

Effects of Atopic Dermatitis on the Morphology and Water Content of Scalp Hair

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ABSTRACT The effects of atopic dermatitis (AD) on scalp hair properties, such as morphology and water content, were investigated using atomic force microscopy (AFM) and thermogravimetric analyzer. Hairs from lesional and nonlesional scalp regions of eight patients with AD were investigated. The severity of the disease, which was evaluated using the SCORing Atopic Dermatitis index, was 48.75 (range, 40–80). Hairs from 15 normal adults were also examined as controls. The surface images were taken in an area of $20 \times 20 \mu\text{m}^2$ with 512×512 pixels and a scan speed of 0.8 line/s. AD affected the cuticle structures and scales of scalp hair. The edges of cuticles were torn and collapsed, and the scales were very thick. The water contents of both types of AD hair were less than the control: $12\% \pm 0.7\%$, $11.7\% \pm 0.4\%$, and $13\% \pm 0.8\%$ for lesional AD hair, nonlesional AD hair, and control hair, respectively. The scalp hair of patients with AD can be characterized by thick and globular scale patterns. The hair of patients with AD has less water content than normal hair showing a good agreement with the property of skin having AD. *Microsc. Res. Tech.* 00:000–000, 2011. © 2011 Wiley Periodicals, Inc.

INTRODUCTION

Atopic dermatitis (AD) is an itchy, chronic, inflammatory skin condition. Since 1960s, there has been a greater than threefold increase in the worldwide prevalence of AD (Schultz-Larsen et al., 2002). The most recent estimates indicate that AD is a major public health problem with prevalences in children of 10–20% in the United States, northern and western Europe, urban Africa, Japan, Australia, and other industrialized countries (Williams et al., 1999). The prevalence of AD in adults is ~1–3%.

AD is characterized by itchy papules that become excoriated and lichenified and typically have a flexural distribution. Clinically, pruritic plaques of the scalp in atopic patients are frequently seen. Shi et al. reported that AD was associated with a high incidence of scalp dermatitis (49.7%; Shi et al., 2011). In the abbreviated version of the Hanifin and Rajka diagnostic criteria, scalp dermatitis is included in the minor features of AD (Hanifin, 1991).

Scalp dermatitis may affect the surface structure and the chemical composition of human hair. Changes in hair morphology can be observed by atomic force microscopy (AFM), a promising tool for observing the surface topographies of biomaterials in two- or three dimensions due to its very high resolution (Binnig et al., 1986; Schaus and Henderson, 1997), which is more than 1,000 times better than optical microscopy. Many studies have demonstrated that AFM is a good technique for the evaluation of surface properties of human hair (Gurden et al., 2004; LaTorre and Bhushan, 2005). Morphological changes induced in hair by diseases such as psoriasis and seborrheic der-

matitis have been observed by AFM (Kim et al., 2011; Shin et al., in press). Thermal analysis is a very efficient method for studying chemical composition and thermal stability of materials. Some studies using differential scanning calorimeters reported that the morphological components of hair are related to thermal stability (Cao et al., 1999; Wortmann et al., 2007). Thermogravimetric analysis (TGA) was used to study the evaporation rate of adipic acid (Wright et al., 2001), the thermal fragmentation of hair (Ehen et al., 2004), and the internal and external water contents of untreated human hair (Barba et al., 2009).

In the present study, the effects of AD on hair, especially on surface topography and water content, were investigated. Using AFM, morphological properties such as pit, scale, and roughness of AD hair were investigated on a nanometer scale, and lesional hair, nonlesional hair, and healthy controls were compared. The water content of AD hair was determined using TGA.

MATERIALS AND METHODS

Hair Sample Preparation

AD was diagnosed according to the criteria of Hanifin and Rajka (1980). The severity of disease was eval-

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uated using the widely accepted SCORing Atopic Dermatitis (SCORAD) index (Stalder and Taieb, 1993). Hairs from regions of lesions and nonlesions of the scalp of eight AD patients were investigated. Shafts of lesional scalp hairs were obtained from scaly erythematous plaques. Shafts of nonlesional scalp hairs were obtained from normal looking scalp 3–5 cm away from lesions. The ages of the patients ranged from 24 to 45 years (mean, 31.0 ± 9.78 years); there were 6 males and 2 females. The average SCORAD was 48.75 (range, 40–80). Patients with other systemic diseases were excluded. Hair from 15 healthy adults (normal controls, 9 males and 6 females) ages 20–54 years (mean 35.4 ± 6.6) were examined in the same way. All hair samples were sonicated for 30 s using 1% sodium dodecylsulfate solution to wash and then were rinsed using distilled water. The hairs were then dried at room temperature for >4 h.

Atomic Force Microscopy

The surface properties of human hair were investigated using AFM, NANOSTATION II (Surface Imaging Systems, Herzogenrath, Germany). The system consists of an AFM scanner ($92.5 \times 92.5 \times 6$ mm³ in *x*, *y*, and *z* directions) and a Zeiss optical microscopy (Epiplan 500 $\times$). The surface topography was taken five to seven times for each sample in noncontact mode. The scan area was 25×25 mm² with 512×512 pixels, and the scan speed was 0.8 line/s. The material parameters of the silicon probe used in the noncontact mode were: frequency of 190 kHz, spring constant of 21–98 N/m, resistance of 0.01–0.02 Ω -cm, and length of 225 nm.

Thermogravimetric Analyzer

The TGA measurements were performed using TA Instruments Hi-Res 2050 TGA thermogravimetric analyzer. The amount of hair used for each analysis varied from about 1.0 mg. The measurements were conducted in an atmosphere of dry N₂. The hair samples were heated from 25 to 205°C at 20°C/min and were kept at 200°C for 30 min to evaporate all the water contained in the hair (Barba et al., 2009). The TGA experiment was done three times for each sample of control, AD lesion, and AD nonlesion hairs.

Pits

A pit was defined as a sharply demarcated depression on the hair surface. Pits were classified into two groups, micro and macro, depending on size. Pits with small diameters (<0.5 μ m) or areas (<0.25 μ m²) were classified as micropits, and pits with large diameters (>0.5 μ m) or areas (>0.25 μ m²) were classified as macropits (Kumar et al., 2008). Numbers of pits were determined per 25×25 mm², which was the size of one image.

Scales

The thicknesses of scales were measured at several different sites for each sample, and all values were averaged.

Roughness

Four surface roughness parameters (Sa, Sq, Sz, and Sku) were calculated at 50 different areas of 3×3 μ m² for each sample. Sa is the average value of roughness,

Sq is the root mean square, Sz is the peak-to-peak value, and Sku is the surface kurtosis, which represent a key feature of the height distribution through the “peakedness” of the profile.

Statistics

Statistical analyses were performed to compare the mean values of physical parameters of hair using a two-tailed Student's *t*-test. *P* values < 0.05 were regarded as statistically significant.

RESULTS

Topography

Figure 1 shows representative AFM images of AD and control hairs. Figures 1a and 1b show the surface topography taken in an area of 20×20 μ m² for the hairs collected from lesion and nonlesion regions of AD, respectively, and Figure 1c shows the control hair. A significant difference in the surface morphology was observed between the AD hairs and control hairs. In both AD hairs of lesions and nonlesions, the cuticle structure was severely damaged and distorted. As shown in Figure 1a, the cuticles of lesional AD hair are not stair-like in shape, like normal hairs. The boundaries and height differences between cuticles are either unclear or are very small. Cracking and miscellaneous damage at the cuticle edge was frequently observed in nonlesional AD hair as shown in Figure 1b. On the other hand, the control hair shown in Figure 1c, shows very clear cuticle structures with consecutive overlapping sheaths (stair-like shape) and smooth edges. The difference in stair-like topography of the cuticles between the AD and control hairs is more clearly seen in three-dimensional images of the surface and the line profiles, which were measured in the position as indicated with white lines in AFM images. The surface of AD hairs was flat or irregularly stepped. In comparison, the control hair shows well-ordered steps between the cuticles with a height of 512 ± 28.9 nm.

Typical Features of AD Hair: Scale, Pits, and Roughness

Scales were observed frequently in all samples of the AD hairs. The distribution patterns of the scale were diverse as shown in Figure 2a. The scales were piled thick on the cuticle (i), hung like icicles (ii), or were spread thin, wide, and evenly (iii). Heights of the scales are listed in Table 1. The scales of AD hair were about six times thicker than those of control hair. The heights were 173.89 ± 142.5 nm and 158.79 ± 107.7 nm for lesional and nonlesional regions, respectively. Control hair had very thin scales with a height of 36.5 ± 5.2 nm.

Pits were observed in AD hair in both lesional and nonlesional regions. The shapes and sizes of the pits were diverse, as shown in Figure 2b. The average numbers of pits were 0.62 ± 0.52 and 0.32 ± 0.28 for the AD hairs in lesional and nonlesional regions, respectively, as listed in Table 1. The hair of lesional regions had more than twice as many pits as the hair of nonlesional regions and control hair. The numbers of pits in nonlesional regions were similar to control hair, and macro and micropits were observed in equal numbers as in controls.

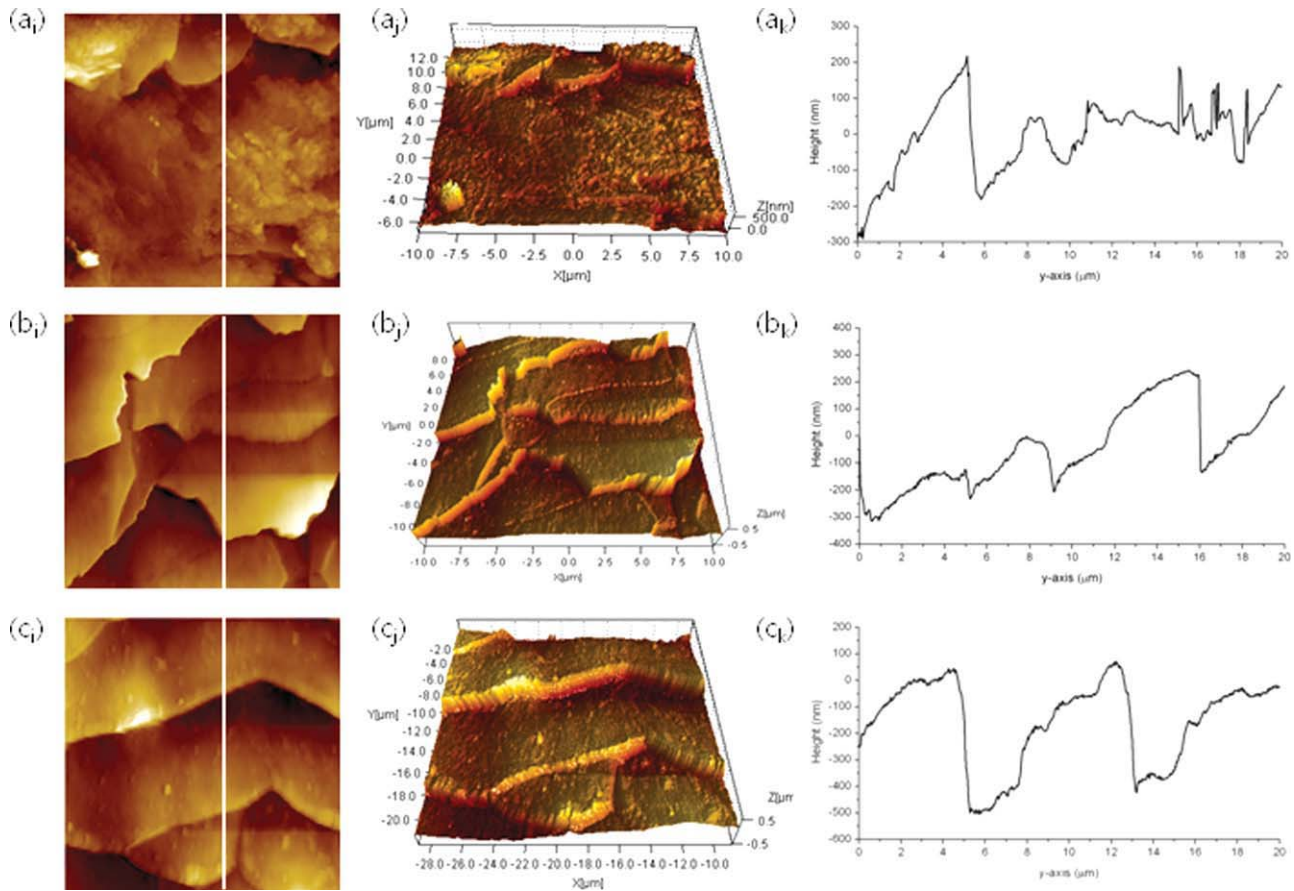


Fig. 1. Representative AFM images of AD and control hair. The colors indicate surface topography: the light and dark colors correspond to the higher and the lower topographies, respectively. (a_i), (b_i), and (c_i) are surface topographies of lesional and nonlesional AD hair and control hair, respectively. (a_{ii}), (b_{ii}), and (c_{ii}) show representative

3D images of (a_i), (b_i), and (c_i), respectively. (a_{iii}), (b_{iii}), and (c_{iii}) are line profiles taken from top to bottom of the topography image. The position taken from the line profile is represented as a white line. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Figure 2c shows an AD hair surface that is not covered with scales. The surface is uneven and rough. The surface of AD hair was rougher than control hair, especially in lesional areas. The four parameters of surfaces roughness that was determined in small areas of $3 \times 3 \mu\text{m}^2$ are listed in Table 1. AD hair in lesional regions had the highest average roughness parameter. The differences between AD and control hairs were statistically significant for all parameters of Sa, Sq, and Sz ($P < 0.05$). The differences between AD hairs in lesional and nonlesional regions were also statistically significant.

Figure 3 shows the results of TGA of both AD hairs and control. All hair samples were heated from 25 to 205°C at 20°C/min in an atmosphere of dry N₂. The first mass loss occurred at the temperature range of 30–200°C, which corresponded to the water content of the hair sample, and the second loss, which started at 201°C, corresponded to the decomposition of the keratin structure. The temperature range of water release was slightly higher than reported results in other studies (Barba et al., 2009). The water contents determined by TGA were $12\% \pm 0.7\%$, $11.7\% \pm 0.4\%$, and $13\% \pm$

0.8% for lesional AD hair, nonlesional AD hair, and control hair, respectively. The water content of both lesional and nonlesional AD hair was less than control.

DISCUSSION

AD affects the morphology of corneocytes. Kashibuchi et al. observed the corneocytes of AD patients using AFM (Shi et al., 2011) and reported that the corneocytes showed a large decrease in flatness index, reflecting their poor differentiation in the stratum corneum due to their enhanced turnover rate. Silverberg demonstrated that hyperkeratosis and prominence of the interkeratinocyte spaces are characteristic skin architecture features through dermoscopy (Silverberg, 2011).

We observed AD effects on cuticle structure and scale by AFM. The cuticle was damaged severely by AD; specifically, the edge of the cuticle was torn and collapsed. Very thick scales were seen in most hairs from patients with AD. Compared to previous reports describing seborrheic dermatitis and scalp psoriasis (Kim et al., 2011; Shin et al., in press), AD scales were character-

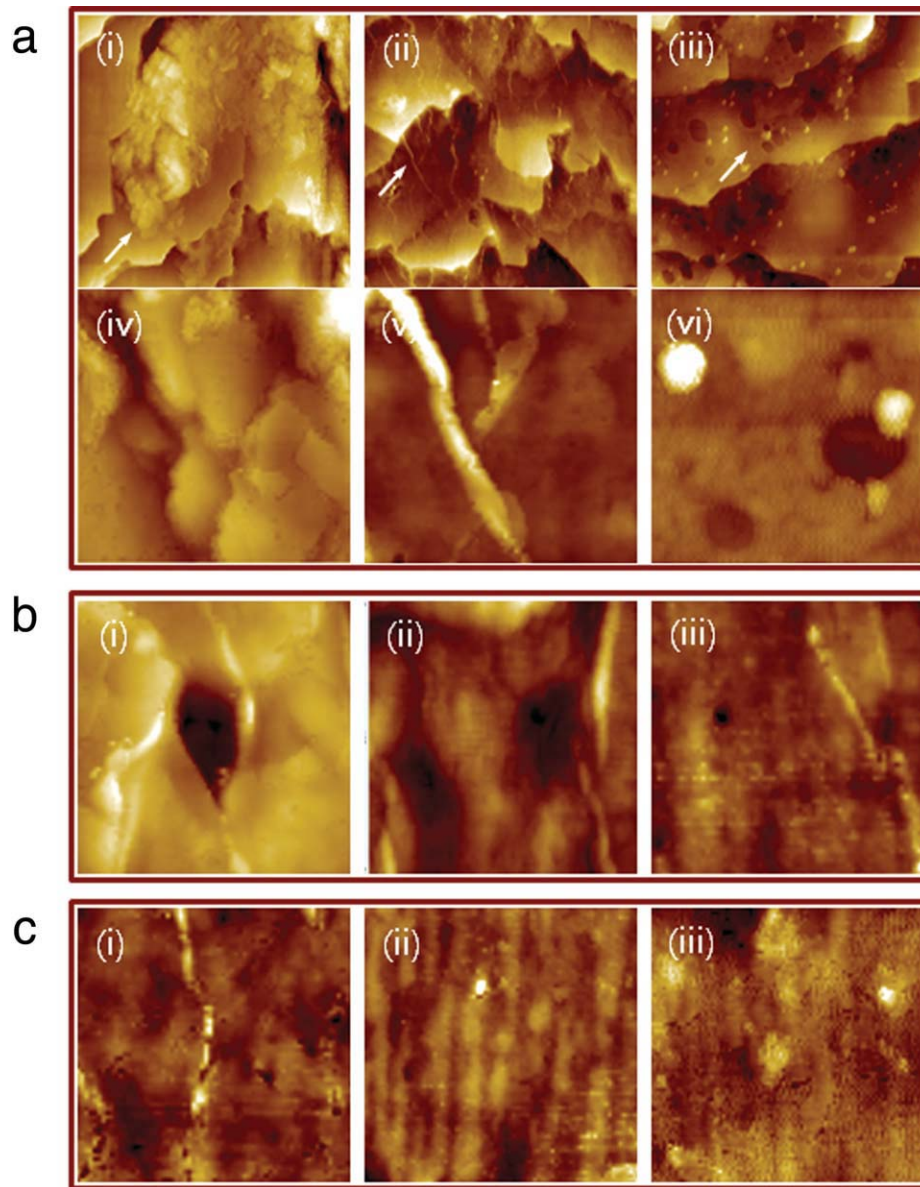


Fig. 2. Representative AFM images of AD hair showing specific features of the surface. Three different types of scale are observed in lesional AD hair as shown in (a). Figs. **a(iv)–a(vi)** correspond to enlarged scale images of [**a(i)–a(iii)**], respectively, indicated by white arrows in detail. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

ized as more elongated in shape, resembling icicles, and/or a globular, such as pepper grains.

Nail pitting is characteristically observed in patients with psoriasis and has also been reported in patients with AD (Nnoruka et al., 2004). We did not observe significant differences in number or size of pits in AD patients compared to control hair. In a previous study, we demonstrated that pits result from crackled corneocytes in psoriatic hair (Kim et al., 2011). In contrast, it seems that the pits in AD hair are secondarily formed by holes in scale. It is suggested that the pits in AD hair originate from different causes than pits in psoriatic hair.

AD affects the water content in the skin. In the pathogenesis of AD, impaired skin barrier function plays a major role. Impaired skin barrier formation allows increased transepidermal water loss (TEWL). TEWL, a sign of impaired epidermal barrier function, is also elevated in active atopic lesions (Gupta et al., 2008). AD is associated with a marked decrease in skin barrier function. The skin barrier defects are observed in both lesional and nonlesional skin (Berth-Jones et al., 2003). Berardesca et al. (1990) reported that uninvolved skin of atopic patients had reduced water content and increased TEWL. In this study, the results of TGA showed that AD hair was drier than control

TABLE 1. Results of pits, scale, and surface roughness of AD and control hairs

		AD hair		Control	P value		
		Lesion	Nonlesion		P value ^a	P value ^b	P value ^c
Scale Pit	Thickness (μm)	149.9 ± 91.1	147.5 ± 66.9	39.4 ± 8.4	0.606	4.28 × 10⁻⁷	1.16 × 10⁻⁵
	Number (N/20 × 20 μm ²)	0.62 ± 0.52	0.32 ± 0.28	0.33 ± 0.26	0.13	0.14	0.917
	Area (μm ²)	0.82 ± 0.46	0.15 ± 0.08	0.58 ± 0.47	0.60	0.62	0.80
Roughness	Macro pit (%)	93	50	56	—	—	—
	Micro pit (%)	7	50	44	—	—	—
	Sa (nm)	22.44 ± 10.94	18.99 ± 9.48	11.92 ± 4.14	0.0007	3.26 × 10⁻⁸	2.12 × 10⁻¹⁶
	Sq (nm)	29.95 ± 15.01	25.13 ± 12.18	15.90 ± 6.07	0.0004	4.62 × 10⁻¹⁵	1.26 × 10⁻⁷
	Sz (nm)	244.99 ± 120.57	219.13 ± 106.97	126.51 ± 53.18	0.006	7.65 × 10⁻¹⁵	6.24 × 10⁻⁹

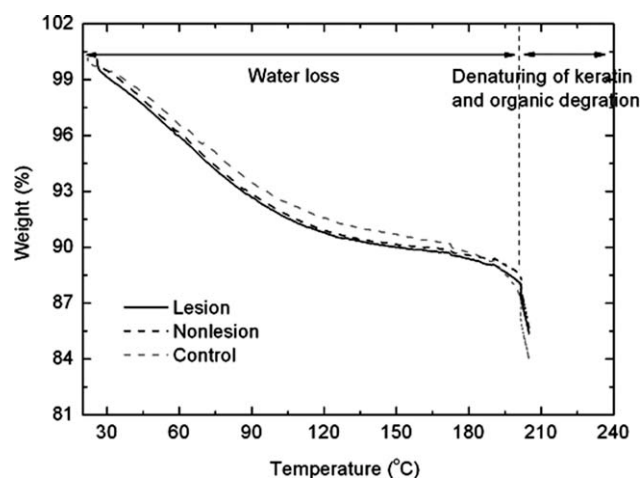
^aP value is the statistical result between the lesional and nonlesional AD hair.^bP value is between lesional AD hair and control hair.^cP value is between nonlesional hair and control hair. Statistically significant results were given in bold.

Fig. 3. TGA for lesional AD hair (black solid line), nonlesional AD hair (black-dashed line), and control hair (gray-dashed line) samples.

hair, which confirms to known AD skin characteristics. The water contents of lesional and nonlesional hairs do not show a statistically significant difference. The decreased water content of hair in AD suggests that atopic patients may have impaired barrier function in the hair as well as skin. In addition, a different pattern was observed for differences in water content between AD hair and control. From 30 to 190°C, both lesional and nonlesional AD hair lost water content more quickly than control hair. The AD hair lost more than 90% of total water content up to 190°C, whereas the control lost only 83% of total water content. The control hair then lost water quickly after 191°C. This behavior also suggests that water molecules may be less strongly linked in AD hair than in control hair because of the impaired barrier function in the hair.

CONCLUSION

Scalp hair in patients with AD is characterized by thick and globular scale patterns. These patterns were observed in both lesional and nonlesional AD hair. Hair in AD also has less water content than normal hair.

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