Preliminary studies on the characteristics of corneocytes using atomic force microscopy (AFM)

Wstępne badania nad charakteryzowaniem korneocytów za pomocą mikroskopu sił atomowych (AFM)

Ida Dulińska-Molak [1/], Małgorzata Lekka [2/], Małgorzata Lewandowska [1/], Monika Pasikowska [3/], Bożena Tyszczuk [3/], Irena Eris [3/]

[1/] Facutly of Materials Science and Engineering, Warsaw University of Technology, Warsaw, Poland
[2/] The Henryk Niewodniczański Institute of Nuclear Physics, Polish Academy of Sciences, Kraków, Poland
[3/] Dr Irena Eris Centre for Science and Research, Warsaw, Poland

Wstęp. Mikroskopia sił atomowych (AFM) jest jedną z najnowocześniejszych technik badawczych, która umożliwia charakterystykę komórek skóry.

Cel. Opracowanie metody pomiaru AFM mechanicznych właściwości komórek warstwy rogowej naskórka przed aplikacją kremu nawilżającego i po jego stosowaniu przez 5 dni.

Materiały i metody. Prezentowane badania dotyczyły pomiarów sztywności, właściwości adhezyjnych oraz topografii korneocytów, przy użyciu mikroskopu sił atomowych Park Systems XE120 AFM. Krem nawilżający stosowano przez 5 dni. Korneocyty zebrano przed i po zakończeniu stosowania kremu, z części wewnętrznej przedramienia za pomocą 'Cuderm tape strips'. W czasie testu nie stosowano innych preparatów kosmetycznych.

Wyniki. Uzyskane wyniki wykazały zmiany właściwości komórek, takich jak moduł Younga i siły adhezyjne, wynikające z reakcji skóry na zastosowany produkt kosmetyczny.

Wnioski. Uzyskane wstępne wyniki wskazują, że technika mikroskopii sił atomowej (AFM) jest precyzyjnym narzędziem do badania zmian właściwości mechanicznych korneocytów.

Słowa kluczowe: *mikroskop sił atomowych, korneocyty, moduł Younga, siły adhezji*

Purpose. Atomic force microscopy (AFM) is a novel technique for skin cells characterization.

Aim. To develop AFM tests for characterization of the outermost epidermis layer, corneocytes. As an example, the effect of moisturizer on the corneocyte properties is studied.

Methods and materials. Topology, rigidity, adhesion properties and friction (between individual corneocytes and AFM probe) of the top layer of corneocytes were measured by means of Park Systems XE120 AFM. Moisturizing cream was applied daily on the forearm for a period of 5 days. The skin flakes were collected before and after the treatment using Cuderm tape strips. No additional treatment of flakes was performed during test.

Results. A protocol for the AFM study of corneocytes is developed. After the treatment, we observed differences of rigidity (the Young modulus) and adhesion properties (pull-off force).

Conclusion. AFM can be used as a very sensitive tool for early detection of changes in corneocytes.

Keywords: *atomic force microscopy, corneocytes, Young 's modulus, adhesion force*

www.kosmet.pl

Nadesłano: 22.02.2012
Zakwalifikowano do druku: 16.03.2012

Adres do korespondencji / Address for correspondence
Dr Monika Pasikowska
Dr Irena Eris Cosmetic Laboratories, Dr Irena Eris Centre for Science and Research, Puławska 107a, 02-595 Warszawa

Introduction

The growing demand with regard to products that improve skin appearance has induced a strong and thriving development of the cosmetic industry in Poland. There is also a growing importance of testing cosmetic products using state-of-the-art technology. To achieve market success, it is not sufficient to simply list product properties resulting from the composition of a cosmetic product. The modern cosmetics industry goes further, complementing tests on a high-quality cosmetic product with the element of its interaction with the biological factor – human skin. This aspect is a scientific challenge for the research sector of the cosmetics industry, as it has developed only recently.

Nanotechnology, used to study the transport of valuable substances deep into the skin, being the natural protective barrier, is the most promising research area. Therefore, it is necessary to monitor the process of molecule penetration at the micro- or even nanometre level to verify the quality of cosmetic products.

Atomic Force Microscopy (AFM) is a technique which enables characterization at the nano- and subnanometre scale. In this technique, interactions between the measuring probe (a tip on a cantilever) and the surface is measured (Fig. 1). The nanomechanical properties of the surfaces may be quantitatively estimated by determining relations between probe-sample distance and forces between them.

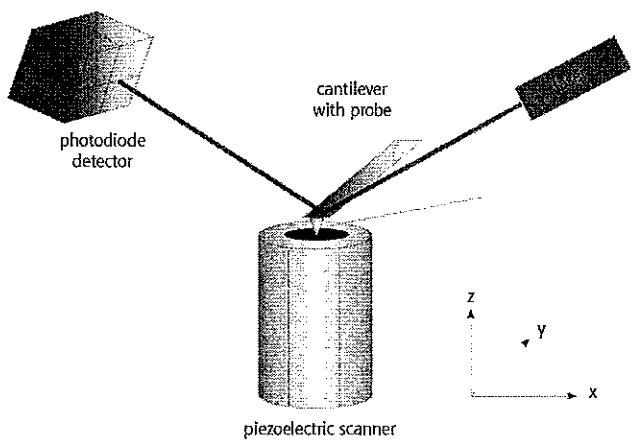

Fig. 1. Schematic of atomic force microscope (AFM)

The main objective of using an atomic force microscope in skin studies was to determine the influence of a cosmetic (moisturising cream) on skin. A method for evaluating changes in the rigidity of cells constituting the stratum corneum (so-called corneocytes) were developed by estimating their Young's modulus values.

Test material

The test material included human corneocytes taken from a person aged over 30. The cells are made up mainly of creatinine. Their diameter was approximately 4 μm with a thickness of approximately 0.1 μm.

Measurements of corneocyte elastic properties were carried out for cells taken after 5 days of using the moisturising product once daily. Non-treated cells taken from the same person provided the control (reference measurement). Corneocytes were sampled from the epidermis using a special adhesive tape (D-squame skin indicator D200, CuDerm Corp, Dalllas, Dx, USA).

INCI

Aqua, Glycerin, Isopropyl Isostearate, Pentaerythrityl Tetraisostearate, Isostearyl Isostearate, Butyrospermum Parkii (Shea Butter), Cetyl Alcohol, Sucrose Cocoate, Sodium Polyacrylate, Rosa Canina (Rosa Canina) Fruit Oil, Sorbitan Stearate, Allantoin, Tocopheryl Acetate, Sodium Hyaluronate, Tin Oxide, BHA, Lecithin, Micrococcus Lysate, Phenoxyethanol, Methylparaben, DMDM Hydantoin, Propylparaben, Parfum, Hydroxyisohexyl 3-Cyclohexene Carboxaldehyde, Butylphenyl Methylpropional, Limonene, CI 77891, Mica, CI 16035, CI 17200.

Theory

The traditional approach when determining Young's modulus values is based on the Hertz model, which describes the deformation of two homogeneous spheres. However, the model does not take into account adhesion forces. When testing the properties of callous epidermal cells, such as corneocytes, it is more appropriate to estimate Young's modulus using a JKR (Johnson-Kendall-Roberts) model [1, 2]. Unlike the Hertz model, the JKR model includes the existence of adhesion forces. The forces act only in the interface area between surfaces, leading to an effective increase in the contact area compared to the area expected from the Hertz model (Fig. 2).

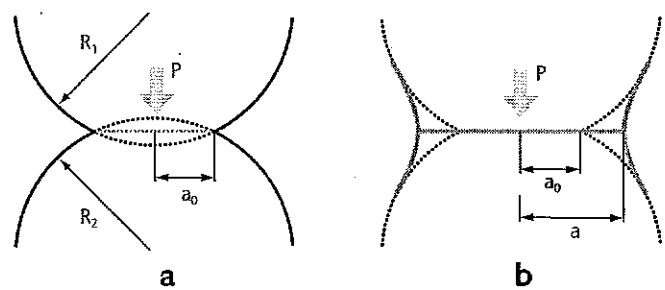

Fig. 2. Diagram of the contact of two elastic globes with a radius of R_1 and R_2 formed under the impact of load P for Hertz model (a) and JKR model (b) [3] (a_0 and a, contact radii for Hertz and JKR models, respectively).

The JKR model is used when small material deflection (or indentation) values occur with relatively large adhesion force values. Furthermore, JKR analysis assumes the existence of a linear strain-deformation ratio and lack of long-distance interactions. That is why JKR model is a proper tool for investigating the rigidity of cells constituting the stratum corneum.

Measurement methodology

Measurements of corneocyte elastic properties were carried out in the air. A flat spring with an elasticity constant value of k=0.6 N/m and a curvature radius of R=50 nm was used for the measurements (Fig. 3).

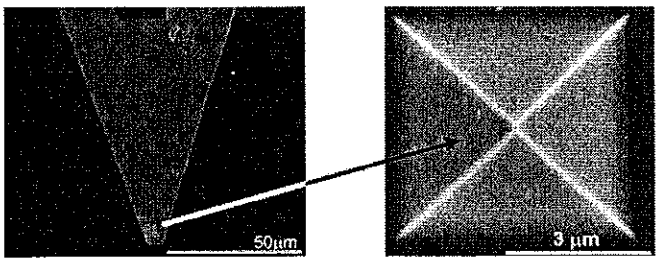

Figure 3. SEM-image of the AFM tip used for measuring corneocyte elastic properties

Ten cells were measured for each sample (control and cream-treated corneocytes). For every single cell, a curve map was recorded in an 8 × 8 configuration which gives 64 force-distance curves.

Data analysis

Young's modulus values for corneocytes were estimated using an atomic force microscope (AFM). The measurement is based on the recording of force-distance curves, or relationships between the deflection of a flat spring and the relative position in the piezoelectric scanner where the test sample is located. Spring deflection corresponds to the force exerted between the end of the sampling tip and the surface of the tested sample. Figure 3 shows a theoretical force-distance curve recorded when moving the tip towards the sample (curve A) and when moving the tip away from the tested sample (curve B).

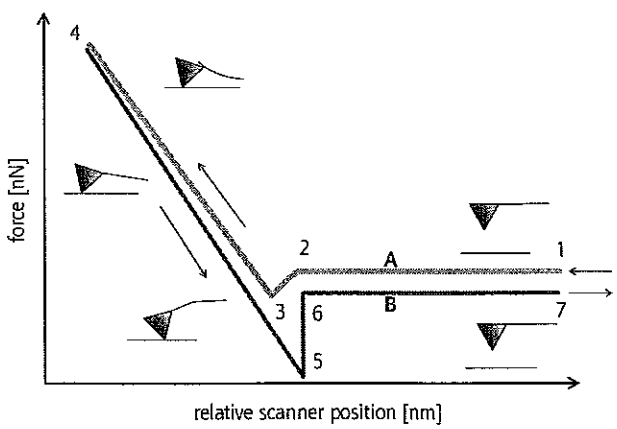

Fig. 4. Diagram of the theoretical curve which describes the relationship between the deflection of a flat spring and scanner location when moving the tip towards (A) and away (B) with respect to the tested surface

The Young's modulus E of the tested corneocytes was determined according to the principles of the JKR model from the following formula:

$$E = \frac{3}{4\sqrt{R}} \cdot \frac{dP_{JKR}}{d(h^{3/2})} \qquad (1)$$

where R is curvature radius of the AFM sampling tip, h is indentation value and P_{JKR} is reduced pressure force which includes adhesion force.

$$P_{JKR} = \frac{1}{\sqrt{3}} P_{off} P_1^{3/2} \qquad (2)$$

and:

$$P_{off} = k \cdot d \qquad (3)$$

$$P_1 = (3 \cdot P_2 - 1) \cdot \sqrt[3]{\frac{1}{9}(P_2 + 1)} \qquad (4)$$

$$P_2 = \sqrt{\frac{Z_{defl}}{d} + 1} \qquad (5)$$

where P_{off} is "pull-off" force, or adhesion force exerted between the AFM sampling tip and the tested surface, k is constant elasticity of the flat spring, Z_{defl} corresponds to the spring deflection value due to the pressure force, and d corresponds to the spring deflection value due to adhesion forces (Fig. 5).

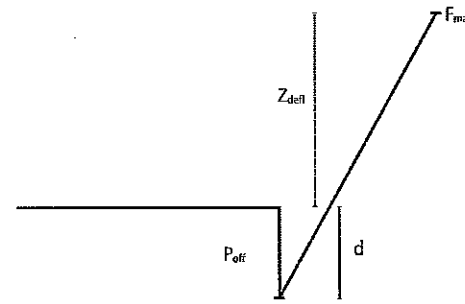

Fig. 5. Illustration of parameters obtained based on the force-distance curve (recorded when moving the tip away from the test surface)

Young's modulus was determined using the JKR model with an assumed indentation depth of 10 nm. The value was selected based on data provided in the article [4] where Young's modulus values determined for corneocytes for various indentation values were shown to fluctuate around the same values, that is, they did not depend on indentation. Mean values for the control sample (that is, corneocytes not treated with the cosmetic) and the corneocyte sample taken after a 5-day treatment with the cosmetic were estimated for approx. 500 curves recorded for each sample.

Study results

As a result of the study conducted, cell images were obtained and Young's modulus values were estimated for two corneocyte types: (i) corneocytes not treated with the cosmetic and (ii) corneocytes taken on the sixth day after five days of skin treatment with the cosmetic once daily.

Corneocyte morphology

Figure 6 shows corneocyte images before and after the 5-day treatment with the cosmetic, recorded using a scanning electron microscope. We did not observe significant differences in the morphology of the cells observed.

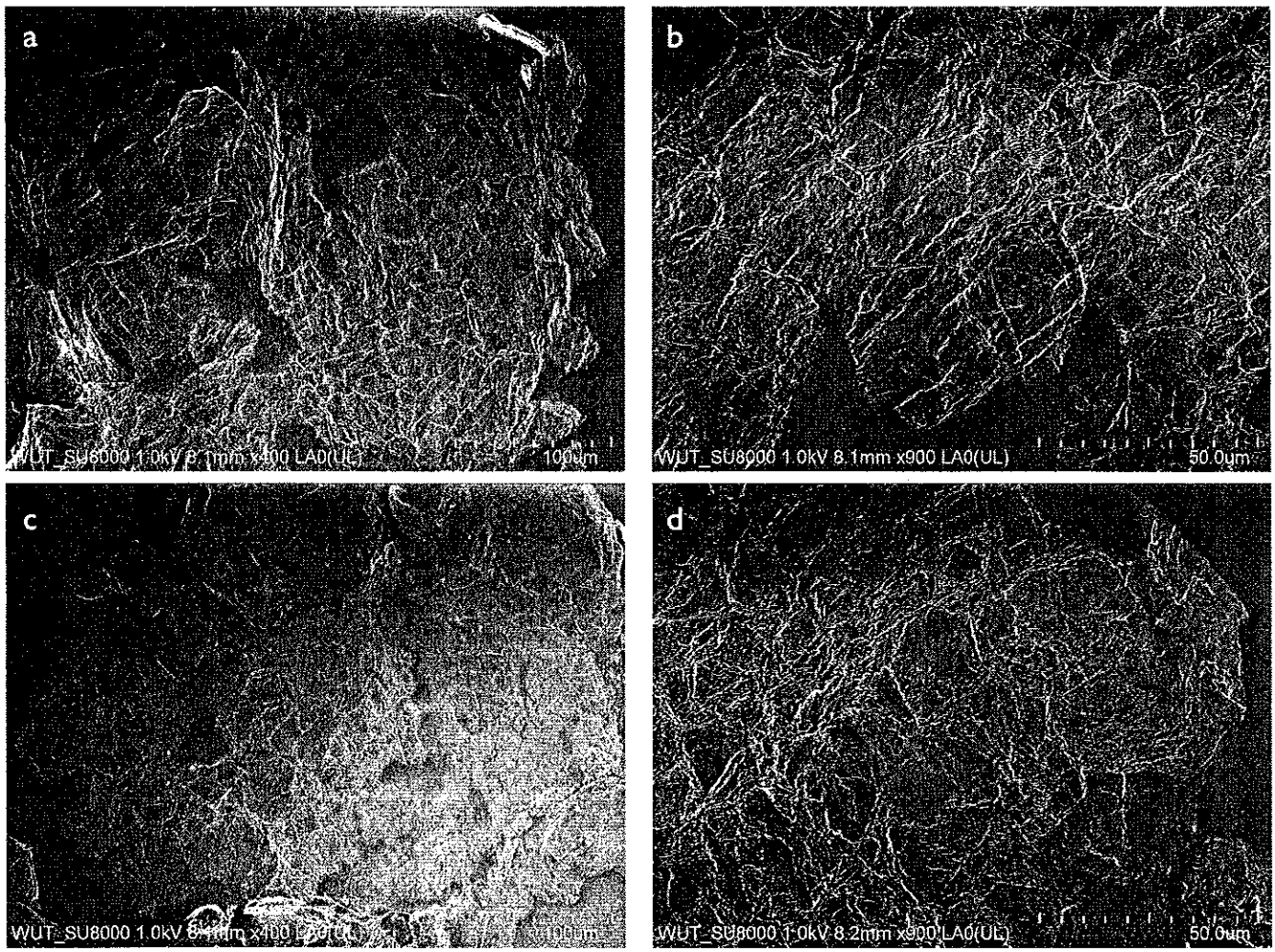

Fig. 6. SEM corneocyte images: before treatment with the cosmetic (a, b) and after a 5-day treatment with the cosmetic (c, d)

Figure 7 shows the surface topography of selected corneocytes recorded in three channels: height (topography data), feedback signal from the controller, and friction. Furthermore, corneocyte images are shown in 3D.

Estimation of Young's modulus values for corneocytes

The mean value of Young`s modulus was determined by fitting Gauss distribution to histograms created from E values calculated for every single curve (Fig. 8 a, b).

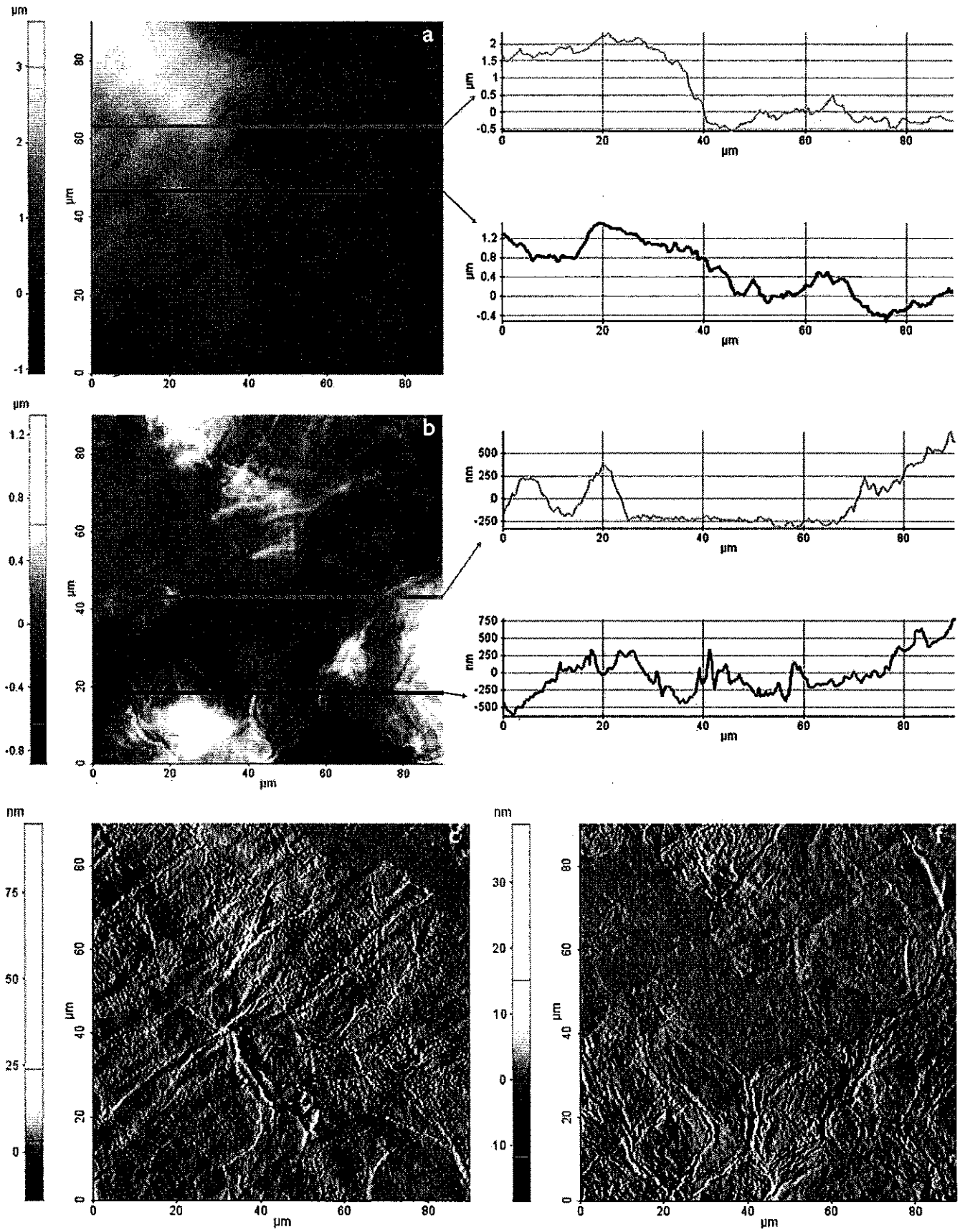

Fig. 7. Surface topography of corneocytes taken from skin not treated with the cosmetic (a, c, d, e) and treated with the cosmetic (b, f, g, h) from the channels: topography, feedback signal from the controller, friction, and as a 3D image

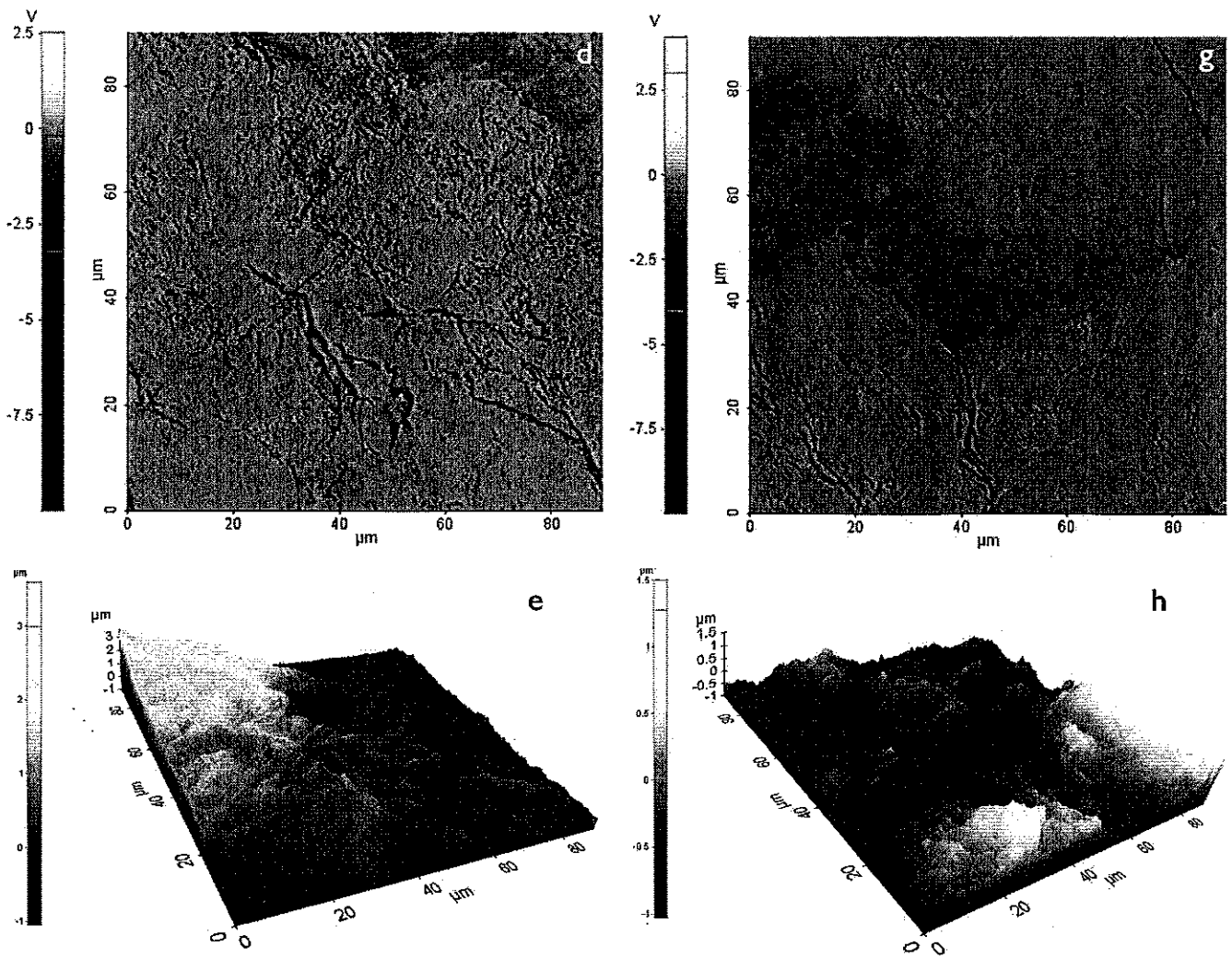

Fig. 7 cont. Surface topography of corneocytes taken from skin not treated with the cosmetic (a, c, d, e) and treated with the cosmetic (b, f, g, h) from the channels: topography, feedback signal from the controller, friction, and as a 3D image

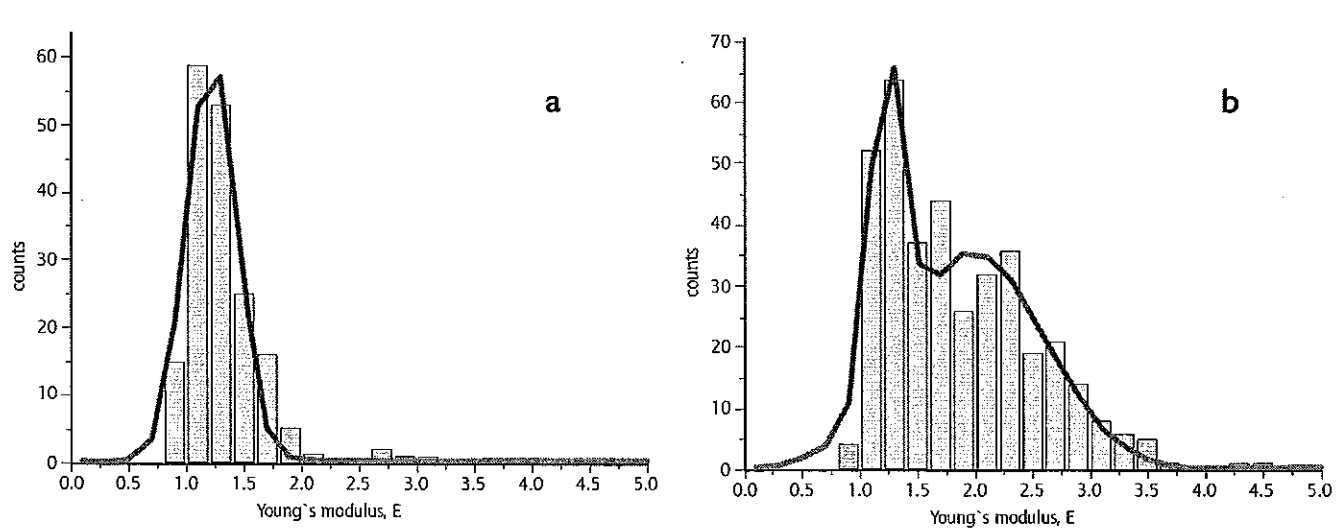

Fig. 8. Young's modulus value distributions for cells not treated with the cosmetic (a) and after 5 days of treatment with the cosmetic (b)

The resulting distributions of Young's modulus values determined from Gauss distribution and estimated using the Student's test (T-test) are shown in Table I.

Table I. Young's modulus values determined according to the JKR model

Corneocytes	Number of curves tested	E value calculated from Gauss function fitting* [MPa]	Mean value, E** [MPa]
control	179	1.30±0.62	1.34±0.46
after 5 days of treatment	371	1.70±1.48	1.87±0.65
		1.28±0.31 1.99±1.23	

* Error in the determination of the centre of distribution is half distribution width at half its height.
** The resulting error is standard deviation.

In summing up the results of the Student's t-test, it can be concluded that statistically:

- Control corneocytes (not treated with the cosmetic) **have different mechanical properties** than corneocytes after the 5-day treatment with the cosmetic at a level of **p=0.00001**.
- Control corneocytes (not treated with the cosmetic) **are more rigid** (that is, they have a fraction with a higher Young's modulus value) than corneocytes after the 5-day treatment with the cosmetic at a level of **p=0.00001**.

Corneocyte adhesive properties

The concept of adhesion force measurement using AFM is based on the estimation of the force needed to detach the AFM tip from the test surface, here the corneocyte surface. The measurement of the "pull-off" P_{off} force requires recording the tip-sample interaction force as a function of relative distance. When analysing curves, the area around point 5 is especially important (Fig. 4). This corresponds to the time point when the measuring tip detaches from the tested surface. This occurs when P_{off} force values become smaller than elastic and repulsive forces. The section between points 5 and 6 determines the adhesion force value between the tip and the tested surface. The resulting distribution of adhesion forces between the AFM sampling tip and the test corneocyte surface is shown in Figure 9.

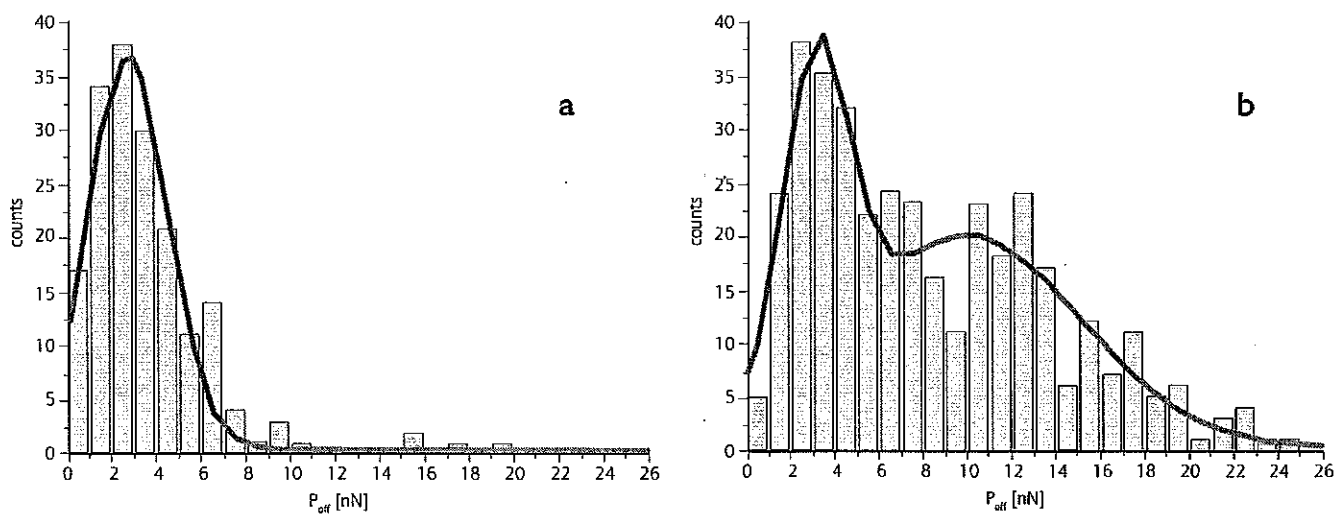

Fig. 9. Pull-off force value distributions for cells not treated with the cosmetic (a) and after 5 days of treatment with the cosmetic (b)

The resulting distributions of P_{off} adhesion force determined from Gauss distribution are shown in Table II.

Table II. Mean adhesion force Poff values

Corneocytes	Number of curves tested	Poff value determined from Gauss distribution [nN]
control	179	2.72 ± 4.20
after 5 days of treatment	371	6.28±14.58
		1.22±0.28 9.94±10.32

Conclusions

The atomic force microscopy technique was used for studying the condition of cells within the external skin stratum corneum (corneocytes). By applying the method, we can observe large differences in rigidity, or adhesion force, of corneocytes taken from the forearm inner area before and after five days of treatment with the cosmetic. It should be noted when analysing the Young's modulus values E for the cells tested that the estimation based on the JKR model shows differences

in elastic properties between cells not treated with the product and those taken after 5 days of treatment. Furthermore, distribution characteristics change. For corneocytes after the 5-day treatment with the cosmetic, two fractions of E values are seen, with rigidity close to that present in corneocytes not treated with the cream (E modulus values: ~ 1.3 MPa) and more rigid ones (E ~ 2.0 MPa). When the product was used, a fraction of spots located on cell surface with higher Young's modulus values formed.

In addition, the tests showed differences in the adhesive properties of the cells tested due to the treatment with the product. The distribution of adhesive forces P_{off} occurring between the AFM tip and cell surface is similar as for Young's modulus values. For cells treated with the cosmetic, an additional fraction of P_{off} values appears, related to stronger tip-treated cell interactions.

To conclude, corneocytes after treatment with the cosmetic become smoother and more rigid, and their adhesion force increases. This is likely to be a reaction to the product used, as its ingredients include materials with moisturising and anti-ageing properties, such as rose oil (*rosa canina*, *rosa mosqueta* or *rosa rubignosa*; all names are commonly used) and low-molecular weight hyaluronic acid [5, 6]. Compounds found in rose oil have strong anti-inflammatory activity and improve water exchange in cells. They stimulate the formation of new skin cells and improve skin tissue regeneration capability by accelerating keratinocyte differentiation. Hyaluronic acid, in turn, does not penetrate into deeper skin layers, but rather coats the skin stratum corneum, maintaining its moisture. Properly moisturised epidermis is flexible and elastic.

When the structure, functions and characteristics of corneocytes are taken into consideration, we conclude that the variation in standard deviations is not an experimental error. Therefore, the resulting mean values can be used as indicators of cell response to the treatment. Therefore, atomic force microscopy can be used in the development of new skin care products.

Piśmiennictwo / References

1. Johnson KL, Kendall K, Roberts AD. Surface energy and the contact of elastic solids. Proc R Soc A 1971, 324: 301-313.
2. Cappella B, Dietler G. Force-distance curves by atomic force microscopy. Surface Science Reports 1999, 34: 1-104.
3. Lee G, Kang S-K, Kwon D. Characterization of elastic modulus and work of adhesion in elastomeric polymers using microinstrumented indentation technique. Mater Sci Eng A 496, 2008: 494-500.
4. Gaikwad RM, Vasilyev SI, Datta S, Sokolov I. Atomic Force Microscopy characterization of corneocytes: effect of moisturizer on their topology, rigidity, and friction. Skin Research Technol 2010, 16: 275-282.
5. Chrubasik C, Duke RK, Chrubasik S. A systematic review on the rosa canina effects and efficacy profiles. Phytother Res 2008, 6: 725-33.
6. Jurzak M, Włodarska K, Garncarczyk A, Gojniczek K. Kwas hialuronowy – glikozaminoglikan o wielokierunkowym działaniu. Dermatol Estet 2008, 10, 4: 240-247.