Atomic Force Microscopy of Human Hair Cuticles: A Microscopic Study of Environmental Effects on Hair Morphology

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We have used an atomic force microscope to provide quantitative real-time analysis of human hair morphologic changes under ambient conditions. This form of microscopy combines the lateral resolution of an electron microscope and the flexibility of a light microscope. Three experiments were performed: a study of hair morphology in air versus water, a kinetic study of hair hydration, and a determination of how pH changes affect hair morphology. The overlapping keratinized cells that form the hair cuticle spread out between 50 and 150% when hydrated, compared to a total shaft diameter change of 10%. This hydration reaches a saturation point within the first few minutes after immersion. Also, hair swells much more at higher pH. J Invest Dermatol 105:96–99, 1995

The growth of hair follicles and the accompanying keratinized cells has been studied for many years and is well understood [1]. However, the mature hair shaft has been the subject of less intense analytical study. The effects of external chemicals on the hair shaft are of great interest in both the medical field and the cosmetic industry. Current methods to analyze these effects consist mainly of macroscopic testing such as touch and tell little about the actual hair morphology.

Light microscopy has been used to analyze macroscopic properties of hair using numerous staining techniques [2]. Electron microscopy has complemented these experiments and has been a useful tool to study microscopic hair morphology [3]. However, these techniques have two very restrictive limitations; light microscopy is limited in lateral resolution whereas electron microscopy does not allow ambient imaging conditions.

We have explored a new method for analyzing hair morphology at high resolution and under ambient conditions. An atomic force microscope (AFM) [4,5] has been used in these experiments to analyze hair morphology as the environmental conditions are changed, allowing the direct study of hydration effects. The AFM combines the lateral resolution of an electron microscope with the environmental flexibility of the light microscope. Also, the height resolution is superior to either technique.

Traditional microscopy utilizes solid lenses to bend light rays and magnify images onto a focal plane. However, due to diffraction, these techniques are limited in resolution to approximately half the wavelength of light. One way to overcome this problem is to turn to alternative forms of radiation that have much smaller wavelengths, such as electron microscopy (EM). However, EM requires that imaging be done in a vacuum, and insulating biologic samples usually requires a metal coating prior to imaging.

Probe microscopy utilizes an alternative imaging mode where a sharp tip is brought very close to a surface and the tip-sample interaction is monitored as the tip is scanned. The resolution for this approach is limited by the size of features on the probe. To image using AFM, a cantilever with a small pyramidal tip is placed over the sample and lowered into mechanical contact. A tube piezoelectric is then used to scan the tip over the sample in sub-angstrom steps. The deflection of the cantilever corresponds to changes in the height of the sample. These motions are monitored by reflecting a laser beam from the cantilever onto a position-sensitive two-quadrant photodiode (see Fig 1).

AFM has been used to study a number of interesting biologic systems. For instance, very high resolution images of DNA, proteins, and DNA-protein complexes have been obtained under physiologic conditions [6–8]. However, true molecular resolution was not observed due to the large tip sizes; this hindrance remains a problem for all AFM experiments. Larger biologic systems have also been studied, such as living neurons and activated platelets [9,10]. However, no quantitative data about hydration rates or pH dependence has been extracted from these studies.

MATERIALS AND METHODS

These experiments have been performed with a Topometrix [11] AFM system with in-house software [12]. A pivoting 75-μm scanner and standard pyramidal tips were used in all of these experiments.

Hair Samples All the hair samples were taken from the heads of undergraduate students at Caltech. We have imaged hair from seven students. The areas of the hair imaged were at least 1 cm away from the scalp. Because this study was designed to demonstrate the power of AFM as an analytical technique for studying hair morphology, we took no precautions about hair condition, care, diet, etcetera prior to acquisition of the samples.

To prepare the samples, we cut the hair, stretched it over a sample stud, and bonded it with five minute epoxy on the ends to hold it in place. The samples were rinsed with water to remove excess contaminants before imaging.

Three different experiments were performed: 1) a comparison of hair morphology in air versus water; 2) a kinetic study to determine the rate of...
hydration of the hair; and 3) a determination of the hair morphology changes versus pH.

For all seven subjects, we took and compared over 200 images of hair in water and 100 images in air. All of the images shown in this report are quite typical and reproducible. To determine the rate at which the hair absorbs water, we imaged an area of a hair in air, added distilled water, and then took another image every 30 seconds for 7 min. The steps in the region imaged were then analyzed and averaged. This procedure was performed on two subjects. We also studied longer term hydration by taking a scan every 2 min for 40 min. We repeated this procedure for four subjects.

To determine the effects of pH on hair morphology, a hair was soaked in distilled water for 25 min. The hair was then immersed in phosphate buffer that had been pH adjusted with hydrochloric acid and sodium hydroxide. Eight different pHs were used. After incubation for 5 min in the buffer, images were taken every 5 min over different regions for approximately 30 min; this procedure was completed for one subject.

RESULTS

Morphology in Air Versus Water  Figure 2a is a typical $63 \times 63 \mu m$ image of hair in air. The image has been shaded with a simulated light source from the right to highlight the structural differences; no other image processing has been performed. The hair cuticle exhibits consecutive overlapping sheaths. These sheaths are layers of imbricated, keratinized cells that make up the cuticle. A linecut was taken to show quantitative height data versus position. The line inserted into the picture shows the location of the corresponding linecut shown in Fig 2b. Throughout this paper, we will refer to the height of one sheath relative to the next as the step height. The average step height of the sheaths in air is approximately 500 nm. Occasionally there will be a larger step in the 1-2-3-4 nm range; less than 5% of the steps measured exhibited this larger size. The average diameter of the hair shafts in air was reproducible and approximately 50 nm.

Figure 3a is a $44 \times 44 \mu m$ image of hair from the same subject as used in Fig 2, after it had been soaked in water for 30 min. The step heights are about 1 $\mu m$, as shown in Fig 3b. For this subject, we observed an average step height of 1200 nm for the hairs soaked in water, approximately a 140% increase. Certain steps were occasionally as small as 500 nm; less than 10% of the steps imaged were this small.

Kinetics of Hydration  Figure 4 is a plot of a single step height for a single subject versus time. The hair was imaged in air, then immersed in water and reimaged. (The microscope requires a realignment procedure once the water is added that takes 1-2 min; therefore the first data point in water is always delayed.) Images were then taken of the same area every 30 seconds for 7 min. The data shown are a single step height change versus time. The majority of the hydration occurs in the first 2 min after immersion. A first-order exponential fit to this data gives

$$\text{step height (nm)} = 680 \text{ nm} - (226 \text{ nm} \times \exp^{-0.0117 \text{ second}^{-1} \times \text{time}}).$$

The rate constant is 0.0117 second$^{-1}$, with a correlation better than 99%. This value was reproducible for scans of the same subject’s hair. The error bars represent the standard deviation in the measurement of the step height.

The kinetic experiment was repeated with the same subject and showed remarkably similar results. Also, experiments have been carried out up to 40 min with data points every 2 min for three subjects; again the majority of the hydration occurred in the first few minutes.

Hair Morphology Versus pH  Figure 5 is a plot of step height versus pH. Each data point is an average of at least 30 steps from hair from the same subject. The first image in each case was taken after 25 min in water followed by 5 min in the buffered solution, well beyond the saturation time determined from Fig 4.

A critical observation is that the step height depends on both the initial and the final pH values. For example, if a hair is immersed in

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**Figure 1.** Atomic Force Microscope. A laser beam is focused onto the back of a flexible cantilever. The reflection then strikes a two quadrant position sensitive photodiode. As the cantilever is raster scanned across the sample, its relative height can be monitored because the beam will strike a new position on the photodetector.

**Figure 2.** AFM image and linecut. a. AFM image of hair in air. The scale bar is 20 $\mu m$; there are 256 $\times$ 256 pixels and the entire image was acquired in 62 seconds. The image is light shaded to accentuate the features. No other image processing was performed. b. AFM linecut. The data represents the quantitative height data following the line drawn in a; from left to right, the two steps are 500 and 400 nm, respectively.
buffer at pH 7 for 25 min and then imaged in buffer at pH 8, a very
different average step height is observed compared to a hair that
is immersed in pH 6 buffer for 25 min, but again imaged at pH 8.
Therefore, each data point in Fig 5 represents step heights observed
for a “fresh” hair sample from the same subject, initially soaked in
distilled water for 25 min, then imaged at the pH level indicated.
The error bars are the standard deviation of measurements of the step
heights. An increasing trend in average step height is shown. A
similar trend was observed with samples from other subjects. At
high pH, not only do the step heights increase dramatically, but
visible degradation (possibly hydrolysis) occurs (see Fig 6).

DISCUSSION

Morphology in Air Versus Water Hair could be more suscep-
tible to damage when wet than when dry. Because hair is a complex
composite of protein fibers and keratinized cells, it may be possible
that adding water hydrates these fibers and weakens their three-
dimensional structure. We have observed a total shaft diameter
increase when hair is immersed in water or buffer (typically about
10%). This swelling has also been observed with light microscopy.

The sheath step height increases dramatically compared to the total
shaft swelling. Two factors could be responsible for this dramatic
difference. The outer layer of cells could be taking up a larger
amount of water than the total hair shaft, or the overlapping cells of
the cuticle could be separating once hydrated. A number of studies
have investigated the binding states of water in keratin [13,14].
However, these studies have been directed at a molecular under-
standing. Our investigation correlates macroscopic morphology to
hydration.

It would be advantageous to protect the hair cuticle from
hydration and swelling while hair is immersed, for instance during
swimming and bathing. If a conditioner or hair treatment could be
devised that inhibited hydration, it could act as a protective coating.
Currently, the effectiveness of these chemical treatments is deter-
moved based on the macroscopic properties of hair and is often
subjective. Quantitative information about relative sheath height
changes may be more pertinent.

Figure 4. Step height versus time. The average step height versus time
of a single hair step for a single subject. Each data point is the average value
of a single step; the error bars represent the standard deviation in the
measurement of the average step height at each point. The first data point
at time 0 represents the step height in air. The solid line in an exponential
fit to the data with a correlation better than 99%.

Figure 5. Step height versus pH. Each data point is an average of many
steps from the same subject at different pH levels; the error bars are the
standard deviation of each. The imaging solutions were phosphate buffer
that was pH adjusted with hydrochloric acid and sodium hydroxide. The
images were taken after soaking the hair for 25 min in distilled water and
then 5 min in the buffered solution, well beyond the saturation time
determined from Fig 4. Images were then taken every 5 min for the next 30
min.
This study has shown that AFM is an excellent analytical technique to provide quantitative information on morphologic changes under ambient conditions. Also, this method eliminates subjectivity that is often used in determining hair care effectiveness. We observe large increases in step heights in certain subjects (see Figs 2 and 3). In other samples, the increase in shear step height in water is not nearly as dramatic. In Fig 4, the single step studied has a height of 460 nm in air. After soaking in water for 5 min it is 675 nm. This is a 47% increase in step height. Still, this opening is much more dramatic than the overall hair diameter change, which is approximately 10%. We have not completed a rigorous analysis of the hair treatment of our sampling group. This study is meant to show the ability of AFM for studying this type of system.

**Kinetics of Hydration**

To take full advantage of the AFM to image hair morphology, we must first be familiar with how hair interacts with water. As can be seen from Fig 4, the hydration of the hair seems to saturate very quickly, after just a few minutes. Therefore, an incubation time of at least 3–5 min should be used before morphologic changes are studied to avoid confusion from simple hydration kinetics.

**Hair Morphology Versus pH**

To design intelligent chemical additives for hair treatment, we must first understand how chemistry affects hair morphology. We have done a simple study on how the average step height is affected by pH changes, providing basic information about the morphologic effects of the hydration process.

Figure 5 shows an increasing trend in the shear step height as the pH increases. This trend has been observed with numerous samples and averaging techniques. The increase is most dramatic at high pH, where the hair is visibly degraded. In Fig 6, the hair begins to lose its cylindrical shape after only 5 min in the pH 11.4 buffer. After a few minutes at this pH, the hair began to fall apart. This could be seen by simply looking at the sample visually. This degradation also occurred at pH 10.4 after about 15 min. It is possible that this degradation is due to the large increase in step height at this pH. The sample soaked at pH 4 did not exhibit this behavior; the three-dimensional structure of the hair at this pH remained completely intact even after 30 min incubation.

A possible explanation for the observed differences in total swelling for various subjects, as well as the observed pH curve, is that cationic surfactants are covering the hair prior to sampling. Empirical observations show that cationic surfactants make good conditioners [15]. An increase in pH would cause the removal of these cationic conditioners, making the hair more susceptible to hydration. At low pH, the cation head groups would remain charged and adsorbed to the hair. This mechanism may also explain why various steps open a different amount under water, because incomplete coverage of the surfactant may occur from one region of the hair to another.

In this study, we have presented a new methodology for studying hair morphologic changes. We believe it is a superior technique to any available; it combines the high resolution of electron microscopy with the environmental and sample flexibility of light microscopy. The dual advantage that AFM affords may make it an ideal choice for studying early stages of certain clinical hair abnormalities that would otherwise go undetected. Also, it should be an effective tool for determining the effectiveness of hair care treatment.

**REFERENCES**

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