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Sub-Microfluidic Setup to Quantify Cell Surface Charge Density

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***Abstract*—The purpose of this study is to create a prototype of an experimental setup for studying the electrical properties of biological cell surface by measuring electrophoretic mobility of cells and estimating zeta potential. We improved the microscopic method of observing cell electrophoresis in sub-microfluidic chamber with real-time video capture. The basic approaches to the analysis of received video images were figured out in order to correctly determine the linear and electrophoretic velocity of cells for further calculation of zeta potential. Automated processing of obtained experimental data allows faster statistical analysis of distribution of parameters within a cell population. Test measurements on erythrocytes and guidelines for the operation of the device have been developed.**

Keywords*—*cell membrane, zeta potential, erythrocytes, microfluidics

I. INTRODUCTION

The determination of morphological and biophysical characteristics of cells is necessary for the unbiased assessment of the functioning of various organs and systems of the body for diagnostic purposes, for the design of new pharmaceuticals, and for fundamental research as well [1]. Nowadays, a number of non-invasive physical methods, including nuclear magnetic resonance, Raman and dielectric spectroscopy, and other techniques are used to study the basic parameters of living cells, both under normal physiological and pathological conditions [2, 3, 4]. Majority of these methods require high costs equipment and sophisticated sample preparation procedure. At the same time in this century less expensive and less workflow consuming microfluidic, lab-on-a-chip and point of care diagnostic devices may provide fast and reliable feedbacks on many pathological conditions or be used for routine monitoring or early diagnostic purposes and so such lower cost easy to use gadgets may improve healthcare accessibility and quality of life and will contribute to the development of telemedicine especially in rural regions and poor countries.

One of the key biophysical parameters of the cell is its surface charge, which depends on the lipid and protein composition of the plasma membrane and the physiological state of the cell. In practice, the surface charge of cells is characterized by their electro-kinetic potential (ζ-potential) [5].

In electrolyte aqueous solutions there is a spatial redistribution of oppositely charged ions on the boundary between the cell and the liquid called the double electric layer. An adsorption layer (Stern layer) is represented by ions, which have high energy of adhesion to the surface; these ions are tightly bounded to the cell surface. Another part represented by the relatively mobile ions that exchange with the ions in the bulk solution forms a diffusive layer. Under the influence of external electric field directed along the boundary there will be a relative displacement of oppositely charged planes of double electric layer, which corresponds to a relative motion of two phases. The potential in the region of the diffuse atmosphere, which moves along adsorption layer with a tangential motion, is called the electrokinetic potential (ζ-potential). For the direct determination of the magnitude of the ζ-potential, it is necessary to know the surface charge density at the boundary of the phase separation and the thickness of the double electric layer. For this reason ζ-potential of big particles is determined indirectly, e.g., by measuring the speed of the movement of cells in the external electric field.

Determination of electrokinetic potential of cells has a great prognostic value in various branches of biomedical sciences. One of the most advanced areas of pharmacology is the "targeted" delivery of drugs to target cells using nano-carriers of various chemical compositions and structures [6]. Estimation of the ζ-potential of cells and nanoparticles allows selecting the optimum nano-carrier among existing or perspective ones for delivering a pharmacological drug to target cells, taking into account their possible electrostatic interactions.

Studies of recent years convincingly suggest that determining the ζ-potential of cells, in particular erythrocytes, can play a significant role in the diagnosis of various pathological conditions in humans. It has been shown that the decrease in the ζ-potential of erythrocytes is closely correlated with hypertension of a various origins, including diabetic macro vascular complications [7]. Acute myocardial infarction is also accompanied by significant changes in the electrokinetic properties of erythrocytes [8]. It was shown that reduction of ζ-potential of erythrocytes may be one of the early diagnostic criteria for pre-eclampsia development in pregnant women [9].

To address the need in measuring zeta potential of red blood cells among other characteristics of blood samples taken for diagnostics by average clinical laboratory we have developed a setup based upon standard and easily available equipment and units. We have designed, produced and tested special sub-microfluidic chamber with the microscopic grid to enable cell velocity calculations, and made a control and measuring unit to set up and monitor voltages between chamber platinum electrodes. Chamber is easy mounted on a standard object stage of a light microscope, could be rinsed fast with the usual procedure, requires microliter quantities of red blood cells and could be adjusted to work with whole-

blood samples to further simplify specimen preparation prior to assay.

Process of cell motion is monitored in a real time with a CCD camera attached to a microscope. Software provided by the camera manufacturers is efficient for our purposes by itself. At the same time to fully automate assays we are developing machine learning approach to measure velocities of a group of moving cells to be able to automatically discriminate drift velocity from electrophoretic mobility and to assess distribution of red blood cells in the specimen by their zeta potential. Taking into account 120 days shelf life of erythrocytes in circulation "older" ones may bear information on hidden changes in blood cells physiology or circulatory system and thus could serve for early diagnostics and in return as possible therapeutic targets for lurking onset of cardiovascular or generalized pathologies.

II. Setup Layout

A. Setup

The principle of operation of the device is based on measuring the linear velocity of cells under the influence of an external electric field using a microscope equipped with a digital camera (Fig. 1).

The installation includes:

1. Sub-microfluidic electrophoretic chamber (Fig. 2, Fig. 3, Fig. 4)
2. Power supply voltage-stabilized unit to operate with voltages in the range from 0 V to 30 V
3. Trinocular optical microscope "MicroMed XS-3330"
4. Multimeter UT61E
5. Digital Camera
6. PC or laptop

Fig. 1. General view of the setup

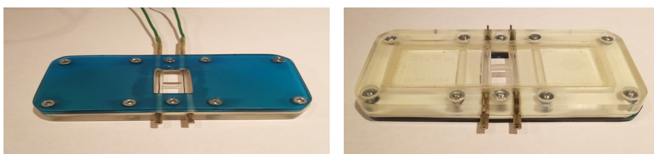

Fig. 2. General view of the microelectrophoretic chamber.

Setup for microelectrophoresis is being assembled, electrodes of chamber attached to the circuit and chamber is placed onto the object stage of microscope. We focus microscope to get the clear image of the coordinate grid of the Goryaev chamber. With the help of a multimeter, the output voltage of the power supply unit was set to 10 V. The 1 ml of erythrocyte suspension was inserted into the microelectrophoretic chamber and the electrical circuit was switched on with simultaneous recording of the video by the the digital camera. ScopeImage 9.0 was used for recording and further analysis of the image. The surface of erythrocytes has a negative charge due to mainly sialic acids of membrane glycoproteins, so native cells always move in the direction of the cathode. For more accurate determination of the mean linear velocity of erythrocytes, electrophoresis was performed in two directions, changing the polarity of the electrodes of the microelectrophoretic chamber (30 seconds in one direction and 30 seconds in reverse). The video was recorded in *.avi format and used for further analysis (Fig. 5).

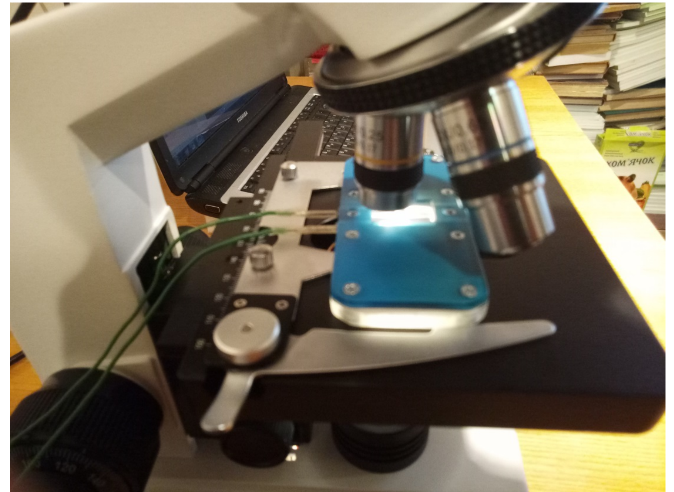

Fig. 3. Microelectrophoretic chamber on the microscope's object stage.

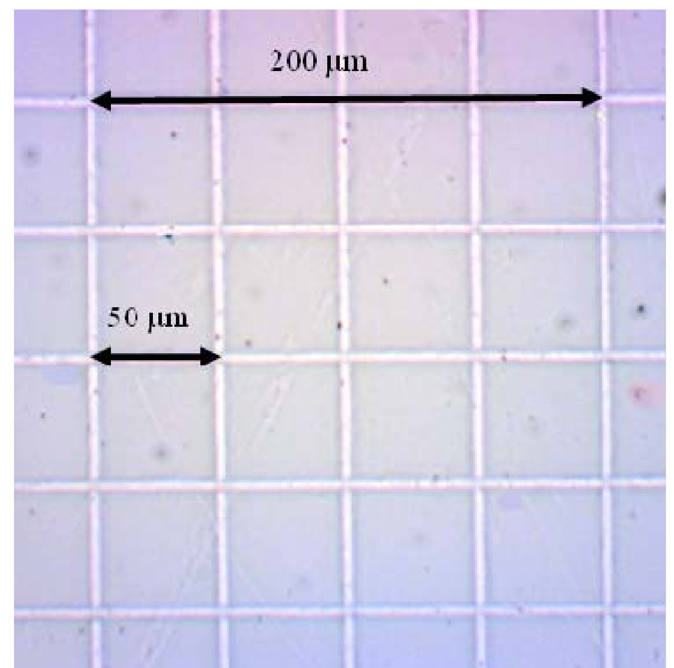

Fig. 4. Coordinate grid of microelectrophoretic chamber (×400 magnification).

B. Technical Characteristics of Microelectrophoretic Chamber

The microelectrophoretic chamber was made on the basis of the cell count Goryaev chamber with two coordinate grids. As electrodes, a platinum wire with a diameter of 0.4 mm and a length of 25 mm was used. The chamber housing is

made of two plates of polymethyl acrylate and silicone gaskets, which are fixed with screw connections (Fig. 2).

Technical parameters of the microelectrophoretic chamber:

Size (L×W×H)	1000×45×10 mm
Volume of electrophoretic cell	1 cm^3
Length of electrodes	25 mm
Diameter of electrodes	0.4 mm
Distance between electrodes	9.5 mm
Coordinate grid:	
big square	200×200 μm
small square	50×50 μm
Weight (without electrodes)	73 g

III. Methodology Of The Determination of Electrokinetic Potential of Cells

To determine the linear velocity of erythrocytes in an electric field with a stopwatch, the time was measured for which a single erythrocyte crosses the large square of the coordinate grid (200 μm).

Linear speed was calculated by the formula (1):

$$v = \frac{S}{t}, \quad (1)$$

here v – is the speed (m/s),

S - distance that the erythrocyte passed (m),

t - time required for the erythrocyte to cover distance S (s).

Calculation of electrophoretic velocity was carried out taking into account the voltage and distance between the electrodes by the formula (2):

$$u = \frac{v \cdot l}{V}, \quad (2)$$

where

u - electrophoretic velocity ($m^2 V^{-1}s^{-1}$),

v - linear velocity (m/s),

l - distance between electrodes (m),

V – voltage (V).

To determine the electrokinetic potential (ζ-potential) of cells the Smoluchowski equation was used:

$$\xi = \frac{4\pi\eta u}{\varepsilon}, \quad (3)$$

here

ζ - electrokinetic potential (V),

η - viscosity of the solution (Pa · s),

u - electrophoretic velocity ($m^2 V^{-1}s^{-1}$),

ε - dielectric permittivity of the solution

To calculate the ζ-potential, the values of η and ε for distilled water were used. In the future, to increase the accuracy of the data obtained, an experimental determination of the viscosity and dielectric permittivity of the buffer solutions could be used.

IV. Studying Zeta-Potential of Rat Red Blood Cells

In this work, 9-month male Wistar rats, obtained from the vivarium of the V.Ya. Danilevsky Institute for Endocrine Pathology of the National Academy of Science of Ukraine were used. Blood, in a volume of 1 ml, was taken from a rat's tail vein in Vacutest Vacuum tube, which contained sodium citrate as an anticoagulant. Red blood cells were obtained by centrifugation for 15 minutes at 200 g and 20°C. The erythrocyte pellet was resuspended in 1 ml of phosphate-buffered saline (1.7 mM KH_2PO_4, 5.2 mM Na_2HPO_4, 150 mM NaCl, pH 7.5) and centrifuged for 5 minutes at 2000g and 20°C. The procedure for erythrocyte washing was repeated three times. The pellet of washed erythrocytes was resuspended in 1 ml of phosphate-buffered saline and stored until the beginning of the study at 4°C for no more than 3 hours. Immediately prior to the study, an aliquot of the erythrocyte suspension was diluted with a buffered solution of sucrose (10 mM HEPES, 240 mM sucrose, pH 7.0) to a final concentration of cells corresponding to 0.1% hematocrit.

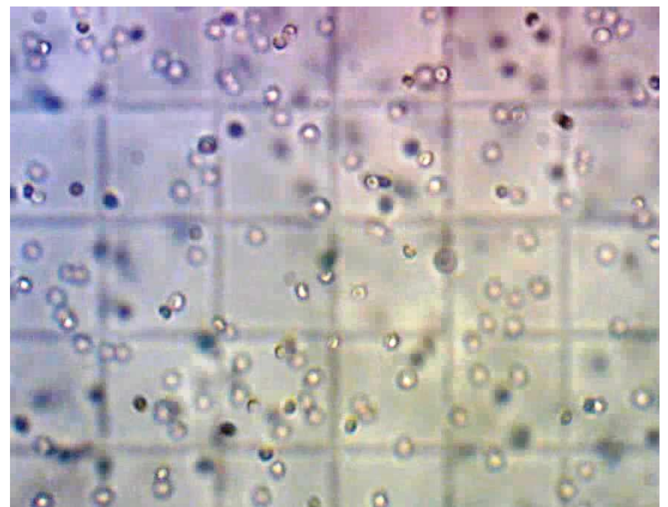

Fig. 5. Erythrocytes on the background of a coordinate grid (×400 magnification)

As a result of the research, it was established that the electrophoretic velocity of red blood cells in rats is in the range of $15.09\times10^{-9} \pm 0.72\times10^{-9}$ $m^2/(V\cdot s)$ (M ± SD, n = 10), and ζ-potential –21.05±1.01 mV (M ± SD, n = 10). The obtained data are in good agreement with the results of other authors who determined these indices in erythrocytes of different types of animals and humans [10, 11, 12].

To further test the reliability of proposed approach to measure cell surface charge under different conditions we have performed series of measurements of zeta potential over a wide range of biologically appropriate values of pH. By determining the electrophoretic velocity in a wide range of

pH values, the ionic spectrum of cells can be obtained, which makes it possible to judge whether the charge on the cell surface is due to ionization of dissociating groups or adsorption of certain ions.

Isoelectric point is a characteristic indicator of the biophysical properties of the cell surface. Depending on the concentration of the potential-determining ions and the specifically adsorbed counter-ions, the value of the electro-kinetic potential may vary from positive to negative, being equal to zero at the isoelectric point. In the absence of specific adsorption of counter-ions, the isoelectric point coincides with the potential of zero charge of the surface.

The magnitude of ζ-potential at given pH and ionic strength of the solution is relatively constant for this type of cell. However, this value may change under the action of various damaging factors on the cell, surface-active substances, antibiotics, etc. Therefore, determining the magnitude of the ζ-potential and isoelectric point can be used to assess the functional state of cells under normal and pathological conditions.

Fig. 6 shows the dependence of the ζ-potential of native rat erythrocytes on pH (electrophoresis buffer: 10 mM K-phosphate buffer, 240 mM sucrose, pH 5.5-8). Best fit approximation allowed us to determine the isoelectric point for erythrocytes that appeared to be located at about pH 4.3. In case of red blood cells, the obtained acidic value of the isoelectric point is largely determined by the dissociation constant of the carboxyl group of the sialic acids of the RBC plasma membrane glycoproteins.

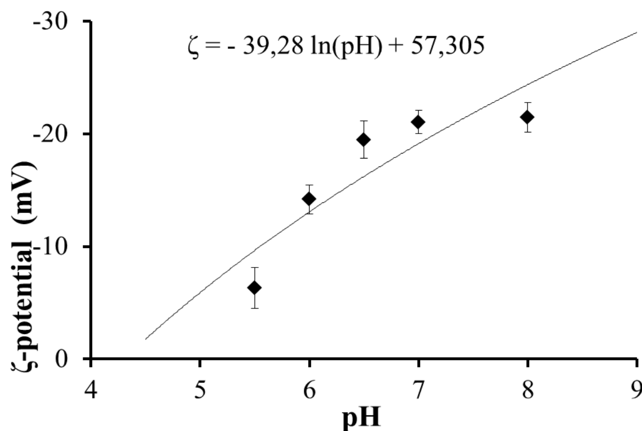

Fig. 6. Zeta-potential of rat erythrocytes as a function of pH. Experimental data are plotted with diamonds as Mean ± SD (n = 5), line is for the best fit approximation with the equation provided in the inset. Calculated isoelectric point of erythrocytes is approximately at pH 4,3.

Further improvement of the device for microelelectrophoresis of cells and methods of its use can become a valuable tool for determining changes in surface charge of cells under various conditions, including pathophysiological processes in acute and chronic diseases, interaction with pharmacological preparations and toxins, and others.

V. CONCLUSIONS

We have improved microscopic method of studying electrophoresis of cells in a microelectrophoretic camera with real-time image video capture. The prototype of a setup for the study of electrical properties of cell surface has been developed and created. The basic approaches to the analysis of received video images are determined in order to correctly determine the linear and electrophoretic velocity of cells for further calculation of zeta potential. Further improvements will include AI automated velocity determination and statistical analysis for a cell population. Conducted measurements of zeta potential of rat erythrocytes are in good agreement with available data. Methodical recommendations for the operation of the device were elaborated.

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