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Human buccal epithelial cell envelope reactions to external micromechanical stimuli

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The reaction of the shell of an adult living cell of human buccal epithelium to an external micromechanical stimulus was studied. The dependence of the magnitude of the reaction on the level of passive or active stimulation, namely, the level of force exposure, was established. It is shown that the cell can exhibit both active and passive reactions to an external micromechanical stimulus, as well as control the level of exposure to adhesive forces.

Keywords: human buccal epithelium cell, shell, micromechanical stimulus, shell elasticity, AFM force spectroscopy.

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The functional state of the human buccal epithelium cells (hereinafter referred to as cells) is strongly informative with respect to the external factors influence on the human body, which makes them a biological object convenient for the life−time diagnostics of the majority of socially important diseases [1–4]. At the same time, the amount of experimental data on the cell shell response to external mechanical stimuli still remains extremely insufficient. In some cases this is connected with experimental conditions, in other cases this is related with unavailability of up−to−date diagnostic techniques providing detection in the same measurement cycle of the entire set of micromechanical parameters of living biological objects (Young's modulus E, longitudinal elasticity coefficient K, adhesive forces F_{adh} and their work function A_{adh}, plastic Δh_{deform} and elastic Δz deformations). Thus, the main task is to develop efficient methods for quantitative monitoring of how the cells percept [5–7] and convert [8,9] external mechanical signals.

In this work we have studied living cells of the human buccal epithelium obtained by liquid−based cytology on the epitaxial silicon surface under normal conditions (Fig. 1, *a*, *b*). The cell viability test by the dye (trypan blue) exclusion method after 2.5 h provided a positive result: the major part of cells was resistant to the dye, which was caused by the presence of the buffer solution (3.03 mM of phosphate buffer (pH = 7.0) plus 2.89 mM of calcium chloride) adsorption layer ~ 100 nm thick remaining on the cell surface after preparing the samples. The cell viability extent was controlled also by their appearance (Fig. 1, *b* and *c*). On the shells of killed cells, their occurred the protein coagulation manifested in the raster image as peptidoglycan dentrits (Fig. 1, *c*).

The cell shell reactions to an external micromechanical stimulus were studied in air under normal conditions using an atomic−force microscope (AFM) NTEGRA SPECTRA; the measurements were performed with cantilever HA_FM/W_2C (with the tip rounding radius of 35 nm) at the Common Use Center „Molecular structure of matter" of the Sevastopol State University. We used the method of force spectroscopy $F_z = F_z(z)$ in the contact scanning mode (the mode of constant force $F_z = F_{const} \approx 20$ nN) in which the approach curves (Fig. 2, curves *1*) and retraction curves (Fig. 2, curves *2*) were measured.

As references, spectra of the approach and retraction curves measured on the free silicon surface were used (Fig. 2, *a*). From them, some features may be distinguished which correspond to the repulsive forces (Fig. 2, *a*, curve *1*, $+F_{\mathrm{VW1}}$, section BC, barrier φ_b) and attractive forces (Fig. 2, *a*, curve *1*, $-F_{\mathrm{VW2}}$ and curve *2*, $-F_{\mathrm{VW3}}$). In both cases, linear section DE describes according to the Hooke's law ($F = Kz$, where K is the elasticity coefficient and z is the coordinate) the cantilever−shell mechanical interaction in the presence of repulsive forces.

It was revealed that, depending on the initial exposure force F_{const}, the living cell response can exhibit both the active and passive shell reaction to the external micromechanical stimulus. As shown by behavior of retraction curves, on the cell shell surface there was a rather thick ($\Delta z_a < 100$ nm) buffer solution adsorption layer (Fig. 2, *b*, inset).

It is possible to relate to the first (passive) type the elastic (an analogue of section DE in Fig. 2, *a*) shell responses initiated by an external passive, i.e. insignificant, impact (Fig. 2, *b*, cases I and II), which were caused only by its elastic micromechanical properties. In case I at $F_{\mathrm{I},const1} = 4\,\mu$N, the elastic strain appeared to be $\Delta z_1 = 69$ nm with $K_1 = 65.7 \pm 0.2$ N/m. In case II at $F_{\mathrm{II},const1} = 6.2\,\mu$N, elastic strain appeared to be $\Delta z_2 = 115$ nm with $K_2 = 67.4 \pm 0.15$ N/m. The approach curve in close proximity of the surface exhibited the absence of repulsive forces (barrier φ_b), while the

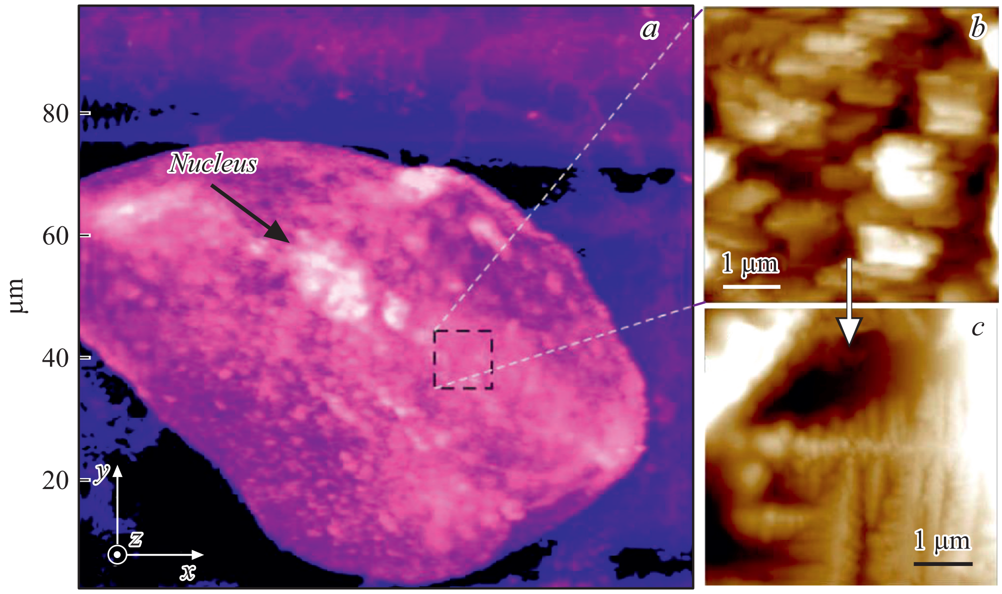

Figure 1. Raster 256×256-pixel AFM images of sections $(100 \times 100\,\mu\mathrm{m})$ of reliefs $h(x, y)$ of the epitaxial Si$\{111\}$ surface on which an adult living cell of the human buccal epithelium is located; the images were obtained after studying by the AFM force spectroscopy the cell itself (a), the $5 \times 5\,\mu\mathrm{m}$ section of its shell surface (b), and the same shell section $(5 \times 5\,\mu\mathrm{m})$ of already dead cell after its protein coagulation in 30 min of staying in air at 40°C (or in > 4 h at room temperature) (c).

retraction curves exhibited arising of adhesive attraction forces $F_{adh} \equiv F_{\mathrm{VW3}} \approx -0.2\,\mu\mathrm{N}$ (the section BD analogue).

The second (active) type of the responses occurred in the case of an active (i.ė., stronger) initial cell shell stimulation ($6 < F_{\mathrm{III},const1} < 21\,\mu\mathrm{N}$, in the case of $F_{\mathrm{III},const1} = 10.1\,\mu\mathrm{N}$ the elastic strain was $\Delta z_3 = 220$ nm with $K_3 = 80.2 \pm 1.18$ N/m, Fig. 2, *b*, case III) and was accompanied by an active shell resistance manifesting itself in the departure from linearity (Fig. 2, *b*, case III, dashed line) and turnup of the approach curve nonlinear section. Emergence of slight plastic deformations Δh_{deform} was accompanied by production of work, which manifested itself in the appearance of hysteresis between the approach and retraction curves (section DE, Fig. 2, *b*, case III, curves *1* and *2*). In this case, the retraction curve passed somewhat lower than the retraction curve but relatively close to it, and the cell shell needed minimum energy to restore its initial condition and return the retraction curve to the previous trajectory. Thereat, the initial value of $F_{\mathrm{III},const}$ began degenerating into $F_{\mathrm{III},const1}$ and $F_{\mathrm{III},const2}$ (Fig. 2, *b*, inset).

Notice that the qualitative (but not quantitative) similarity in the behavior of the approach and retraction curves was observed by authors of [3] (but without description) in studying by the AFM force spectroscopy the elasticity of local sections $(20 \times 20\,\mu\mathrm{m})$ of shells of human intestinal cells Caco-2.

The third (passive) type of responses took place at further enhancement of the initial cell stimulation to above $F_{const} \geqslant 21\,\mu\mathrm{N}$ and was accompanied by already decreasing shell resistance, namely, passive resistance (Fig. 2, *b*, case IV, curve *1*). As the approach curve shows, elevation of the initial force impact level F_{const} also resulted in the departure from linearity, but in this case the nonlinear section (the section DE analogue) deviated from the linear dependence not upward but downward (Fig. 2, *b*, case IV, dashed straight line). Thereat, the increase in plastic deformation sizes $\Delta h_{deform4} = \Delta z_4 - z_{deform4} = 101$ nm ($\Delta z_4 = 321$ nm with $K_4 = 84.5 \pm 0.22$ N/m) was accompanied by a reduction rather than by an enhancement of external force impacts caused by the cell shell passive reaction. In other words, at the initial force impact $F_{const} > 21\,\mu\mathrm{N}$ the human buccal epithelium cell shell is no longer able to exhibit sufficient resistance to external impacts.

The behavior of the retraction curves shows that, in the force exposure cases III and IV, gradual decrease in the load is followed, beginning from a certain level of external exposure ($F_{(\mathrm{III,IV})const} \approx 5\,\mu\mathrm{N}$), by self–assisted recovery of the cell shell shape (Fig. 2, *b*, curves *2*). For instance, in the ordinary classical variant, gradual removal of load from the plastically deformed body would bring the retraction curve to point $z_{deform4}$ defining the plastic deformation size $\Delta h_{deform4}$ (Fig. 2, *b*, case IV, curve *2*). In reality, the retraction curve behavior shows that at, e.g., $F_{\mathrm{IV},const} < 5\,\mu\mathrm{N}$, it returns to its initial trajectory, and the shell begins restoring its mechanical and geometric characteristics. Being measured again, the approach and retraction curves behaved in the same way. It is clear that such an unconventional shell response to the external mechanical exposure cannot be reduced to a simple mechanical process and needs consumption of internal energy and information resources of the cell. The absence of holes in the AFM images obtained

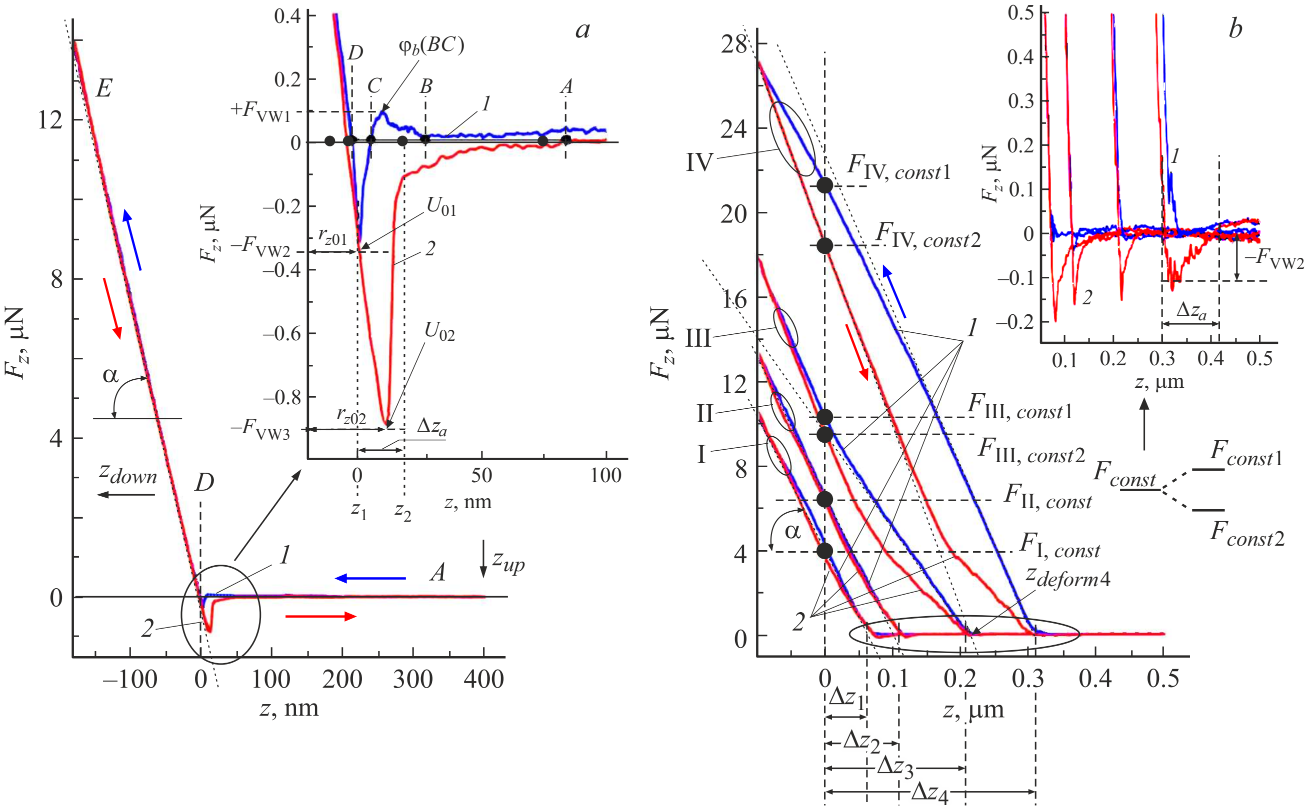

Figure 2. Approach curves (*1*) and retraction curves (*2*) (the AFM force spectroscopy method) measured on the epitaxial silicon surface (*a*) and on the surface of human buccal epithelium cell shell at the following magnitudes of the initial force exposure: I — $F_{\mathrm{I},const1} = 4\,\mu$N, II — $F_{\mathrm{II},const1} = 6.2\,\mu$N, III — $F_{\mathrm{III},const1} = 10.1\,\mu$N, IV — $F_{\mathrm{IV},const1} = 21.6\,\mu$N (*b*). The insets present more detailed images in the adsorption layer region.

after force exposure indicates that the shell was not distorted by the force impacts (Fig. 1, *b*).

Almost total absence of barrier φ_b in the approach curves in cases III and IV evidences for the absence of the shell hydrophoby. The negative retraction curve sections in the near–surface region indicate the presence of adhesive attraction forces (an analogue of section *BD* in the Fig. 2, *a* inset). This fact evidences for relatively low hydrofility and almost absolute absence of hydrophoby in the cell shell initial state. Since the cell wettability is low relative to that of the substrate, the cell is able to freely move in the environment; the absence of hydrophoby allows them to easily withstand the surface tension forces.

Thus, it was established that the human buccal epithelium cells can exhibit not only passive but also spontaneous active reactions to external micromechanical stimuli, and control the level of exposure to adhesive forces.

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Ethical practices

All the first–in–human trials performed in the framework of this study comply with Ethical Standards of the institutional and/or national research ethics committee, as well as with Declaration of Helsinki (1964) and its subsequent amendments or matched ethical norms. The permission for studies with the buccal epithelium sampling was obtained by the SSU Ethics Committee (study № 3; July 15, 2021). The buccal epithelium was sampled according to the RF Regulations for First–in–Human Trials. An informed voluntary content was obtained from each trial participant.

Conflict of interests

The authors declare that they have no conflict of interests.

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