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# Biosensor Scheme for the Determination of Intracellular Pressure of Erythrocyte

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The paper presents a scheme of the biosensor for determining the intracellular pressure of erythrocytes. The possibility of measuring of the volume and area of the erythrocyte is provided in a biosensor to determine the value intracellular pressure. In MEMS this creates flow that enters into Coulter capacitive sensor through the rate control system and then in the system of signal transmitting. The definition of erythrocyte volume and calculation of intracellular pressure occur in the computer system.

**Keywords**: Micro-electromechanical system, Biosensor, Intercellular pressure, Red blood cells.

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## 1. INTRODUCTION

Theoretical analysis of the morphology of erythrocyte [1-4] and numerical calculation of its dependence on intracellular pressure $\Delta$P [5, 6] have allowed to develop a method for determining $\Delta$P based on atomic force microscopy (AFM). State of membrane erythrocyte, as well as the efficiency of the ionic channel of membrane determine its elastic properties [6-9]. The calculations show that the intracellular pressure of erythrocyte is determined by reactive pressure created by the membrane elasticity [5, 9]. Therefore, for the experimental data analysis of AFM scans of erythrocytes we use the following relations [10-12]:

$$\Delta\mathrm{P[kPa]} = 9.1\cdot\left(\frac{V}{V_0}-1\right)+1.054\cdot\left(\frac{V}{V_0}-1\right)^2 \qquad (1)$$

$$\frac{V}{V_0}=\frac{V/\mathrm{S}}{V_0/S_0},\quad \text{provided}\quad S=S_0 \qquad (2)$$

where $\Delta P$ – intracellular pressure, $V$ – volume of erythrocyte as measured, $V_0$ – erythrocyte volume from erythrocyte collection in Norma, $S$ – area erythrocyte as measured, $S_0$ – erythrocyte area from erythrocyte collection in Norma.

Three-dimensional surface of the erythrocyte, obtained during AFM measurements, allows you to determine volume cells [10-12], in which it is possible to calculate $\Delta P$. Numerical calculations have shown when changing the volume under the influence of the reactive pressure of the erythrocyte membrane the surface area practically is unchanged. In case of violations of the membrane ionic channels the value of the reactive pressure $\Delta P_r$ will change, which in turn will affect on the biomechanics of erythrocyte as a whole, as a result the intracellular pressure $\Delta P$ also will change. Thus the value of $\Delta P$ will reflect the membrane state and the efficiency of its ionic pumps.

It is seen that for the application of the formulas (1) and (2) according to the erythrocyte AFM you must first determine the initial value $V_0$ for each erythrocyte on the scan. As a result it should turn out an initial sampling of erythrocytes and the distribution of volumes in Norma. Moreover, this initial sampling will be different for different species and age. In the derivation of equations (1) and (2) it didn't say about biological species separately and other parameters such as age. Accordingly the normalization or the initial sampling is taken as the Norma, it is necessary to perform for the erythrocytes under otherwise identical conditions. Since in biological and medical research they carry out statistical comparison with the control group, it is impossible to determine the initial volume erythrocyte before change its morphology in the experiment. Thus in accordance with numerical calculations the intracellular pressure is determined by the degree of bending in the center membrane, that gives reason to calculation $\Delta P$ values depending on the relative change of volume normalized to the its area according to formula (2).

On the other hand we have known Coulter method for determination of cell volume while passing the microchannel between the capacitor plates [13]. The aim of this work is to develop the scheme of biosensor based on microchannel silicon to measure the volume and the intracellular pressure of erythrocytes.

## 2. THE SCHEME OF THE BIOSENSOR

Fig. 1 shows a flowchart of the biosensor for determination of erythrocyte intracellular pressure, which consists of several elements. The first element is a MEMS for the flow creation, which allows you to create a microflow with erythrocyte. The linear sizes of erythrocytes can vary from 5 microns to 12 microns therefore microfluidics MEMS contains microchannels with the sizes of not less than 20 microns. The MEMS structure should have an pump, for example, based microchannel silicon and sensor for flow control. The control system should be set and measure the flow rate of fluid to properly carry out the measurement of cell volume.

The flow rate transmits to the system of transmission signal (Fig. 1), so we can take into account the rate of passage of cells through the capacitive sensor. The signal from the sensor is transmitted into a computer, in which the magnitude of the capacitance change is recalculated into the cell's volume in accordance with the rate of fluid flow.

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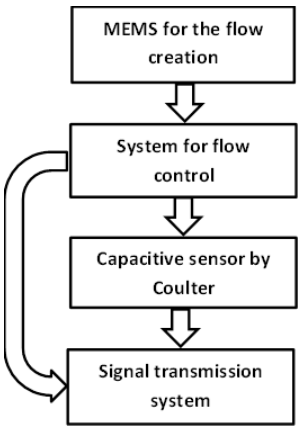


**Fig. 1** – Flowchart of the biosensor for determining the intracellular pressure of erythrocyte

Signal processing is performed in several stages since the for the calculation of according to the formulas (1) and (2) it is necessary to first determine the initial volume $V_0$ and surface area $S_0$. For this the dynamic signal of the timing capacitance change is compared with the flow rate. Indeed the signal duration of capacitive sensor determines the period of passage of cells through the capacitor. If we know the flow rate it's easy to determine the linear size of erythrocyte, which determines the surface area $S$ of the cell base. The magnitude of the capacitance change is defined by cell's volume, however it is required the precise normalization of sensor for calculation. Further from the initial sampling that is stored in the computer memory, the value of $V_0$ is selected according to the value $S = S_0$ and formula (2) is the ratio of the $V/V_0$ and then the formula (1) determines the intracellular pressure of erythrocyte. During the measurement and data accumulation the construction of the distributions of the volumes and the linear sizes of erythrocytes in the sampling are occurred as well as the determination of their intracellular pressure.

Fig. 2 shows a scheme of the biosensor for determining the erythrocyte intracellular pressure. The circuit comprises a fluid flow input containing erythrocytes, a MEMS pump, a flow rate sensor, two capacitances and flow outMEMS pump can be realized by the technology of the two chambers and the piezoelectric membrane.

The implementation of the sensor of liquid flow rate is possible using the results of paper [14]. The sensor for flow control is produced on the basis of the structure with the asymmetry of silicon microchannels, in which fluid flow rate depends on the applied voltage and the orientation of the matrix with respect to the pumped over volume. The flow rate is determined by the built-in electric field that occurs in the interface layer of silicon oxide on the surface of micropores.

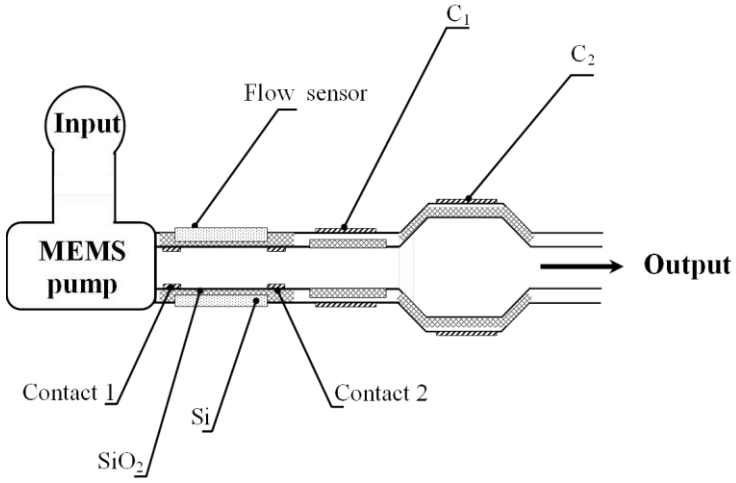


**Fig. 2** – Scheme of biosensor for determining the erythrocyte intracellular pressure, which shows a flow rate sensor and capacitors $C_1$ and $C_2$

In accordance with paper [14] the realization of a flow rate sensor is based on the effect of the emergence of charge at boundary of the silicon oxide and the silicon substrate. During the motion of fluid through microchannels the ions are transferred from contact 1 to contact 2, creating a potential difference $\Delta U$ [V] $\approx 0.3 \cdot \Delta P$ [kPa]. In the scheme a connection between the contacts are not shown, but it is clear that isolation takes place by means of silicon oxide. Using a flow rate sensor allows us to fix flow rate and the passage of cells through the capacitances 1 and 2 by means feedback through the MEMS pump.

Fig. 2 shows that in the biosensor we use two capacitances for cell recognition and determination of their volume. At a fixed time span the cell flow rate through the container 1 will be known, and the start and end of the pulse will depend on the passage of time and linear dimensions of the cells. Similarly, in the container 2 the duration of flight will depend upon the flow rate and size of the cell, but also the shape of the cell. Indeed during entering the cells into the container 2 from the container 1 takes place a flow deceleration. As a result duration of the passage through the container 2 will be more than container 1. In the future it is possible to simulate the process of passing through the cell capacitance system, which allows to determine cells by using their behavior in the microchannel. As a result, the shape of capacitance signals 1 and 2 from time to time will determine several factors: firstly, the linear dimen-

sions of the cells and, consequently, to calculate its volume; secondly, cell is classified to one of types (erythrocytes, leukocytes, monocytes, and coalesced erythrocytes, etc.); thirdly, to retard flow if the difference signal is insufficient or on the contrary, to increase the flow rate.

The learning process of recognition system of cells from the time dependence of capacitance contains several steps. First, the red blood cells by centrifugation for analysis are selected, they are conducting a study on the biosensor, looking for patterns of signals for red blood cells and enter the data in the recognition system. Similarly conduct training on monocytes, lymphocytes and other cells.

## 3. CONCLUSION

The paper proposed a scheme for measuring the intracellular pressure of erythrocyte. The circuit includes several functional elements - MEMS system for creating flow with erythrocytes, the control system of flow rate, Coulter capacitive sensor and signal transmit system to the computer. According to calculations the intracellular pressure depends on the ratio cell's volume to its initial value. The distribution of the initial volumes stored in the computer, so the definition of the erythrocyte volumes distribution at the current moment will provide information for the calculation of the intracellular pressure of erythrocyte.

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