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MODERN METHODS AND DEVICES FOR ELECTROMANIPULATION IN CELL ENGINEERING

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The article briefly describes modern methods and devices for electromanipulation with living cells for purposeful changes in their biophysical properties during the implementation of cell engineering processes.

Formulation of the problem. Electromanipulation is a complex of methods of influence by harmonic and/or pulsed electric fields (EF, PEF) on living cells with the help of specialized equipment for the purpose of reversible or irreversible electroporation (electrical breakdown) of their membranes (fusion, stimulation, therapy), increasing the permeability of membranes for the transport of nano- particles and macromolecules (permeabilization), ensuring the transfer across the membrane and embedding genes, DNA, RNA, viruses into the cell (transfection), cell convergence before fusion, diagnosis, sorting, rotation (dielectrophoresis), etc. [1]. These methods are used, in particular, in cell engineering, which is one of the most important scientific directions and has not only fundamental, but also great applied importance in animal husbandry, crop production, biomedicine and other fields of knowledge [1].

The purpose of the article. The main purpose of the article is to give a brief description of the two most modern methods and equipment of electromanipulation, which are successfully used to solve problems of cell engineering, especially to study the dynamics of the membrane electroporation process.

Basic research materials. The development of methods of cell engineering in animal husbandry using electromanipulation, for example, to solve the problems of embryo reconstruction (cloning, chimerization, hybridization by dielectrophoresis, electrofusion and electroporation) is mainly determined by the level of development of modern devices used for these works. The accumulated world experience and experimental data on the development of new methods and devices for electromanipulation with living cells have already reached a stage where it is possible to widely implement the automation of cell engineering processes. This is facilitated by the fact that the basic processes of cell activity, even without external influences, have an electrical nature and are determined mainly by electrical forces. The interaction of charges in the membrane and the cell at the level of molecules and ions, their transport across the membrane, the formation of transmembrane potential, ion cytoplasm homeostasis, membrane polarization and electrical oscillations of its layers, energy exchange in mitochondria, etc., - the biophysical quantities describing these processes are either directly electrical or mediated through the movement and interaction of electric fields and charges, always implicitly present even in non-electrical quantities such as viscosity, heat-mass transfer, or biochemical reactions. Therefore, hardware methods of electromanipulation are relatively easy to integrate into automated measurement and control cell complexes, due to the fact that the information signal already has an electrical nature and does not need to be further converted.

Among the new methods of electromanipulation, high-voltage electroporation in the nanosecond range is interesting and promising. Fundamental studies of cell electroporation in this range have been started relatively recently [1, 2]. It turned out that short and high-voltage electrical pulses are able to affect the infrastructure of the cell, in contrast to the parameters of conventional membrane electroporation. It was found that the high PEF intensity (10-300 kV / cm) combined with the short pulse duration (7-300 ns) can affect only the internal organelles of the cell, in particular, the nucleus, leaving the outer membrane unchanged [2]. This effect is useful from the point of view of the possibility of manipulating the state of cell organelles for various applications, for example, point to deactivate individual cells in the tissue by depressing the nucleus or by irreversible electroporation of its membrane, or by introducing cells into apoptosis. It can be also modify genes inside a cell.

One of the promising methods of electromanipulation in the aspect of application in cell engineering is pulse conductometry of a living cell in a voltage-variable PEF [3]. This method is based on the phenomenon of cell membrane electroporation. This method allows not only to implement almost all known methods of electromanipulation on a single hardware and methodological basis, but also to carry out lifetime diagnostics of cells, pulse stimulation of oocytes development outside the body, to determine the state of the cell membrane in various solutions, including cryoprotectors, as well as to perform a number of other techniques for conductometry of liquids of different origin [4, 5].

The block diagram of the device for the implementation of the method of pulse conductometry in a voltage-variable PEF is shown in Fig. 1. The numbers are: 1 - a rectangular pulse generator with variable amplitude, 2 - microelectrodes (a box-less conductometric cell), 3 - decade divider of precision resistors, 4 - digital two - channel storage oscilloscope and 5-stabilized power supply. When measuring conductivity, a series of rectangular voltage pulses increasing in amplitude with a given step is fed from the generator 1 to the microelectrodes 2 covering a living cell and immersed in a drop of liquid medium. The oscilloscope 4 simultaneously measures the amplitude of the pulses at the output of the generator 1 and at the resistor 3. Further, the obtained primary data are processed according to a given algorithm and the result is a graph of the dependence of the conductivity of the cell or the liquid medium between the electrodes on the PEF strength. Then the resulting conductivity dependence can be analyzed, for example, for the presence of extremes or sharp changes in curvature (the rate of conductivity growth) by standard procedure of mathematical analysis of curves [3, 5].

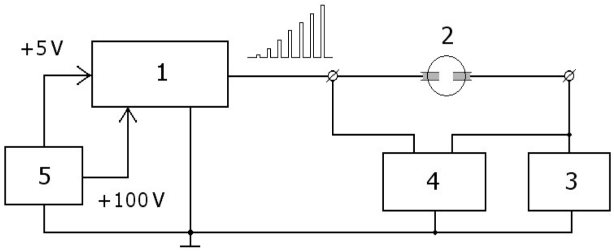

Figure 1 – Block diagram of the pulse conductometer

With the help of the conductivity dependence on the field strength, it is possible to determine all electroporation parameters of the cell membrane of different species of animals. Figure 2 shows as an example the conductivity curve of the mouse oocyte, which was in 0.3 M aqueous sucrose solution. Below for comparison is the dependence of the conductivity of the 0.3 M sucrose solution, which does not dissociate in water and is therefore a good dielectric.

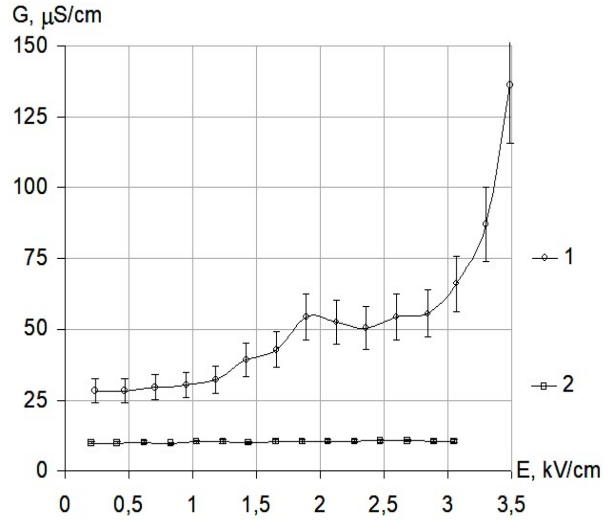

Figure 2 – Dependence of the conductivity of mouse oocyte (2) and 0.3 M sucrose (1) on the field strength.

The graph shows that the first phase of reversible electroporation of the membrane is noticeable at field strength of 0,8-1,8 kV/cm. This can be used, for example, for the transfer of nano-molecules into the cell that does not penetrate the membrane normally (drugs, cryoprotectors, etc.). With an increase in the field strength in the range of 1,9-2,5 kV/cm, a phase of membrane stabilization occurs - a quasi-linear region [5].

This is leads to the fact that the cell conductivity is almost unchanged – the second phase of reversible electroporation of membranes occurs. In this case, the cytoskeleton is most likely destroyed and the cell enters a state of electrical hyperstimulation. When the field strength is more than 2,8 kV/cm, the last phase, but already irreversible elec-troporation with electric breakdown of membranes and cells lyses takes place.

Conclusion. Thus, the above technologies with the use of methods and devices of electromanipulation are among the modern promising areas of cell engineering (especially second), but are not limited to the above list. Probably, the development of methods and applications of cell engineering currently taking place in the world will allow to form other promising directions and applications that are potentially capable of implementation on the basis of electromanipulation methods.

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Аннотация

СОВРЕМЕННЫЕ МЕТОДЫ И ПРИБОРЫ ДЛЯ ЭЛЕКТРОМАНИПУЛЯЦИИ В КЛЕТОЧНОЙ ИНЖЕНЕРИИ

Шигимага В. А.

В статье кратко описаны современные методы и приборы электроманипуляции с живыми клетками для целенаправленного изменения их биофизических свойств в ходе реализации процессов клеточной инженерии.

Анотація

СУЧАСНІ МЕТОДИ І ПРИСТРОЇ ДЛЯ ЕЛЕКТРОМАНІПУЛЯЦІЇ В КЛІТИННІЙ ІНЖЕНЕРІЇ

Шигимага В. О.

У статті коротко описані сучасні методи і пристрої електроманіпуляції з живими клітинами для цілеспрямованої зміни їх біофізичних властивостей в процесі здійснення клітинно-інженерних процесів.