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Measuring of Adhesion Force in the Cell—Cell System Based on Atomic Force Microscopy Technology

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We developed and tested a method for fabrication of a biosensor chip based on native human whole blood lymphocyte and a titanium tipless cantilever. The biosensor can be used for measuring intermolecular adhesion forces in the cell—cell system by using atomic force spectroscopy. The developed biosensor chip was applied for measuring adhesion force between lymphocyte and granulocyte and between lymphocyte and erythrocyte in healthy individuals and in patients with acute lymphoblastic leukemia (before treatment, during standard treatment, and during relapse). It was found that adhesion force between lymphocyte and granulocyte and between lymphocyte and erythrocyte increased almost twice during relapse, which is an important diagnostic marker of early cytological abnormalities indicating progression of the disease.

Key Words: *biosensor chip; atomic force microscopy; blood cells; adhesion*

Adhesion is a key mechanical property of living cells essential for immune reactions, migration, thrombus formations [1], cells invasion during tumor progression [6], and local inflammation in the vascular wall [3]. In molecular and cell physiology, various experimental approaches are used in studies of adhesion properties of cells. For instance, a popular method of liquid disintegration implies blood sampling, cell isolation and incubation in a microchamber, and estimation of the percentage of adherent forms after washing with buffer solution. A disadvantage of this approach is rough estimation of the number of adherent cells and impossibility of measuring the force of cell adhesion to the glass substrate. Moreover, the obtained information does not provide objective picture of cell—cell interactions. In biological systems, adhesion force are largely determined by the expression of adhesion molecules on contacting cells, while cell adhesion to glass substrate depends on not only its receptor apparatus, but also on the incubation medium containing protein complexes.

The use of atomic force microscopy in biological studies allows developing new methodical approaches to assessment of adhesion forces at the molecular level using single cells, cell monolayers, and polymer microspheres immobilized on tipless cantilevers [5]

Our aim was to measure adhesive forces in the cell—cell system by using a biosensor chip based on native lymphocytes.

MATERIALS AND METHODS

Whole blood lymphocytes were used for preparing a biosensor chip. Blood samples were obtained from patients with lymphoblast leukemia at the stages of primary diagnosis (N=4), therapy (N=4), and relapse (N=4). Blood samples from healthy donors (N=4) were used as the control. The blood was collected into vacuum tubes. The leukocyte and erythrocyte fractions were separated by 5-min centrifugation at 1500 rpm; the plasma and leukocytic ring were collected. Leukocyte viability was evaluated by *in vitro* staining with 0.4% trypan blue in PBS (pH 7.2-7.3); dead cells (stained blue) were counted under a light microscope. Cell suspensions with viability ≥95% were used in the experiments.

The cell biosensor was constructed on the basis of a standard tipless cantilever CSG 11/tipless treat-

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ed with 2% gelatin during scanning. To this end, a drop of gelatin solution was placed in the center of a degreased glass slide and erythrocyte and leukocyte suspensions were placed on each side. The preparation was transferred to a humid chamber [4]. Force spectroscopy was performed under an Integra Vita (NT-MDT) microscope. A standard cantilever CSG 11/tipless fixed in a probe holder of a measuring head of the microscope was brought to the gelatin drop. The scanning area was shifted to the side with leukocyte suspension. Under optical control, an area with single lymphocytes was chosen and the cantilever was brought to a single lymphocyte. As a result of force microcopy and pressing on the cell, a biosensor was prepared; this biosensor was then used for measuring adhesion forces in the lymphocyte-granulocyte and lymphocyte—erythrocyte systems. Using the prepared biosensor, force curves for at least 15 granulocytes from each sample were recorded by the force spectroscopy mode. Then, the scanning area was shifted to the erythrocyte suspension, where force curves for at least 15 erythrocytes in each sample were recorded.

The obtained force curves were processed using Nova software. The force-distance curves were transformed from D-z to F-ΔHeight coordinates, where D is photodiode mismatch current and z is change in piezotube length along Z axis. The force of probe adhesion to the sample was calculated by Hook's law:

$$F=k\times\Delta Height$$

where F is adhesion force (nN), k is spring constant (N/m), and ΔHeight is the change in piezotube length along Z axis.

The data were processed statistically using Microsoft Excel. Significance of differences between healthy individuals and leukemia patients was evaluated by the Mann—Whitney *U* test at $p<0.01$.

RESULTS

Measurements revealed differences in the adhesion forces between the groups of patients with acute lymphoblast leukemia at different stages of the disease and control individuals (Table 1). In the control group, adhesion force between lymphocyte and granulocyte was by almost 1.6 times higher than that between lymphocyte and erythrocyte. In the group of patients with acute lymphoblast leukemia, lymphocyte—granulocyte adhesion force at the stage of diagnosis increased by 37% ($p<0.01$) and at the stage of relapse by 117% ($p<0.01$) in comparison with the corresponding parameter in healthy controls; during therapy, adhesion force surpassed the control value by 18% ($p<0.01$). Lymphocyte—erythrocyte adhesion force significantly increased at the stage of leukemia diagnosis (by 46%) and during relapse (by 49%); however, no significant differences in this parameter were found during therapy.

TABLE 1. Adhesion Force (nN) in the Cell—Cell System (n=60; $M\pm m$)

Group	Lymphocyte—granulocyte	Lymphocyte—erythrocyte
Healthy individuals	75.6±1.1	46.5±0.9
Acute lymphoblast leukemia		
primary diagnosis	120.8±2.3*	86.9±1.4*
therapy	92.5±0.3*	44.6±0.3
relapse	164.1±0.4*	91.8±0.4*

Note. n: number of analyzed cells. *$p<0.01$ in comparison with the control (*U* test Mann—Whitney).

Thus, lymphocyte—erythrocyte adhesion force did not differ from the control during therapy, but considerably increased during relapse (by almost 2 times). Lymphocyte—granulocyte adhesion force increased at all stages of the disease, especially, during relapse and debut. It can be hypothesized that this increase in adhesion properties of the cells during the development of tumor process in the blood system is determined by greater number of terminal sialic acid residues in the O- and N-type carbon chains on the cell surface [2]. Hence, the proposed approach provides data about adhesion of blood cells in normal and during the development of pathophysiological conditions, *e.g.*, leukemia. Parameter of cell—cell adhesion can be used as an early marker for detection of residual leukemic cells after treatment, which will help to solve an important scientific and practical problem related to the choice of individual treatment protocol and to predict and detect early cytological signs of disease relapse.

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