Measurement of cell membrane permittance by the dielectrophoresis method

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Based on the equivalent electric circuit of a cell, the equation for determination of the cell membrane permittance has been justified. The contactless method for measuring the permittance with the use of the inhomogeneous alternating electric field has been developed. The method consists in determination of the equilibrium frequency being a boundary frequency between zones of positive and negative dielectrophoresis in a cell. Human erythrocytes were used as the cells to be examined. The measurements showed that for intact erythrocytes the membrane permittance C_m was constant in all the electrolytes used in the experiment and equal to $(2.4 \pm 0.5) \cdot 10^{-13}$ F, which was in a good agreement with the data obtained by other methods. The measurements of the membrane permittance of human erythrocytes depending on the parvovirus concentration demonstrated that the value of the membrane permittance could be used as a quantitative characteristic of the cell biological activity.

Introduction

It is well-known that the atmospheric surface layer contains a wide diversity of viable microorganisms.[1] Investigation of the composition and activity of these microorganisms by biological methods is very expensive and time-consuming because of a low concentration of the microorganisms in the atmosphere. Therefore, development of a physical method allowing identification of a cell and determination of its biological activity from several easily measured parameters is an urgent problem. In this paper, we propose a method for measuring one of such parameters.

The literature data are indicative of a high sensitivity of cells to impacts of various passive (acids, alkalis) and active (bacteria, viruses) ambient agents. Even insignificant concentration of some of them (viruses, proteins, toxins) can have a fast and specific effect on the biological activity of a cell and, as a consequence, on a wide spectrum of its electrophysical characteristics.[2] One of the important electrophysical characteristics of a cell is its membrane potential taking part in regulation of intracellular processes and reflecting the cell interaction with the ambient medium. The value of the membrane potential is connected with the value of the membrane permittance. Thus, the membrane permittance is one of the parameters that can be used for quantitative estimation of the biological activity of a cell. In connection with the above-said, it is obvious that development of contactless methods for measuring this parameter is quite urgent.

The aim of this paper was to develop a contactless method for measuring the membrane permittance of a cell using inhomogeneous alternating electric field (IAEF).

In IAEFs we can observe a motion of biological particles: cells, bacteria, viruses. The character of the motion is determined by the type of the electric field and the properties of particles and the ambient medium. The translational motion of biological particles in IAEF is called dielectrophoresis (DEP). Dielectrophoresis can be positive and negative. Positive DEP is characterized by the translational motion of cells toward the increasing gradient of the electric field, while the negative one is characterized by the opposite direction of the cell motion.

Dielectrophoresis allows biological particles of one kind to be separated from particles of other kind or intact particles to be separated from defective ones.[3] Dielectrophoretic separation of cancer cells from blood is described in Ref. 4. Moreover, the sensitivity of the DEP method allows studying the first stages of the virus–cell interaction.[5]

It is convenient to represent a cell in IAEF as a complex electrophysical object, whose components can be characterized by electric parameters. Therefore, the DEP phenomenon can be described with an equivalent electrical circuit of a cell[6] (Fig. 1).

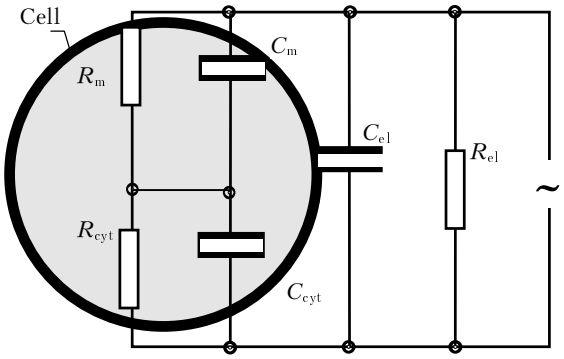

Fig. 1. Equivalent electrical circuit of a cell.

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From analysis of this circuit, we can get the following equations for complex resistance of the cell and the medium:

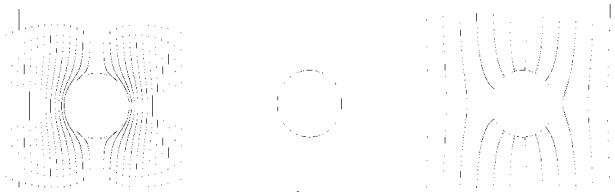
$$Z_c(\omega) = Z_m(\omega) + Z_{cyt}(\omega) = \frac{R_m}{1 + i\omega R_m C_m} + \frac{R_{cyt}}{1 + i\omega R_{cyt} C_{cyt}};$$
$$Z_{el}(\omega) = \frac{R_{el}}{1 + i\omega R_{el} C_{el}}, \quad (1)$$

where Z_c is the complex resistance of the cell; Z_m, R_m, C_m are the complex resistance, active resistance, and permittance of the membrane, Z_{cyt}, R_{cyt}, and C_{cyt} are those of the cytoplasm, Z_{el}, R_{el}, C_{el} are the same parameters of the electrolyte; ω is the cyclic frequency of IAEF.

It is obvious that at some ω_{equ}, a situation can take place that

$$Z_c(\omega_{equ}) = Z_{el}(\omega_{equ}). \quad (2)$$

Such ω_{equ} is called the equilibrium frequency. At this frequency, the currents traversing the cell and the electrolyte are equal (Fig. 2).



a *b* *c*

Fig. 2. Current streamlines through a cell and electrolyte at low (*a*), equilibrium (*b*) and high (*c*) frequencies.

Thus, the cell is equivalent to the ambient electrolyte and dielectrophoresis at this frequency is absent. In other words, the equilibrium frequency is a boundary between the frequency ranges of positive and negative DEP.

The working frequency range ω for DEP is ~ 10^5–10^8 Hz. In this range, for most cells and electrolytes the following inequalities are valid:

$$Z_m >> Z_{cyt};$$
$$\frac{1}{\omega C_m} >> R_m; \quad (3)$$
$$R_{el} >> \frac{1}{\omega C_{el}}.$$

With allowance for these inequalities, Eq. (2) can be written as:

$$\frac{1}{\omega_{equ} C_m} = R_{el}. \quad (4)$$

Since at the equilibrium frequency the current through the cell is equal to the current through the electrolyte (see Fig. 2), it is quite logical to take the electrolyte resistance R_{el} equal to the resistance it would have in the volume occupied by the cell. In other words,

$$R_{el} = \rho_{el} \frac{l_c}{S_c}, \quad (5)$$

where l_c is the cell length along the axis of cell orientation in IAEF; S_c is the area of the cell cross section across this axis; ρ_{el} is the specific resistance of the electrolyte. From Eqs. (4) and (5) we obtain the equation for determination of membrane permittance:

$$C_m = \frac{1}{\omega_{equ} \rho_{el}} \frac{S_c}{l_c}. \quad (6)$$

Experiment

In general, the conditions of DEP for measuring the membrane permittance follow the well-known similar experimental schemes of dielectrophoresis.[3] However, in measurements with electrolytes having low specific resistance, one should keep in mind two principally important points.

First, electrolysis of water is observed at low frequencies, and the lower the specific resistance, the higher the minimal frequency, at which electrolysis is still absent. Therefore, it is worth looking for the equilibrium frequency starting from high frequencies (about 1–5 MHz) toward the lower ones.

Second, as the electric conductivity of the electrolyte increases, electrode heating giving rise to convective vortices increases, while the DEP force decreases. Therefore, it is necessary to regulate the voltage applied to the electrodes in order to achieve the maximum DEP force and avoid appearance of convective vortices.

As the cells under examination we used donor human erythrocytes. The erythrocytes were twice washed from conducting ingredients by low-rate centrifugation in a tenfold volume of 5.5% glucose solution. The specific resistance of the solution was set through adding the needed amount of NaCl and measured with the LCR E7-11 meter. The specific resistance of electrolytes was varied from 1 to 100 Ω·m.

To study the effect of the biological activity of cells on the membrane permittance, we have conducted experiments with erythrocytes infected by a parvovirus (CPV "Laika" L1040H). Cells were infected through the following procedure. In the first hole of a plane-table 100 μl of the cell suspension were mixed with 100 μl of the virus solution. Then 100 μl of the mixture were placed in the second hole and again mixed with 100 μl of the cell suspension, and so on. Thus, the concentration of viruses in each next hole was halved as compared to the previous one. The virus concentration in the first hole was estimated by the physical titer method; it was 1 virus per a cell. A total of nine holes were used in this procedure.

Results and discussion

The measurements conducted have shown that for intact human erythrocytes the product $\omega_{equ}\rho_{el}$

was almost unchanged for all electrolytes used in the experiment and equal to $(2.5 \pm 0.1)\cdot 10^7$ $\Omega\cdot m\cdot s^{-1}$. This fact confirms Eq. (6) for finding the membrane permittance, since the membrane permittance must be independent of the specific resistance of an electrolyte. Thus, the membrane permittance of human erythrocytes was $C_m = (2.4 \pm 0.5)\cdot 10^{-13}$ F, which agrees with the data obtained by other methods.

Variation of the characteristic length l_c and area S_c of erythrocytes contributes to the error in determination of the membrane permittance, therefore the error was about 20%.

We have also conducted the measurements of the membrane permittance and the equilibrium frequency of human erythrocytes depending on the parvovirus concentration in the electrolyte solution with the specific resistance of 10 $\Omega\cdot$m. The results of measurements are summarized below in the Table, where we can see that the low virus concentration (1 virus per 2^8 cells) changes the equilibrium frequency. However, we failed to notice some variation of the membrane permittance because of the large measurement error. However, already at the concentration of one virus per 2^4 cells the erythrocyte membrane permittance halved. Consequently, our assumption that the membrane permittance can be used as a quantitative characteristic of the biological activity of a cell is confirmed.

Thus, we have developed a contactless method for measuring the cell membrane permittance and justified the use of the membrane permittance as a quantitative characteristic of the biological activity of cells. We plan to check the developed method with other cells.

Membrane permittance and equilibrium frequency of human erythrocytes depending on the concentration of virus CPV "Laika" L1040H

Concentration of virus particles	No virus particles	One virus particle		
		per 2^8 cells	per 2^4 cells	per one cell
Equilibrium frequency $\omega_{equ}/2\pi$, MHz	0.39	0.40	0.82	1.55
Membrane permittance, $\times 10^{-13}$ F	2.4	2.4	1.2	0.6

Acknowledgments

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