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CENTRE FOR SCIENCE AND RESEARCH

Atomic force microscopy characterization of corneocytes

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Atomic force microscopy (AFM) is a novel technique for ex vivo analysis of epidermal stratum corneum cells (corneocytes). In this study, AFM was used to evaluate topography, elasticity and adhesion of corneocytes before and after treatment with moisturizer. Cream was applied daily on the forearm for a period of 5 days. The material for test was collected from the skin surface of volunteers using Cuderm tape strips before and after the treatment. First, changes in the morphology of the cells were evaluated. It was found that corneocyte surface was smoother after the application of the cosmetic product. The analysis also included Young's modulus measurements. Young's modulus is a measure of elasticity (flexibility) of the tested material. The study results prove the existence of statistically significant differences in the mechanical properties of corneocytes taken after cosmetic treatment compared to the control sample. Control corneocytes were more rigid than corneocytes taken after the 5-day cosmetic treatment. The measurements of adhesive forces was based on the estimation of the force needed to detach the AFM tip from the corneocyte surface. The results revealed a relatively wide distribution of adhesive forces in similar way to Young's modulus values. A bigger fraction characterised by stronger tip-surface interactions was recorded for samples taken after the cosmetic treatment. The AFM analysis of corneocytes taken by tape-stripping proved that they became smoother, less rigid, with stronger adhesive bonds between them after the cosmetic treatment. In conclusion, AFM can be used as a very sensitive tool for early detection of changes in corneocytes after moisturizer usage.

AIM OF STUDY:

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

MATERIALS AND METHOD

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.

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.

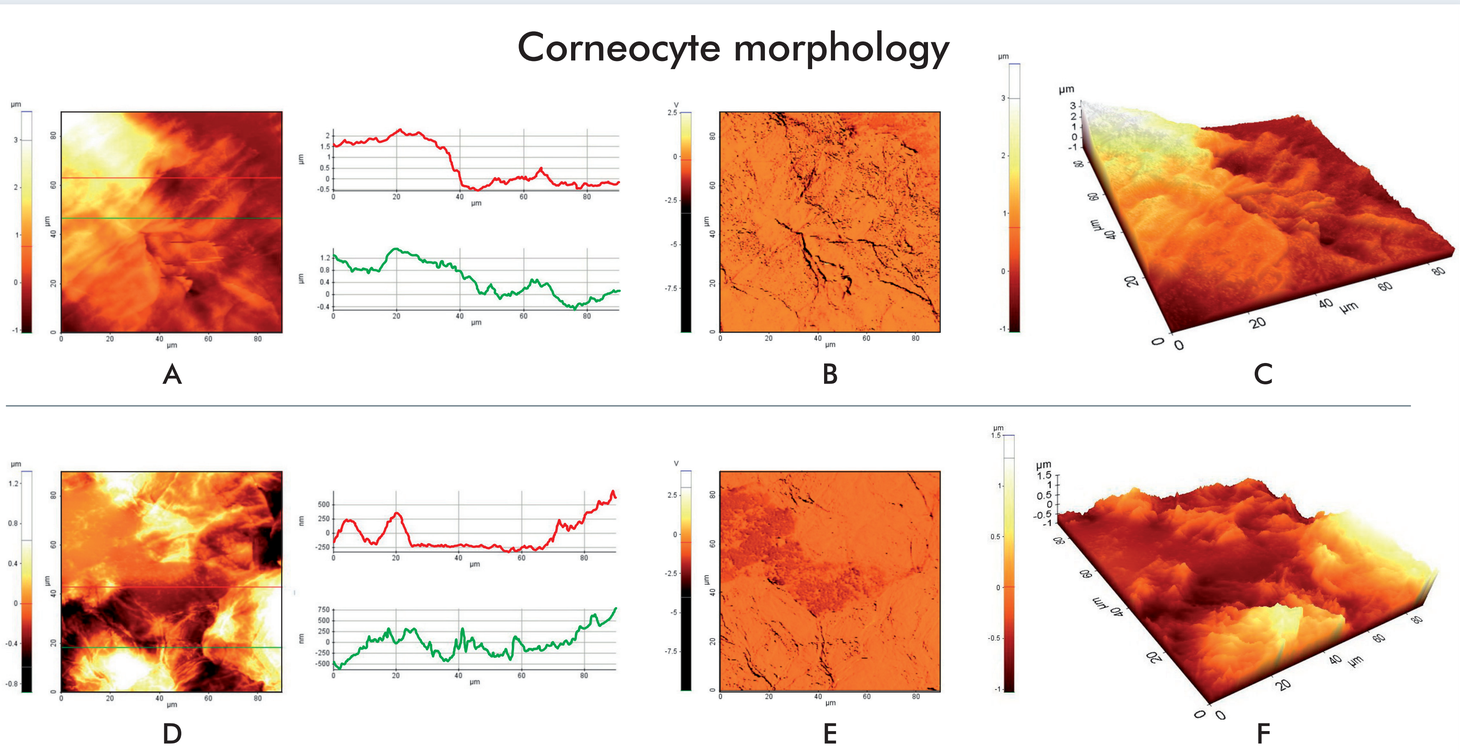

Figure 1. Surface topography of corneocytes taken from skin not treated with the cosmetic product, **A, B, C** and treated with the cosmetic product, **D, E, F** from the channels: topography, feedback signal from the controller and friction, 3D image

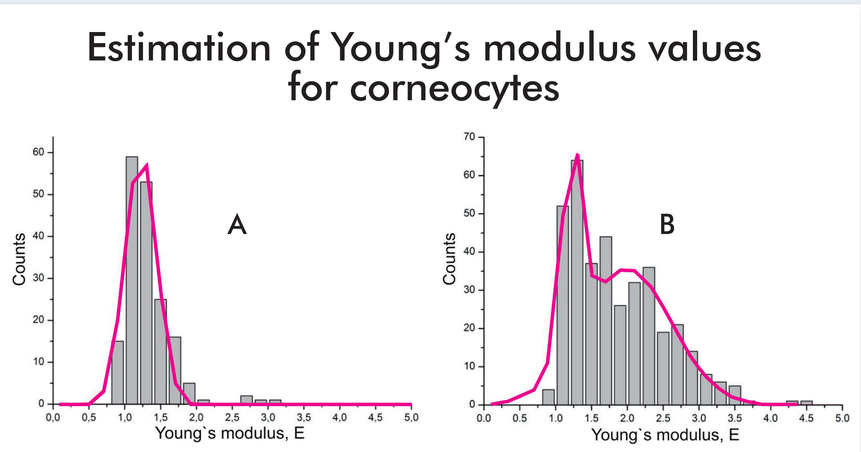

Figure 2. Young's modulus value distributions for cells a) not treated with the cosmetic product and b) after 5-days of treatment with the cosmetic product
The resulting distributions of Young's modulus values determined from Gauss distribution and estimated using the Student's test

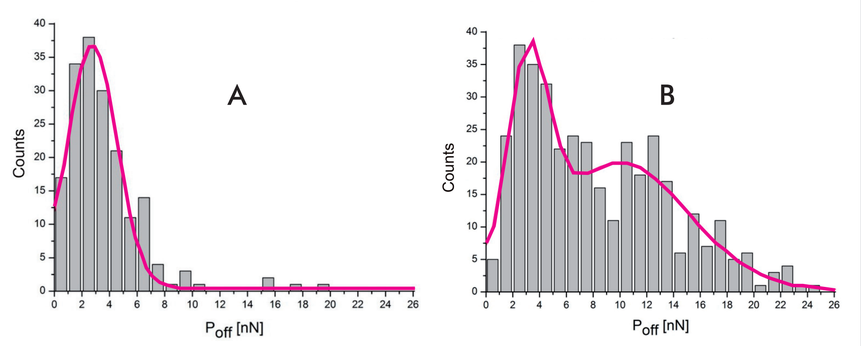

Figure 3. Pull-off force value distributions for cells a) not treated with the cosmetic product and b) after 5-days of treatment with the cosmetic product
The resulting distributions of P_{off} adhesion force determined from Gauss distribution

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

Corneocytes	Number of curves tested	E value calculated from Gauss function fitting E *[MPa]		Mean value (T-test) E **[MPa]
Control	179	1,30 ± 062		1,34 ± 0,46
After 5 days of treatment	371	1,70 ± 1,48		1,87 ± 0,65
		1,22 ± 0,28	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.

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. 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. 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 Poff 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 Poff values appears, related to stronger tiptreated cell interactions. To conclude, corneocytes after treatment with the cosmetic become smoother and less rigid, and their adhesion force increases. Therefore, atomic force microscopy can be used in the development of new skin care products.