Charge density alterations in human hair fibers: an investigation using electrostatic force microscopy

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Synopsis

A new method for high-resolution analyses of hair surface charge density under ambient conditions is presented in this paper. Electrostatic force microscopy (EFM) is used here to analyze changes in surface charge density in virgin hair, bleached hair, and hair treated with a cationic polymer. The atomic force microscopy technique is used concomitantly to analyze morphological changes in hair roughness and thickness. The EFM images depict exactly how the polymer is distributed on the surface of the hair fiber. The EFM's powerful analytical tools enabled us to evaluate the varying degrees of interaction between the hair fiber surface charge density and the cationic polymer. The surface charge density and the polymer's distribution in the hair fibers are presented in the light of EFM measurements.

Résumé

Ce travail présente une nouvelle méthodologie pour analiser la densité de charge superficielle à température ambiante avec une grande resolution. La microscopie à force électrostatique (EFM) a eté utilisée pour mesurer la densité de charge supeficielle des cheveux vierges, décolorés et traités avec un polymer cationique. La technique de microscopie à force atomique (AFM) a eté utilisée simultanément pour évaluer les changements de morphologie, de rugosité et d'épaisseur des cuticules. Les images EFM nous ont permis de visualiser la dègradation et la distribution du polymère sur la superficie des cheveux. Grâce à la puissance analytique de l'image EFM, les différents degrés d'altération des fibres capilaires avec le polymère ont pu être observés. Les images de la densité de charge superficielle et de la distribution du polymère sur la surface des cheveux ont été présentées à la lumière de l'EFM.

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Introduction

Constant investigations for the technological development of hair care products that surpass the consumer's expectations have long represented a challenge for researchers and scientists. Many investigations have focused on developing effective cosmetic products for human hair, and scientists have employed a variety of methods in their attempts to understand the alterations and prove the cosmetic efficacy of these products [1].

Human hair is structured in highly organized strata that are highly resistant to external stimuli. However, morphological changes can occur through daily hair care routines [2]. Hair damage leads to a change in the physical properties and modifications in the surface charges of hair.

Mechanical and electromechanical stresses induce the formation of submicrocavities within polymer materials [3]. Electrons can then move

without scattering within the submicrocavities, leading to further degradation. This raises the possibility of the formation of significant amounts of carbon and oxygen anions and cations following polymer stressing, which would then be trapped in the dielectric [4].

Cationic ingredients in general are highly substantive to hair because of its low isoelectric point, which is approximately pH = 3.67 in cosmetically unaltered hair, and even lower in bleached hair. Therefore, at any pH above the isoelectric, the surface of hair bears a net negative charge and positively charged (cationic) ingredients are attracted to it [5]. Most often, the active component of the conditioning formulation is a polymeric (such as polyquaternium 6®) or monomeric (such as trimethyl ammonium bromide, CETAB) cationic quaternary compound, which has a great affinity for the negatively charged hair fiber surface. These conditioners adsorb strongly by electrovalent interaction with sulfonic acid groups on the hair surface. Interaction between the conditioner and the keratin fiber can lead to different degrees of interaction on the surface of the hair fiber [6]. Jachowicz et al. [7] studied the generation of static charge on intact and modified human hair fibers in the rubbing mode, using a variety of metal and polymer contact probes.

Andre et al. [8] studied the adsorption of conditioning polymers employing atomic force microscopy (AFM) to characterize the polymer/sufactant complex. Pfau et al. [9] used the AFM to characterize the adsorption and desorption behavior and morphology of a set of polyquaternium polymers on human hair. Results were in line with a simple model of coulombic interaction between hair and polymer and were interpreted on this basis.

Electrostatic phenomena in insulators have been known for the past four centuries, but many related questions are still unanswered, for instance: what is the distribution of electric potentials across an organic polymer or ionic non-conducting material, and how does it contribute to the mechanical, optical, adhesion, and electrically insulating properties of the solid? [10].

A new possibility to address these questions was created recently, thanks to advances in analytical electron microscopy [11]. The electrostatic force microscope (EFM), for instance, maps the variation and potential energy difference between a tip and a sample arising from non-uniform charge distributions and local variations in surface work function [12].

An up-to-date text on dielectrics emphasizes a fourth component of the electrical polarization of a dielectric or insulator. This component is interfacial polarization, the result of a local accumulation of charges resulting from the migration phenomena and concentrating around imperfections such as impurities, defects, grain boundary, cuticle boundary, and others [13].

An analysis by EFM was performed to detect electrostatic forces. Differences induced by electrostatic forces on the surface of hair fibers during scanning are detected and give a qualitative measurement of the local charge density 'J.S. Vasconcelos et al., unpublished data'.

When a voltage is applied between the tip and the sample, an interaction occurs between the charges and energy variations in the sample's stored capacitances. The force the hair fiber is subjected to under an applied dc bias is due to the charge–charge interaction, and changes in capacity energy, which is given by:

$$\text{Force} = \frac{q_s q_t}{4\pi\varepsilon_0 z^2} + \frac{1}{2}\frac{dC}{dz}\left(V_{applied} - V_{contact}\right)^2$$

were q_s is the surface charge, q_t is the charge induced on the tip, z is the tip–sample separation, C is the capacitance between the tip and the sample, $V_{applied}$ is the applied voltage, and $V_{contact}$ is the contact voltage 'J.S. Vasconcelos et al., unpublished data'.

In the present work, we evaluate the surface charge density of virgin hair and bleached hair measured by EFM. AFM is used simultaneously to study the topography of the fiber's surface. In the same way, we evaluate the different degrees of charge density interaction of the cationic polymer (PQ-6) on the hair fiber surface.

Experimental

Materials

Untreated black Caucasian hair was obtained from De Meo Brothers, New York, USA. Hair tresses weighing approximately 1.0 g and a length of 25.0 cm each were prepared for this work. The hair samples were pretreated with 3% lauryl ammonium sulfate (LAS) at 25°C and then air-dried.

Bleaching

The bleach solution was prepared by mixing a 6% hydrogen peroxide (H_2O_2) solution with a

concentrated ammonium hydroxide solution and ammonium persulfate powder in a 2:1:1 weight proportion, respectively. The tresses were left in the bleach solution for 30 min at 25°C. After bleaching, the tresses were rinsed in abundant distilled water, washed out with a solution of 3% LAS, and air-dried. This treatment turned the tresses light brown with a reddish tone.

Treatment

The virgin and bleached hair tresses were washed five times with a solution containing 3% of PQ-6. Figure 1 shows the structure of the monomer of the PQ-6 polymer. The PQ-6 polymer is a homopolymer with an average molecular weight of approximately 100 000 and a charge density of approximately 126.

Two hundred microliters of the solution were left for 30 s in contact with the hair fibers, which were then rinsed in distilled water for 1 min and air-dried at 25°C. This procedure corresponded to one washing.

Atomic force microscopy

Atomic force microscopy studies were performed using a Digital III-Instrument under atmospheric conditions at room temperature. Imaging was obtained in the contact mode, using silicon nitride profile tips mounted in a cantilever at a 3.6 nN force constant. The AFM images were processed with Nanoscope® software (Digital Instruments, Inc., Santa Barbara, CA, USA), which was also used to measure the roughness and thickness of the cuticle layers.

Electrostatic force microscopy

The measurements by electrostatic force microscopy were taken as function of tip voltage and characterized by the contrast of the EFM signal, which is altered with variations in the tip bias. The EFM signal is obtained by changes in the resonant frequency in the oscillating tip. Bias
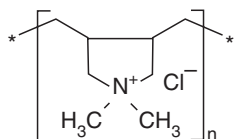

Figure 1 Polymer PQ-6.

voltage was applied to the tip and the hair fibers were grounded. The tip–sample separation, z, was set at 25 nm. The EFM and AFM measurements were taken simultaneously.

Statistical treatment

The roughness and thickness values of the cuticle layers were treated statistically by means of the Prism® 2.01 software (GraphPad Prism version 4.00 for Windows, GraphPad Software, San Diego, CA, USA), using Tukey's multiple comparison test. The symbol P indicates the probability that one hair is statistically equal to or different from another hair. One minus P represents the level of confidence that the sets of hair were really different. These data confirmed the varying level of confidence that the sets of hair were really different [1].

Results and discussion

Figure 2 shows the AFM and EFM images of virgin hair, and virgin hair treated with PQ-6. Note that the virgin hair (Fig. 2A) has uniform, regular-shaped cuticle layers oriented longitudinally and aligned along the length of the fiber.

The EFM images show negative charges varying from magenta (more negative) to red (less negative), and positive charges varying from green (more positive) to yellow (less positive). In this instance, the bias was switched to 0.0 V. Figure 2(B) presents the EFM image of a virgin hair with an overall homogeneous positive and negative charge distribution on the surface and a slight accumulation of negative charges at cuticle edges. These charges were attributed to the structures of the hair's amino acids. Cuticle edges are more susceptible to the accumulation of charges because they are interfacial regions and therefore prone to concentrate defects.

Figure 2(C) shows the AFM images of virgin hair treated with PQ-6, revealing a morphology similar to that of Fig. 2(A), although the roughness of the fiber is reduced, as shown in Fig. 3. This reduction may be attributed to the deposition of polymer on the surface of the hair fibers. The mean roughness, Rm, is defined as the arithmetical average of the absolute values of the surface deviations, measured from the mean plane within the box cursor [1]. The hair's surface roughness revealed in the various AFM images was calculated for three regions, using AFM's Nanoscope® software, which was also used
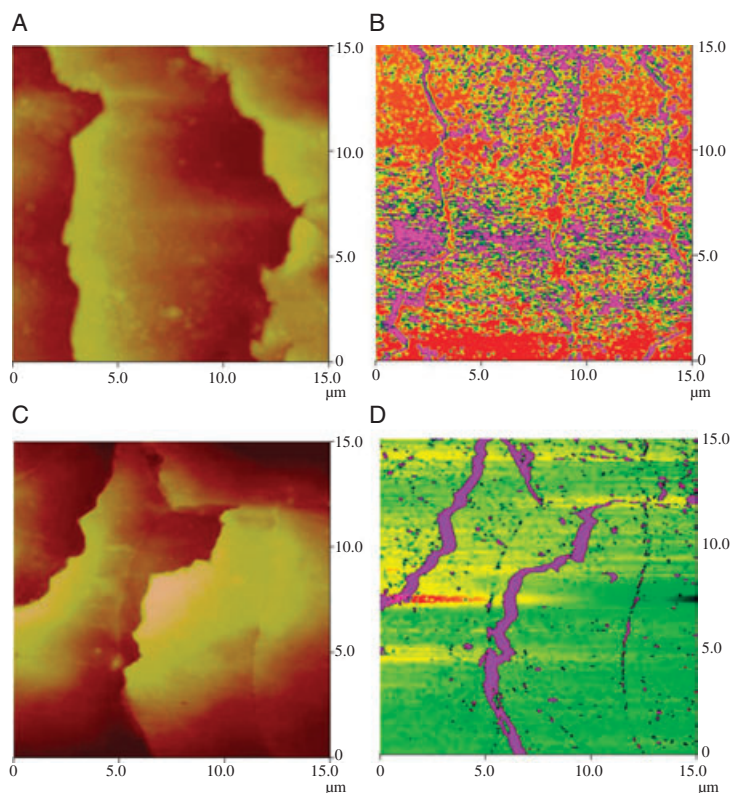
Figure 2 AFM images of virgin hair (A, B) and EFM images of virgin hair treated with PQ-6 (C, D).
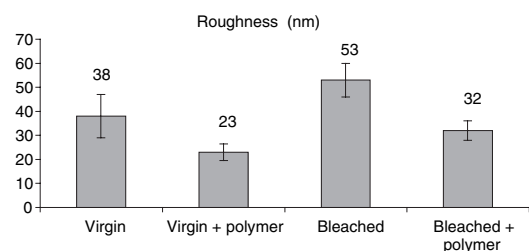

Figure 3 EFM images of cuticle surface roughness of hair subjected to different treatments. Tukey's multiple comparison test: virgin vs. virgin + polymer ($P < 0.001$); bleached vs. bleached + polymer ($P < 0.001$).

to evaluate cuticle thinning based on cross-sectional analysis. Three cross-sections of each region were prepared for analysis, and revealed in different profiles according to the various treatments. The cuticle thickness values were calculated by measuring the maximum peak distance in each cross-section of the different profiles (Fig. 4). The procedure of cross-sectioning is given in Fig. 5. The lesser thickness of virgin hair treated with polymer was likely evidence of the deposition of a polymeric film on the surface of the treated hair. However, further information is

required to define how the deposition of the polymer on the hair's surface occurs, i.e. does the polymer become homogeneously distributed, forming a polymeric film on the hair's surface, or is the deposition site-specific?

The EFM image in Fig. 2(D) depicts the hair fiber treated with polymer and may provide an answer to this question. As can be seen, the image differs significantly from Fig. 2(B). The deposition of the polymer on the hair's surface completely altered the charge distribution in the fiber. This image reveals two very distinct regions: at the cuticle edges there is an accumulation of negative charges (magenta), whereas the cuticle surface shows an accumulation of positives charges (green). The accumulation of negative and positive charges indicates the presence of effective barriers in the material 'J.S. Vasconcelos et al., unpublished data'. The commercial polymer PQ-6 is cationic and interacts with the anionic regions of the hair fibers, appearing green in the EFM image. The regions where cationic polymer was not deposited appear to be negatively charged. This finding leads to the assumption that, in the regions where polymer was not deposited, a migration of charges
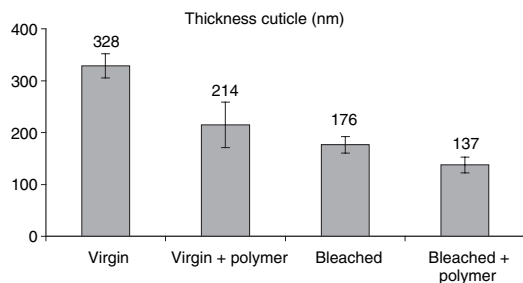
Thickness cuticle (nm)

Figure 4 AFM images of cuticle thickness of hair fibers subjected to different treatments. Tukey's multiple comparison test: virgin vs. virgin + polymer ($P < 0.001$); bleached vs. bleached + polymer ($P < 0.001$).

occurred due to the intensification of negative charges in those regions. Those regions appear to be the cuticle edges. The explanation for this apparent discrepancy, as the cuticle edges have a tendency to concentrate negatively charged defects and should therefore interact preferentially with the cationic polymer, is that there may be a physical obstacle to the polymer's adherence. This would make the polymer's adsorption difficult in the sheath of cuticles edges due to the cuticle's

topography and thickness of virgin hair (close to 330 nm, Fig. 4).

Figure 2(D) depicts exactly how the polymer is distributed on the surface of the hair fiber, clearly indicating that, on the surface of the cuticles, the polymer forms a discontinuous and positively charged film.

Figure 6 shows AFM and EFM images of bleached hair, and bleached hair treated with PQ-6. The morphology revealed in the AFM images indicates that the cuticle layer in the bleached hair (Fig. 6A) underwent a process of chipping, extraction and erosion, resulting in dryness and greater susceptibility to further damaging action, which may involve large segments or sections of scales being ripped from the hair. The surface roughness (Rm), which was calculated as described earlier, increased with the bleaching process, as demonstrated in Fig. 3. The cuticle thickness evaluation results showed lower values for bleached than for virgin hair. This observation is consistent with the destruction of the cuticle structure and layers due to the deterioration of protein, which causes reductions in cuticle thickness.
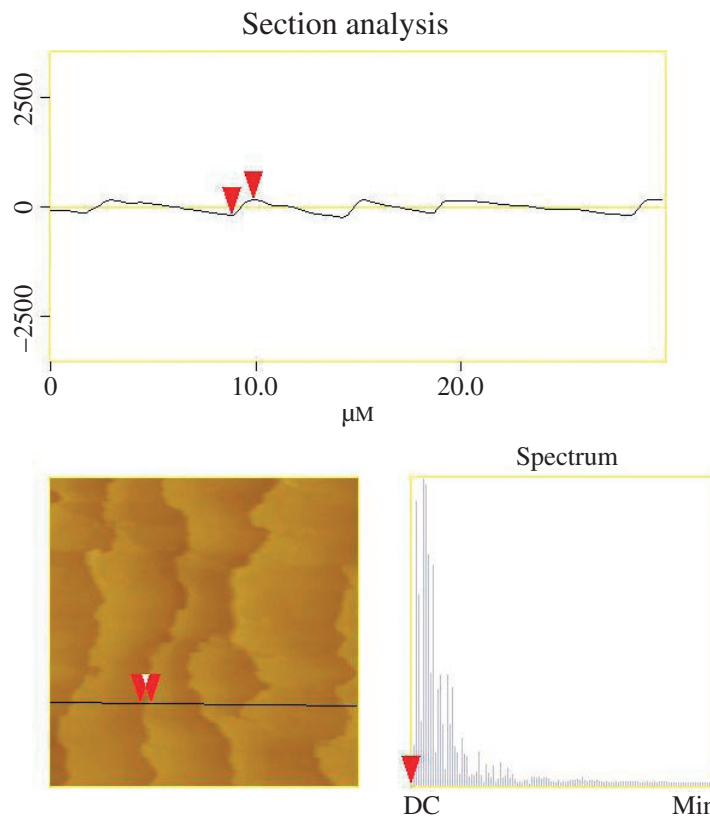

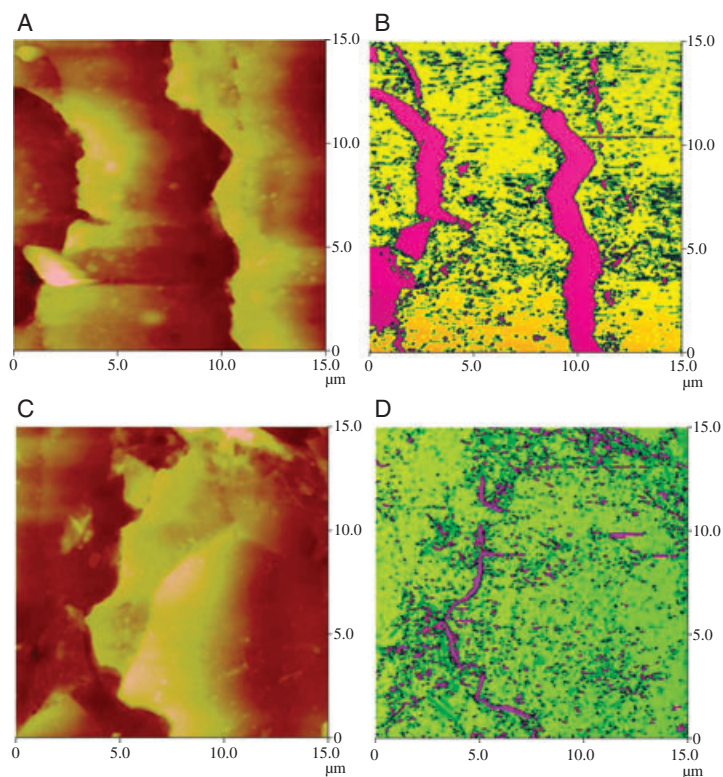**Figure 5** Proceeding of cross-section analysis of AFM images.

Figure 6 AFM images of bleached hair (A, B) and EFM images of bleached hair treated with PQ-6 (C, D).

The whole-fiber amino acid composition of human hair when bleached is described in the literature. These data suggest that the primary chemical differences between extensively bleached hair and unaltered hair are lower cystine content, higher cysteic acid content, and lower amounts of tyrosine and methionine in bleached hair. These findings suggest that the reaction of bleaching agents with human hair protein occurs primarily in the disulfide bonds [5].

Figure 6(B) depicts an EFM image of bleached hair, revealing that the bleached fiber contains a higher concentration of negative charges at the cuticle edges. This indicates that cystine degradation occurs preferentially at the cuticle edge, which is an interfacial region. The concentration of charges in cuticle edges is visual confirmation of this degradation. The negative charges are anionic sites on the fiber's surface and evidence of cysteic acid and other products of degradation.

When the bleached hair fibers were treated with polymer, the general appearance of the cuticle's integrity seemed improved (Fig. 6C) as a result of the deposition of polymer on their surfaces. The adsorption of polymer in hair fibers reduces their roughness (Fig. 3) and hence, their porosity. The

lower thickness values (Fig. 4) of treated hair result from this deposition. Again, the AFM images and cuticle thickness and roughness values do not provide sufficient information about the distribution of polymer on the fiber.

The EFM image (Fig. 6D) indicates a considerable change in charge distribution, with an almost total absence of negative charges, due to the interaction of the cationic polymer with the anionic site created in the bleaching process. The recovery of polymer in the fiber is practically complete. The deposition is homogeneous but not continuous, with a few negative charges distributed punctually along the film. Unlike the virgin hair, the bleached hair cuticle is less thick (close to 175 nm, Fig. 4) and shows a considerable accumulation of negative charges at the edges, physically and chemically facilitating the adhesion of polymer in the cuticle layers. The penetration of low-molecular-weight components of the cationic conditioning compounds into the intercuticular regions leads to plasticization of the cuticular sheath [6]. Therefore, polymer treatments are more effective in degraded hair fibers, which have a greater number of anionic active sites for interaction, lower cuticle thickness, and greater roughness.

Conclusions

The results of this work successfully demonstrated that EFM techniques can be used to detect local variations in charge densities in nano- to micron-sized domains of hair fiber. However, the values of cuticle thickness and roughness provided by the AFM images provide insufficient information about the distribution of polymer in the fiber. On the other hand, the EFM images clearly reveal the degradation and distribution of polymer in the hair fibers.

The changes in the surface charges of hair fibers subjected to different treatments can be efficiently monitored through this technique. We demonstrated that the distribution of polymer varies depending on the initial charge distribution on the surface of hair fibers and on the initial conditions of roughness and cuticle thickness. The treatment is more efficient in damaged hair fibers because they have more numerous active anionic sites to interact with the polymer and their topography facilitates its adsorption to the cuticle layers.

The advantages of EFM may render it the ideal choice for studies of charge distribution, interaction of cosmetic products with hair fibers, and for viewing cosmetic components on hair surfaces.

Acknowledgements

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