Physical-mechanical image of the cell surface on the base of AFM data in contact mode

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Abstract. Physical and mechanical properties of the cell surface are well-known markers of a cell state. The complex of the parameters characterizing the cell surface properties, such as the elastic modulus (E), the parameters of adhesive (Fₐ), and friction (Fᵢ) forces can be measured using atomic force microscope (AFM) in a contact mode and form namely the physical-mechanical image of the cell surface that is a fundamental element of the cell mechanical phenotype. The paper aims at forming the physical-mechanical images of the surface of two types of glutaraldehyde-fixed cancerous cells (human epithelial cells of larynx carcinoma, HEp-2c cells, and breast adenocarcinoma, MCF-7 cells) based on the data obtained by AFM in air and revealing the basic difference between them. The average values of friction, elastic and adhesive forces, and the roughness of lateral force maps, as well as dependence of the fractal dimension of lateral force maps on Z-scale factor have been studied. We have revealed that the response of microscale areas of the HEp-2c cell surface having numerous microvilli to external mechanical forces is less expressed and more homogeneous in comparison with the response of MCF-7 cell surface.

1. Introduction

Physical and mechanical properties of the cell surface representing the layered composite material consisting of glycocalyx, lipid bilayer, and cytoplasmic layer with cortical cytoskeleton are well-known markers of a cell state. Basic cellular element contributing the mechanical properties of cell surface is the cortical cytoskeleton as a cell bearing support. The complex of the parameters characterizing such cell surface properties as the elastic modulus (E), adhesive (Fₐ) and friction (Fᵢ) forces can be measured using atomic force microscope (AFM) in a contact mode. This complex of AFM parameters forms namely the physical-mechanical image of the cell surface that is a fundamental element of the cell mechanical phenotype [1]. The mechanical phenotype (“mechanotype”) of cells is considered by many researchers to be a potential biomarker for cell types ranging from pluripotent stem to cancer cells [2, 3]. The complex of the parameters forming the physical-mechanical image of the cell surface includes not only the averaged characteristics of elastic, adhesive, and friction properties, but also the spatial distribution of these properties over the cell surface.

The paper aims at forming the surface physical-mechanical images of two types of cancerous cells (human epithelial cells of larynx carcinoma, HEp-2c cells, and breast adenocarcinoma, MCF-7 cells) based on the data obtained by AFM and revealing the basic difference between them.
2. Materials and methods

2.1. Preparation of cell samples for AFM

Samples of human primary skin fibroblasts, larynx carcinoma cells (HEp-2c cell line), and breast adenocarcinoma cells (MCF-7 cell line) were kindly provided by D. R. Petrenyov (Institute of Radiobiology, NAS of Belarus, Gomel, Belarus). The cells suspensions were placed on the specially prepared glass slides in Petri dishes with growth medium and incubated during 24 h at 37 °C and 5% CO₂. Then the cells were fixed with 0.5% glutaraldehyde (30 min) and washed with deionized water. The cells were dried in tilted position (75-85°) in air laminar stream (0.42 m/s) at room temperature.

2.2. Atomic force microscopy

The scanning was performed with an AFM (“NT-206”, MicroTestMachines Co., Belarus) using standard probes (MikroMasch) in air under room conditions. CSC38 AFM cantilever type (lever B, nominal force constant of 0.03 N/m) was used. Topographic images and lateral force maps were recorded. Lateral forces determined mostly by the friction forces between the AFM probe tip and surface were assessed by measuring the cantilever's torsion value and represented in arbitrary units. Using the lateral force maps, the statistical parameters of the surface roughness (Rq) were assessed. The microscale AFM images of the cell surface without apparent defects were used for analysis. The cell surface regions for AFM analysis were chosen between the nucleus and peripheral region. The elastic modulus (Young’s modulus) and adhesive force were assessed by force spectroscopy (force-curve analysis) [4] using blunted NSC11 (MikroMasch) cantilever tips (radius 76-81 nm, force constant 3.0 N/m). The indentation depth was 10 nm.

2.3. Fractal dimension estimation

Fractal dimension (Df) was calculated for lateral force maps of the cell surface (2.5×2.5 µm²) using the following formula (box counting dimension):

\[ D_f = \lim_{\varepsilon \to 0} \frac{\lg N(\varepsilon)}{\lg \frac{1}{\varepsilon}} \]  

where N(ε) is the number of cubes with edge ε that together included the studied surface.

The area with the studied surface was divided by cubic lattice with cube edge length ε that was initially set as half of the area size. Then the number of cubes that included at least one point of the surface was calculated. After that, cube edge length ε was reduced by half and the process was repeated in loop until cube edge length became less than a certain constant depending on the AFM scanning step. The fractal dimension of the surface was estimated as a maximal slope coefficient of the obtained dependence between lg(N(ε)) and lg(1/ε). In the used modified box counting algorithm, the surface was initially divided into 8 equal fragments. After calculation of the dimension for each fragment, the fractal dimension of the whole surface was calculated as the mean value and limits of 95% confidence interval. To analyze the dependence of Df on the Z-scale factor, X- and Y-data of the AFM image were not changed but Z-data was multiplied by t that was changed in a broad range. The fractal dimension was calculated using the box counting algorithm for each t value and the dependence Df(t) was plotted and analyzed.

2.4. Statistical analysis

The correlation of data to normal distribution was checked with the Shapiro-Wilk’s W test. Results are represented as limits of 95% confidence interval (CI). The sample means were compared with Student’s t test.

3. Results and discussion

The basic morphological parameters of the studied cells (human fibroblasts and cancerous epithelial cells of lines: HEp-2c and MCF-7) are represented in Table 1. Figure 1 shows the typical lateral force maps of the cell surface.
Figure 1. Typical lateral force images of cell surface areas in the middle part of (a) fibroblast, (b) MCF-7 cell and (c) HEp-2c cell. Image area: 2.5 × 2.5 μm².

Table 1. Morphological parameters of cells.

| Cells      | D₁, μm   | D₂, μm   | h, μm   | D₂/D₁ | D₁/h  | D₂/h  |
|------------|----------|----------|---------|--------|--------|--------|
| Fibroblasts| 60.4±26.6| 22.3±6.9 | 1.5±0.3 | 0.39±0.15 | 40.7±12.8 | 16.3±8.7 |
| MCF-7 cells| 33.1±11.6| 14.9±1.8 | 1.5±0.4 | 0.51±0.18 | 22.6±6.5  | 10.5±2.6 |
| HEp-2c cells| 28.1±2.4| 11.1±2.5 | 1.0±0.3 | 0.40±0.08 | 30.0±9.0  | 12.0±4.1 |

D₁ and D₂ are the maximal and minimal sizes of cells measured in orthogonal directions in the plane of the cell contact to the glass surface; h is the maximal value of the cell height; D₂/D₁ is a parameter characterizing the cell polarization; D₁/h and D₂/h are parameters characterizing the cell spreading on a solid surface. Data are represented as the limits of 95% CI. n=5-13.

Though the cell sizes differ for the different cell types, especially in the case of fibroblasts and cancerous epithelial cells, nearly all the parameters showing the cell ability to spreading and polarization are the same.

To form the physical-mechanical images of the surface of the studied cells we used the complex of AFM parameters obtained in a contact mode. The complex included the statistical parameters (the mean and standard deviation) of the elastic modulus (Young’s modulus, E), adhesive force (Fₐ) measured in different locations of the cell surface, the roughness (Rₚ) of lateral (friction) forces assessed over microscale lateral force maps of the cell surface, the fractal dimension (Dₖ) of microscale lateral force maps of the cell surface and its dependence on Z-scale factor (t). Parameter Dₖ=f(t) characterizes the structure of the distribution of the mechanical properties of the cell surface more comprehensive than a single value of the fractal dimension calculated for the AFM image of the cell surface as well as the roughness of the same AFM image [5].

Averaged values of the Young’s modulus and adhesive force for two types of cancerous epithelial cells were significantly less than those for fibroblasts. The obtained result is in accordance to literature data that states the lower stiffness for cancerous cells compared to the stiffness of the cells of normal tissues [6]. For both analyzed cancerous cell types, the parameters were close to each other, but the significant shift (p<0.05) of their values to the lower value interval for HEp-2c cells was fixed (Fig. 2).

Figure 2. Frequency polygons of (a) relative elastic modulus and (b) relative adhesive force.
Figure 3. Spatial distribution of the cell mechanical properties. (a) Roughness ($R_q$) of lateral forces measured over microscale area of the cell surface. (b) Dependence of the fractal dimension ($D_f$) of microscale lateral force maps for the cells on Z-scale factor ($t$).

Table 2. Fractal dimension of the lateral force maps for different cell types at two Z-scale coefficients. Data are represented as the limits of 95% CI. n=6-9.

| Cell type      | $t = 0.0183$ | $t = 2.7183$ |
|----------------|-------------|-------------|
| Fibroblasts    | 2.27±0.07   | 2.32±0.04   |
| MCF-7 cells    | 2.07±0.04   | 2.15±0.08   |
| HEp-2c cells   | 1.93±0.06   | 2.02±0.05   |

The parameters related to the distribution of the mechanical properties of the cell surface were also different for the different cell types. The roughness of lateral force maps for HEp-2c cells was significantly less in comparison with those of both fibroblasts and MCF-7 cells (Fig. 3a).

The parameter $D_f(t)$ for all studied cell types differs both quantitatively and qualitatively (Fig. 3b, Table 2). The fractal dimension of the lateral force maps for HEp-2c cell surface is lower at the coefficients $t$ corresponding to the maxima of dependence $D_f(t)$ for fibroblasts (Table 2) and the shape of the curve $D_f(t)$ is smoother than one for other cell types and has one maximum (Fig. 3b). These facts support more homogeneous distribution of the mechanical properties of the studied surface areas for HEp-2c cells than for MCF-7 cells.

Relative elastic modulus ($E/E(FB)$) and relative adhesive force ($F_a/F_a(FB)$) are calculated with respect to the mean values of elastic modulus and adhesive force for the samples of fibroblasts.

In contrast to the surface of MCF-7 cells and fibroblasts, there are many microscopic cellular membrane protrusions (microvilli) on the surface of the HEp-2c cells (Fig. 1). The microvilli effectively increase the cell surface area and are useful for such cell absorption and secretion. Adherent cancerous cells as a rule do not have a developed actin cap, but have many short microvilli on their surfaces [7] and the increased presence of microvilli correlates closely with the growth potential and metastatic ability of cancerous cells [8]. On the one side, microvilli can unify the mechanical properties of the cell surface at the microscale, because the cell surface is covered completely with one type of actin structures. On the other side, these superstructures are more flexible than the cellular surface itself, which can change the response of the cell surface with microvilli to mechanical stress. Our experiments were performed in air, thus, the behavior of the microvilli in biological liquids can differ. It is known that microvilli are covered with glycoproteins, which supports the adhesion between cells. However, the general features of mechanical response of the cells surface with microvilli may be expected to be the same as we have revealed in our work.
4. Conclusion

Based on the data obtained with atomic force microscope in contact mode, the physical-mechanical images of the cell surface were obtained for two types of the cancerous epithelial cells in glutaraldehyde-fixed and dried states. They included the averaged values of the parameters characterizing elastic, adhesive, and friction forces and the parameters characterizing the spatial distribution (at the nano- and microscales) of these forces. They are the Young’s modulus, adhesive force, roughness, and fractal dimension of lateral force maps and its dependence on Z-scale factor. The study of the mechanical properties of cell surfaces has shown that the response of microscale areas of the HEp-2c cell surface with numerous microvilli to external mechanical forces is less expressed (lower elastic, adhesive, and friction forces) and more homogeneous in comparison with the response of MCF-7 cell surface.

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