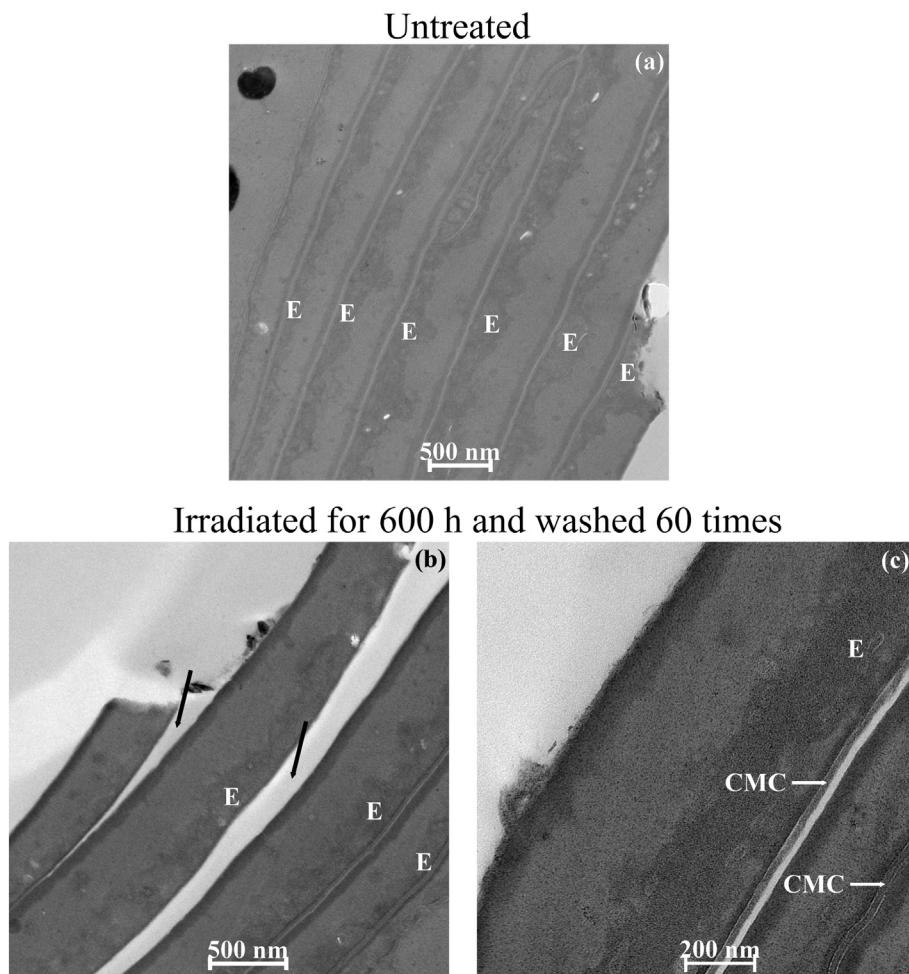


Fig. 10. Images of ultra-thin sections of the cuticle cells in dark brown hair: (a) untreated hair and (b) and (c) irradiated for 600 h with a mercury lamp and washed 60 times, showing cuticle cells of the same hair strand. Index E indicates the endocuticle and the arrows show the cell membrane complex (CMC).

or complete removal of the endocuticle or the entire cuticle cells. The association of this two treatments (irradiation and washings), which is frequent in daily life, intensify the damages to hair cuticle, for instance, peeling of the cuticle layers and complete cuticle removal, thus contributing significantly to hair fragility and opacity.

Acknowledgements

The authors thank CAPES for financial support, also the LME/LNNano/CNPEM and the INOMAT/IQ/UNICAMP for the technical support during the electron microscopy analyses.

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