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Effect of photodamage on the outermost cuticle layer of human hair

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ABSTRACT

The surface of the hair is the region most exposed to solar radiation and to the environment in general. Many of the well-known damaging effects of sun exposure on hair must start or even be restricted to the most external cuticle layers. As such, this work investigates morphological, ultrastructural and chemical changes in the outermost cuticle layer of dark brown hair, using atomic force microscopy (AFM), field emission scanning electron microscopy (FESEM) and attenuated total reflectance Fourier transform infrared spectroscopy (ATR-FTIR). The results showed that after 230 h of irradiation with a mercury lamp, small bumps with globular shape (heights lying in the 1–5 nm range) appeared on the cuticle surface and their size increased with increasing irradiation times. In addition, the enlargement of pre-existing holes was also observed (holes increase around 350% in depth) and the height of the steps formed between the edges of two cuticle scales increased around 65%, as a result of 500 h of irradiation. The damages in hair strands were accurately identified by analyzing exactly the same surface region before and after irradiation by AFM images. Finally, the results were discussed in terms of the chemical differences between the non-irradiated and the irradiated hair, for instance, the increased level of cystine oxidation as a consequence of photodegradation.

1. Introduction

Changes in hair color due to sunlight exposure are a well-known effect in hair science [1,2,3]. Also, the damaging action of irradiation on hair chemical components, such as proteins, lipids and melanin pigments [4,5,6,7] has been reported. Hoting et al. [5] using amino acid analyses, showed that photodamaging is more pronounced in amino acid residues from the outermost layers of the hair strand than on those from the inner layers. These authors assigned this effect to the absence of melanin, which is the pigment responsible for photoprotection, in the outer hair layers. In a previous paper [1], we also showed that the photobehavior of white hair, irradiated with a mercury vapor lamp or with solar radiation, is dependent on the wavelength range of the incident radiation and on the initial shade of yellow in the sample.

Concerning the changes in hair morphology and ultrastructure due to photodegradation [8,9,10] there are only a few studies addressing this topic. Since the cuticle is the most external layer of the hair strand, it is the one most exposed to chemical and environmental damages. Cuticle cells are closely arranged forming 5 to 10 overlapping layers from the root towards the tip of the hair and provide a chemically resistant sheet protecting the internal hair components, such as the cortex and the medulla. The cuticle is formed by amorphous material and each cuticle cell is divided into four subunits that have a distinct chemical composition [11,12]: 1) The endocuticle, which is the most internal layer, is made up of non-keratinous material (≈3% *w*/*w* cystine) and is hydrophilic; 2) The exocuticle and 3) The A-layer are both hydrophobic and more reticulated, due to the higher cystine content (≈15% and ≈ 30% *w*/*w*, respectively); and 4) The epicuticle, which is the most external layer of the cuticle cell, and is thin (≈2.5 nm), hydrophobic and cystine-rich (≈12% *w*/*w*). The cystine groups of the epicuticle are bound to a lipidic layer (18-methylenicosanoic acid), forming a proteolipidic membrane that covers the cuticle [13]. The study of radiation effect on the cuticle is remarkably important, due to its protective role for the hair internal structures.

Weigmann et al. [8,9] used field emission scanning electron microscopy (FESEM) to analyze the UV radiation effect on hair surface and concluded that the cuticle is the region most affected by photodegradation, due to its high cystine concentration. They also observed that humidification cycles, intercalated with the UV irradiation periods, intensified the damages, since they caused thinning of the cuticle cell. The thinning of the surface cuticle was also observed by Longo et al. [10] that used scanning electron microscopy (SEM) and atomic force microscopy (AFM) to investigate the morphology of Caucasian black hair irradiated for 160 h with a xenon lamp. These samples were irradiated under high relative humidity and temperature (50–100% and 60 °C, respectively) and the most important photodegradation effects observed were the extraction of the cuticle scales and the formation of bubbles (≈200 nm) on the cuticle surface. Both papers studied the photodegradation action by comparing different strands in blended hair sample (mix of strands of many heads) [8,9,10].

It is a great challenge, however, to distinguish the photodamage effects on hair, because of the intrinsic morphological and chemical variations in hair strands. These differences can be found between strands

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from different heads (genetic variability), or between strands with distinct degradation levels from the same head or even between the root and the tip in a single hair strand. Dubief [14], for instance, showed a drastic increase in the cysteic acid content detected by infrared spectroscopic (IR) between the roots and the tip ends of virgin hair. This author suggests that this effect was caused by photo-oxidation, since cysteic acid is known to be a product of cystine photodegradation [15]. Also, Swift and Smith [16] showed that the hair surface is significantly changed along the hair length. While the scale edges present a smoother and more regular contour close to the strand root, as the distance increases towards the strand tip, the scale contour becomes more uneven, rougher and partially broken.

To overcome the intrinsic differences in hair strands before irradiation, it is important to obtain statistically representative images by analyzing: many strands from the same sample; and many different regions from each strand. The third interesting approach is to acquire images from the same region in the same sample before and after irradiation, so that the photodegradation effects on hair can be isolated from the pre-existing differences [17,18]. AFM is a very promising technique for such approach, because it is non-invasive and it does not require sample preparation (such as sputtering, carbon evaporation, microtome cutting or staining), allowing the samples to be analyzed in their natural state. Luengo and collaborators applied this methodology of analyzing the same sample area to investigate chemical properties of the human hair cuticle and its changes following a bleaching process. Chemically modified AFM tips, terminated with CH_3— and NH_2— groups, were used to achieve chemical contrast between hydrophobic and hydrophilic regions on the surface [18].

In the present work, the three approaches were used to investigate the photodegradation action on the morphology of hair cuticles. AFM is used to evaluate morphological changes taking place in exactly the same area on the sample surface before and after irradiation with a mercury lamp for periods up to 500 h. To our knowledge, this is the first time this methodology is applied with this purpose. Besides this, we used FESEM to scan different strands in each sample and different areas in the same strand, so that the results could be considered statistically valid. FESEM is a very convenient technique for sample mapping, because it allows a broad range of magnifications that can be easily and quickly adjusted during scanning. Finally, the chemical changes as a consequence of photodegradation were evaluated using attenuated total reflection (ATR).

2. Experimental

2.1. Samples

Dark brown hair was purchased from De Meo Brothers Inc. located in New York, USA (called “blended brown hair” along the text). Dark brown hair was also collected from one volunteer with no history of chemical treatments (called “brown hair from single-donor” along the text).

Prior to the experiments, the hair samples were washed with a 2.0% *w/w* sodium lauryl sulfate aqueous solution, according to the following steps: 1) hand-washing with 1 mL of the solution for 1 min; 2) rinsing with tap water at room temperature for 30 s; and 3) repetition of steps 1 and 2, but with rinsing for 2 min. The samples were then dried at room temperature and stored in plastic bags.

2.2. Radiation Sources

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 (Fig. 1), in addition to emitting very low infrared (IR) radiation. It is thus very different from the continuous solar spectrum. The overall procedure for irradiation with a mercury vapor lamp is described elsewhere [3]. 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.

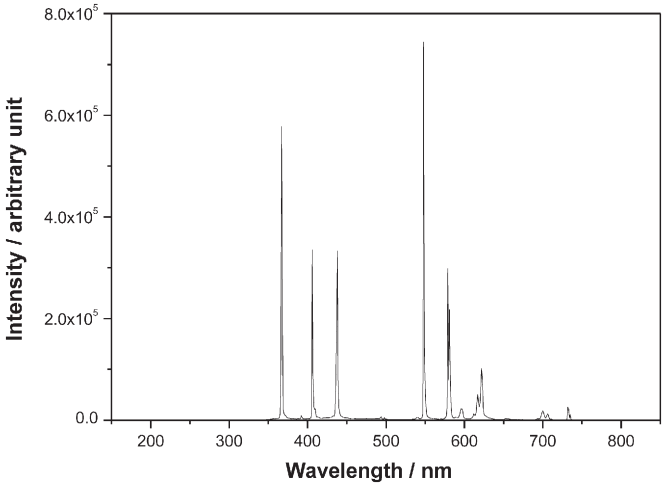

Fig. 1. Emission spectrum of the mercury vapor lamp (125 W).

2.3. Sample Irradiation

Hair strands were daily irradiated using a mercury lamp full-spectrum (UV, Vis and IR) for a 10 h period, which was followed by a 14 h period in the dark (inside a laboratory cabinet), after which the sequence was repeated cyclically. Irradiation was carried out inside a fume hood, as described elsewhere [3], for periods of 230, 500 or 600 h. The temperature and the relative humidity inside the fume hood were monitored daily and kept under 29.3 ± 0.9 °C and 44 ± 4% average values, respectively. The temperature data indicate that the mercury lamp emitted very low IR radiation. The doses of radiation intensity obtained for the mercury vapor lamp during the 10 h exposure were: 1.6 J cm^{-2} (UVB), 56.0 J cm^{-2} (UVA), 11.0 J cm^{-2} (Vis) and 313.0 J cm^{-2} (IR).

2.4. Atomic Force Microscopy (AFM)

AFM images were obtained in non-contact mode, using a Park NX10 Scanning Probe Microscope from the National Laboratory of Nanotechnology (LNNano/CNPEM), located in Campinas, SP, Brazil. The temperature was controlled at approximately 24 °C and the relative humidity was kept under 15% by a N_2 flow. Measurements were carried out using SiN_4 probes (NANOWORLD), NCHR model, with a 320 kHz nominal resonance frequency, a 42 N m^{-1} constant force and a typical tip diameter around 15 nm. Images with improved resolution were obtained with a sharper tip (SSSNCH model, with approximately 3 nm diameter). Blended hair strands were fixed on the sample holder by their ends, using silver adhesive. Images were obtained in three different strands of the sample (2 areas per strand, 10–60 images per strand). The same areas were imaged before and after irradiation. The software Gwyddion 2.36 was used for data treatment and to obtain the root mean square (RMS) roughness from topography images. RMS roughness is defined as the standard deviation of the height (z values) within the selected area (1 μm × 1 μm):

$$\mathrm{RMS} = \sqrt{\frac{\sum (Z_n - Z_{ave})^2}{N}} \quad (1)$$

where z_{ave} is the average of the z value within a specific area, z_n is the z value for a given point within this area and N is the number of measured points.