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Shevchenko, Tatyana S.; Skorkina, Marina Yu.; Zhernakova, Nina I.; Shentseva, Elena A.

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Artículos

The measurement of adhesion forces in the system «cell-cell» by using atomic force microscope

La medición de las fuerzas de adhesión en el sistema «célula-célula» utilizando un microscopio de fuerza atómica.

Tatyana S. Shevchenko
Belgorod State University, Rusia
shevchenko@bsu.edu.ru
http://orcid.org/0000-0001-5378-9082

Marina Yu. Skorkina
Belgorod State University, Rusia
http://orcid.org/0000-0002-4052-6627

Nina I. Zhernakova
1Belgorod State University, Rusia
http://orcid.org/0000-0001-6196-5576

Elena A. Shentseva
Belgorod State University, Rusia
http://orcid.org/0000-0002-0150-1099

Redalyc: https://www.redalyc.org/articulo.oa?id=170262877006

Abstract:

: The objective was to develop a biosensor chip based on lymphocytes for studying the adhesion properties of the cells; to measure the adhesion forces between blood cells in normal and pathological conditions. In research standard cantilever CSG 11/tipless series (USA) was treated with 2% gelatin solution. After that, the lymphocyte was attached to it from the prepared leukocyte suspension placed on the glass. The preparation of the sensor was done in the power spectroscopy mode during the scan by single pressing on the cell. The patients' peripheral blood with acute lymphoblastic and acute myeloblastic leukemia was used for measuring adhesion forces among cells at the treatment and recurrence stage. The obtained data shows that the objective criteria of the beginning state of the recurrence of the lymphoproliferative processes is an increase in the forces of the intercellular interactions in the lymphocyte-granulocyte system, while the recurrence of myeloid lineage proliferation of haemopoiesis causes a decrease in adhesion forces in the same system. This result gives the possibility to predict and identify early micro-rheological changes at the beginning of decompensation of the remission state at the cellular level during the development of malignant proliferative processes in the blood system.

Keywords: biosensor chip, blood cells, adhesion force, lymphoblastic leukemia, acute myeloblastic leukemia.

Resumen:

El objetivo era desarrollar un chip biosensor basado en linfocitos para estudiar las propiedades de adhesión de las células; Medir las fuerzas de adhesión entre las células sanguíneas en condiciones normales y patológicas. En la investigación, la serie CSG 11 / tipless en voladizo estándar (EE. UU.) Se trató con una solución de gelatina al 2%. Después de eso, se unió el linfocito de la suspensión de leucocitos preparada colocada en el vidrio. La preparación del sensor se realizó en el modo de espectroscopia de potencia durante la exploración presionando una vez en la celda. La sangre periférica de los pacientes con leucemia mieloblástica aguda y linfoblástica aguda se usó para medir las fuerzas de adhesión entre las células en la etapa de tratamiento y recidiva. Los datos obtenidos muestran que los criterios objetivos del estado de inicio de la recurrencia de los procesos linfoproliferativos es un aumento de las fuerzas de las interacciones intercelulares en el sistema linfocito-granulocito, mientras que la recurrencia de la proliferación del linaje mieloide de la hemopoyesis causa una disminución en la adherencia. Fuerzas en el mismo sistema. Este resultado ofrece la posibilidad de

Author notes

shevchenko@bsu.edu.ru

predecir e identificar cambios micro-reológicos tempranos al comienzo de la descompensación del estado de remisión a nivel celular durante el desarrollo de procesos proliferativos malignos en el sistema sanguíneo.

Palabras clave: chip biosensor, células sanguíneas, fuerza de adhesión, leucemia linfoblástica, leucemia mieloblástica aguda.

Introduction

The promising direction in the field of cellular technologies is the creation of fundamentally new approaches in the study of adhesive properties of cells based on biosensor chips which can be used as probes for an atomic force microscope. In particular, atomic force spectroscopy (AFS) makes it possible to quantify the local modulus of elasticity, as well as the adhesion force, by visualizing the shear elastic characteristics of biological tissues.

The sensitivity of the AFS method depends on the characteristics of the cantilever. It was recommended to use probes with a "soft beam" for the stiffness constant of which is in the range of 10^{-2}-10^{-1} N/m and the rounding radius is 10 nm for the work with inert objects in the AFS mode [1]. However, these methods have the main problem, the small radius of the AFM probe (10 nm), which easily breaks the integrity of the biological membrane is damaging it with its tip.

The AFS mode allows measuring not only the elasticity but also the intermolecular adhesion forces, arising between the modified metal probe and the cell2. However, this method has a number of limitations. One of the insurmountable characteristics is the interaction force between the cell and the probe, which increases with the extension of the scan time due to the formation of bonds. The consoles used to measure for the cells' adhesion is very rigid in comparison with cells and tissues. Besides that, for "soft samples" (for example, cells with reduced mechanical rigidity), it is difficult to separate the relative contribution of surface forces to the adhesion and deformation of the sample. By now, various AFM techniques have been developed with using sensors, such as inert quartz balls immobilized on the tipless, attached to the middle of the beam, and single cells, cellular monolayers [3,18].

In view of this, the research objective was to develop a technology for creating a biosensor chip based on native cells (erythrocytes and lymphocytes) and to measure the adhesion forces between blood cells in normal and pathological conditions.

Materials and Methods:

Design of a biomechanical sensor

A number of conditions were chosen for technology development of creating a biomechanical sensor

1) The sensor must have the properties of a native cell;
2) To measure the adhesion forces between the single cells;
3) Reduce to a minimum of possible artifacts during measurements.

In order to fulfill the condition of compliance of the tipless type being developed to the properties of the native cells, a standard cantilever of the CSG 11/tipless series (USA) was used to prepare the biosensor which was treated with a 2% gelatin solution and the lymphocyte from the prepared leukocyte suspension placed on the glass was attached (fig.1). The preparation of the sensor was done in the power spectroscopy mode during a single scan by single pressing on the cell.

FIG. 1.
Preparation of biosensor chip: the procedure of landing of tipless to the cell

Fig. 1. Preparation of biosensor chip: the procedure of landing of tipless to the cell

Before the procedure, a suspension of leukocytes was prepared by centrifugation of the whole heparinized blood at 1500 rpm for 5 minutes (without washing in phosphate buffer). Then the viability of leukocytes in vitro tests was checked by staining the cell suspension with 0.4% trypan blue in phosphate -salt buffer (pH 7.2-7.3) and the subsequent counting of the dead cells on a light microscope. The dead cells are dyed blue and the living cells are not stained. In the experiment, leukosuspensions was selected with the viability of at least 95% for the sensor preparation.

2% gelatin solution was prepared, which was applied to the middle of a non-fat glass slide. A droplet of leukocytes was put on one edge of the glass at one side of the applied solution and a drop of erythrocyte was placed on the other edge at another side. The specimen was put into a dampening chamber saturated with water vapor. A force microscopy procedure was carried out at the place of the specimen where a gelatin drop is located by landing carrying the standard cantilever of CSG 11/tipless series into the drop. Then it was changed by moving of the scanning area to the opposite edge of the specimen, where the leukosuspension was located. A site with separated lymphocytes was selected and the cantilever was brought to a single lymphocyte under the control of the optical system of the microscope.

The advantages of the proposed method

1. Obtaining objective data on adhesion forces among cells due to the use of a living cell – lymphocyte as a scanning part of the biosensor.

2. Simulation of conditions as close as possible to ex vivo conditions when the interaction takes place between two native cells that carry various families of adhesion receptors on their surface that interact with biological molecules in the body.

3. Working with the viable cells which are supported by using the dampening chamber which creates optimal conditions for preserving the native shape of the cells.

4. The combination of the preparation method of the biosensor and the procedure for measuring adhesion forces in a single scanning procedure allows reducing the research time and increases the speed of object manipulation due to the rapid change of the scan areas.

Measuring adhesion forces

The patients' peripheral blood with acute lymphoblastic (ALL) and acute myeloblastic (AML) types of leukemia at the stage of treatment and recurrence of the disease was used in the experiment. In the group of patients with ALL 15 samples were taken at the stage of treatment and 3 blood samples were used at the stage of recurrence. In the group of patients with AML 17 samples were taken at the treatment stage and 2

samples were used at the recurrence stage. The blood of healthy people was used as a control (n=12). Blood was obtained by venous puncture leukosuspension was isolated by centrifugation. The samples were used with the cells viability 95% or more in the experiment.

The adhesion force between the lymphocyte and leukocyte, lymphocyte and red blood cell, lymphocyte, and lymphoblast, lymphocyte and myeloblast were measured after biosensor chip preparation based on the native lymphocyte. Measurements were carried out the force curves by taking from their surface in the force spectroscopy mode.

Measurement of force curves was performed from the surface of at least 15 cells from each sample. The force curves obtained in the experiment were processed using the Nova software. The adhesion force of the biosensor to the sample was found on the basis of Hooke's law. The results of the experimental studies were processed using the methods of statistics. The reliability of the differences between the samples was determined using the U test at $p < 0.01$ for nonparametric data. The paper is presented the average values (M) and the statistical error of the mean (m).

Results

The adhesion force between lymphocyte and granulocyte was increased by 21.5% ($p < 0.01$) but no significant differences between lymphocyte and erythrocyte were found in the group of patients with ALL during treatment and using standard chemotherapy regimens compared to cells of healthy people (table).

Table. Adhesive forces in the "cell-cell system"

TABLE
Adhesive forces in the "cell-cell system"

Groups	Adhesive forces, nN		
	"lymphocyte-granulocyte"	"lymphocyte-erythrocyte"	"lymphocyte -blast"
Control	75.5 ± 0.7	46.5 ± 0.6	-
ALL treatment	91.8 ± 0.3*	45.0 ± 1.1	74.0 ± 0.4
ALL recurrence	164.1 ± 0.4*	91.8 ± 0.4*	-
AML treatment	52.4 ± 0.2*	29.9 ± 0.2	60.7 ± 0.5
AML recurrence	40.7 ± 0.2*	21.9 ± 0.3*	-

Note: ALL – acute lymphoblastic leukemia, AML – acute myelogenous leukemia, n= the number of measured cells

*Statistically significant differences among data of the cells of sick and healthy people by U test at $p < 0.01$

At the same time in the group of patients with ALL at the stage of treatment in the peripheral blood blasts were identified. The adhesion force between blast and lymphocytes was reduced by 1.2 times compared to that between the lymphocyte and granulocyte and increased by 1.6 times as compared with lymphocyte and red blood cell.

At the stage of recurrence of the disease the adhesion force between the lymphocyte and granulocyte a significant was increased by 117.5% ($p < 0.01$) and between the red blood cell and lymphocyte was observed by 97% ($p < 0.01$) as compared with the blood of healthy people at the stage of recurrence of the disease.

In the group of patients with AML at the treatment stage, the adhesion forces between the lymphocyte and granulocyte were decreased by 44% ($p < 0.01$) as compared with the control. Significant changes in the adhesion force between the lymphocyte and the red blood cell were not found, although there was a tendency

to weaken their intercellular interaction. However, the adhesion force between the lymphocyte and the blast was increased by 1.2 times as compared with the extracellular interactions in the lymphocyte-granulocyte system and by 2 times in comparison with the lymphocyte-erythrocyte system.

Discussion

According to the obtained data, it is clear that the objective criteria of the beginning state of the recurrence of the lymphoproliferative processes are an increase in the forces of intercellular interactions in the lymphocyte-granulocyte system. Simultaneously, the adhesion forces in the same system decrease in the stage of recurrence of the proliferation of myeloid lineage of haemopoiesis.

According to the literature data, increases in the adhesive properties of tumor cells are due to a large number of terminal sialic acid residues on carbohydrate chains of O- and N-types on their surface. [4]. It is obvious that the developing state of recurrence in the group of patients with ALL indicates a change in their receptor apparatus and an increase in the intercellular interactions between them and other blood cells when there are still morphologically normal lymphocytes in the peripheral blood.

The development of an acute myeloblastic type of proliferation is accompanied by the accumulation of defective myeloblasts in the bone marrow followed by their release into the peripheral bloodstream that leads to a violation of humoral and cellular immunity links [5]. Especially, it was found that patients with the AML had the low phagocytic ability of neutrophils, the intensity of adaptive immunity, and impaired neutrophil receptor apparatus at the level of the bone marrow. These features are closely related to the blast cell phenotype (CD16, CD64) and associated with reduced complementary activity [6,16,17,20].

It is possible that the main cause of the observed decrease in the intercellular interaction in the lymphocyte-granulocyte system is a violation of the receptor apparatus of granulocyte hemopoietic cells both during treatment and at the recurrence stage when there are no myeloblasts in the blood. At the same time, an increase in the adhesion force between the lymphocyte and the blast during the treatment stage indicates co-stimulatory signals which are necessary for immune cells for intercellular signaling and for triggering cytokine cascades of regulation. In particular, the literature presents data according to which the use of various treatment protocols is accompanied by the development of toxic reactions in the human body [7,14,15,19]. A number of researchers have noted an increase in the concentration of such cytokines as IL-1, IL-6, IL-8 and TNF-α of blast cells of the myeloid line in patients with AML [8-10]. Researchers suppose that IL-6 induces excessive proliferation of myeloma cells if receptors are located on the cell surface to this cytokine [11-13].

Conclusions

Thus, using a constructed biosensor chip the objective data included the cell adhesion in normal conditions and during the development of malignant lympho- and myeloproliferative processes in the blood system was obtained. The proposed approach will make it possible to predict and to identify early microrheological changes at the beginning of decompensation of the remission state at the cellular level during the development of malignant proliferative processes in the blood system. The obtained experimental data reflects the changes in the adhesive properties of the lymphocyte population at the stage of disease recurrence and can be used as one of the diagnostic criteria for the early detection of abnormal subpopulations of leukemic cells after treatment.

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