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TREATING SKIN WITH USE OF ELECTROPORATION

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Abstract. Since the opening of the electroporation method, E. Neuman was passed a lot of time. This method allows penetrating inside the cell of high-molecular compounds, by increasing the permeability of the membrane using an electric field. At present, this method is used both as a reversible and as an irreversible process in biotechnology and medicine. Electroporation is used in different variety of areas, for example, in medicine for the treatment of cancer, in biotechnology to create hybrids, in cosmetology for the delivery of drugs to the cells. Wherever this method is used, it is always based on the effect of electromagnetic pulses on the cell membrane to form pores of the required dimensions. Recently, the electroporation method has become widely used in cosmetology, as a method of non-injection mesotherapy. During electroporation in the bilayer lipid membrane, a local restructuring of the structure occurs, leading to the appearance of reversible through water channels. This method promotes faster penetration of necessary substances (drugs, vitamins) inside. This technique increases cell permeability by 400 times. In this case, about 90 - 100% of the active substances penetrate.

Keywords: electroporation, electrical breakdown, treating of skin defects, treating cancer, permeabilization of membrane.

Introduction. An electrical breakdown or electroporation of a biological membrane is a temporary or irreversible phenomenon of destabilization of a cell membrane under the action of a pulse, which leads to an increase in the electrical conductivity of the cell membrane [1,4-7,12-16]. During destabilization, the membrane becomes highly permeable even for large molecules. An electrical breakdown is carried out by the formation of hydrophilic pores in the membrane.

The method of electroporation was invented in 1970 by Eberhard Neumann. In a biomembrane under the action of pulse of different voltages can be created pores. [3]. Depending on the conditions of the

electroporation, it is divided into reverse and irreversible. The main task of this method is to create in the membrane of cells open pores of sufficient size to ensure that the necessary substances enter the cell. These substances, depending on the purpose, can kill the cell, or restore its life. Depending on the duration of the pulse and the magnitude of the electric field, the cells get an appropriate effect [1].

Such a method is used in biotechnology to introduce macromolecules (usually DNA or RNA) into mammalian cells, bacteria or plants.

Creating pores in cells were started to use in different areas. This allowed solving a number of issues, such as: obtaining hybrid animals, interspecific chimaeras, sterilization of nutrients, transdermal transfer of drugs in the human body, treatment of cancer.

Materials and methods.

1. Types of electroporation

There are few types of electroporation: reversible and irreversible (Fig.1). Depends on these two types they are found their own places in different areas, as genetic engineering, cosmetology, medicine and others.

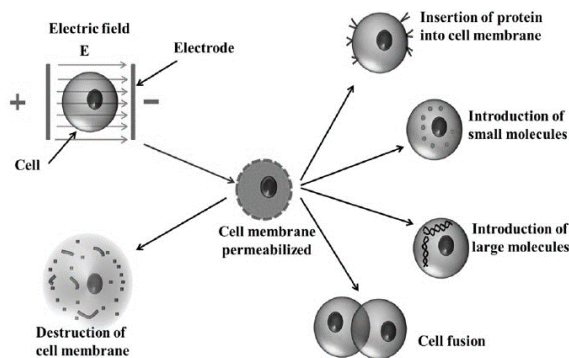


Fig. 1. Types of electroporation [3]

An electrical breakdown or electroporation in genetic engineering, as one of the methods for delivering macromolecules to cells, is a temporary violation of the barrier function that occurs when the membrane potential exceeds a certain threshold value. The cells are placed in a solution with the necessary macromolecules, and then act on them a pulse of high voltage for a short period of time (microseconds). Pores in the cell membrane remain open for about 30 seconds [4]. This time is enough for penetration into the cell of the introduced material. After the termination of the electric pulse, the pores in the membrane disappear.

In cosmetology, first of all, electroporation is used as a method of mesotherapy without injection for the treating of acne, wrinkles, cellulitis, stretch marks. With the help of an apparatus of electroporation, which generates pulses of high frequency (3000 Hz), pores are formed and cell permeability is increased by 400 times. Due to this, necessary preparations such as vitamins, collagen penetrate into cells.

Electroporation has shown its effectiveness in solving a variety of problems, such as: age changes in the skin, treatment of acne, restoration of elasticity and water balance of the skin, treatment of cellulite.

Also, this method can be used in prosthetics: in the preparation of tissues before surgery, or in post-operative treatment. When using electroporation in postoperative treatment will help in the fighting against pain syndrome, as well as improve the limb after amputation, feeding the skin of the amputating limb with the necessary elements.

In clinical pharmacotherapy, the use of erythrocytes as carriers of medicinal substances is considered promising. Electrocorporation is used to load red blood cells with drugs. Studies of erythrocytes loaded with anthracycline drugs showed that the lifetime of such erythrocytes-carriers, after their introduction into the bloodstream is 3-4 weeks, which is tens of times longer than the circulation of the drugs in the bloodstream if they are injected directly into it. In addition, the use of loaded erythrocytes reduces the side effects of drugs.

With electroporation, the medicine is applied locally and then the pathological region undergoes electroporation. Since the electroporation expands the pores and increases the permeability for a short time, the drug substance that penetrated the cell. falls into it as a trap, due to the fact that the permeability of the cell membrane is quickly restored. This method allows to increase the concentration of the drug in cells / tissues which is many times higher than the level of traditional methods of treatment. Thus, the amount of the necessary drug can be reduced, as the concentration of drugs in the pathology area increases.

In the field of cellular engineering, an irreversible electroporation for cellular fusion is used as a means of cloning of animals and plants valuable for the agriculture; obtaining hybrid animals and interspecific chimaeras. This method is simple, universal, non-invasive, less toxic and more secure since the cell genome is not damaged.

The phenomenon of irreversible electroporation is also used for the sterilization of food products, nutrient media for the cultivation of cells and tissues [3].

In recent years, more attention has been paid to irreversible electroporation, in particular its use for the treatment of cancer. As with reversible electroporation, a powerful external electric field is used in the irreversible. In contrast to the application of this technique in cosmetology, when the effect of electric

current is single and short-term, in oncology short impulses act on the same place of cells many times. The power of the pulse and its duration exceed the threshold level of stability of the cell membrane, which leads to the formation of a large number of nanopores, which under the action of the electric field remain permanently open [2]. Such changes lead to cell death due to the inability of the membrane to return to its original state.

2. How it works

The cell is a cytoplasm surrounded by a membrane. The cytoplasm is a conducting medium, the membrane is a dielectric.

The membrane protects the cell from external factors, including effects of electric current. For protection in the membrane is present a two-layer lipid layer 5-7 nm in thickness, which is a dielectric [3].

There are ions in the cell itself and in the intercellular fluid, they serve to control the functioning of the cell. The exchange between ions is provided by means of special channels located in the cell membrane. Thus, there is a certain potential difference, under which the cell membrane exist constantly. The cell membrane has a certain resistance R of the electric current I , which remains constant at a potential difference $\Delta\varphi$ between the two sides of the membrane [1]. Thus, for the membrane Ohm's law is preserved:

$$I = \varphi / R. \quad (1)$$

Electroporation arises if the induced transmembrane potential $\Delta\varphi_m$ is above a critical value of the breakdown potential of the membrane $\Delta\varphi_{cr}$, that is, the condition is satisfied: $\Delta\varphi_m > \Delta\varphi_{cr}$.

The value of $\Delta\varphi_{cr}$ is determined by the properties of the membrane.

The induced transmembrane potential is determined by the value of the intensity of the external electric field by Maxwell's equation (2):

$$\Delta\varphi_m = 1,5 r E_0 \cos\theta, \quad (2)$$

where r is a radius of the cell; θ is a tangle between radius vector and strength vector of electric field E_0 .

The entire transmembrane potential difference is applied to the dielectric membrane

For the implementation of electroporation, it is necessary to apply to the solution of cells a considerable voltage of a pulsed electric field in the order of 1000-1200 V / cm. This is due to the need to create a sufficient voltage difference on the cell membrane. The duration of the pulse varies depending on the purpose of conducting electroporation.

From the experiments, the scientists found out that the law keeps up with a potential difference not exceeding 300 mV [3]. This potentials' difference is a boundary. If to act at the membrane of the cell with a potential difference above 300 mV, it will lead to a sharp increase of the current passing through the membrane. This will lead to the appearance of pores in the cell's membrane. This phenomenon is called electrical breakdown or electroporation. When an external action is stopping the difference, potentials goes to normal and pore sizes are reduced on the membrane (Fig. 2).

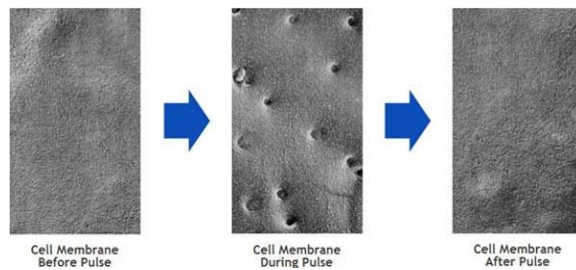


Fig. 2. Electroporation [5]

During destabilization, the membrane becomes highly permeable even for large molecules. In the bilayer lipid membrane, a local restructuring of the structure, leading to the appearance of reversible through water channels.

Cells have their own electrical potential, when Pulse reach the critical value of membrane potential, membrane have pores (Fig.3).

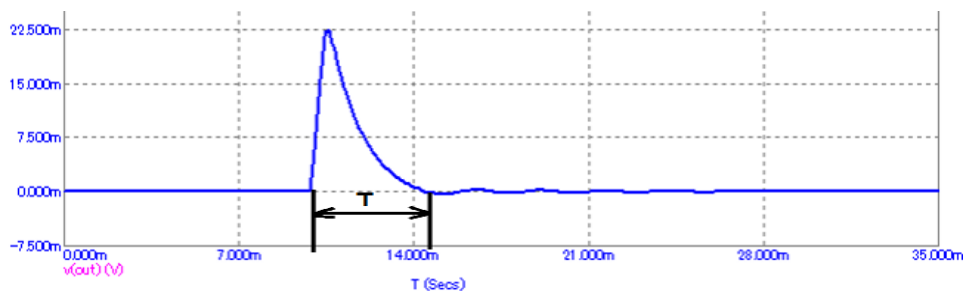


Fig. 3. Temporal electroporation of cell membrane

With the help of electroporation, about 90-100% of the necessary substances fall more rapidly inside of the cells.

But with the increase in the potential difference or the duration of the pulse action, after the energy barrier has been overcome, pore formation becomes spontaneous. This leads to the formation of a multitude of nanopores and, as a result, to the destruction of the cell (Fig.4).

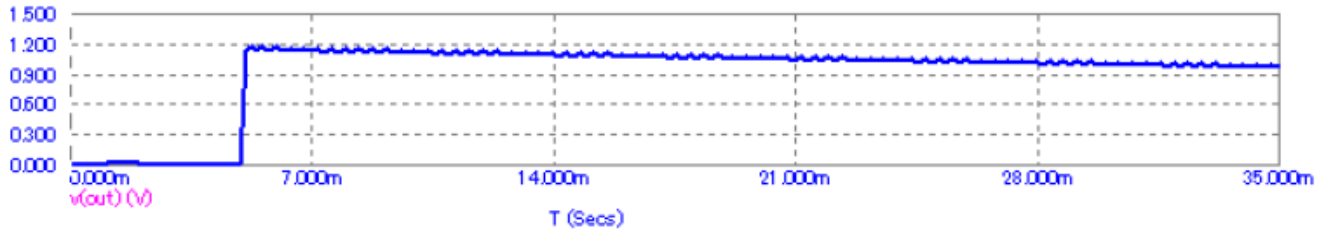


Fig. 4. Breakdown of membrane

The potential at which the formation of pores occurs is called the breakdown potential. This potential differs between species of the cells and depends on many factors. In particular, the breakdown potential depends on the lipid and protein composition of the membrane.

Electroporation of skin is very popular because it can effect at all layers of skin (epidermis, dermis, subcutaneous layer) (Fig.5).

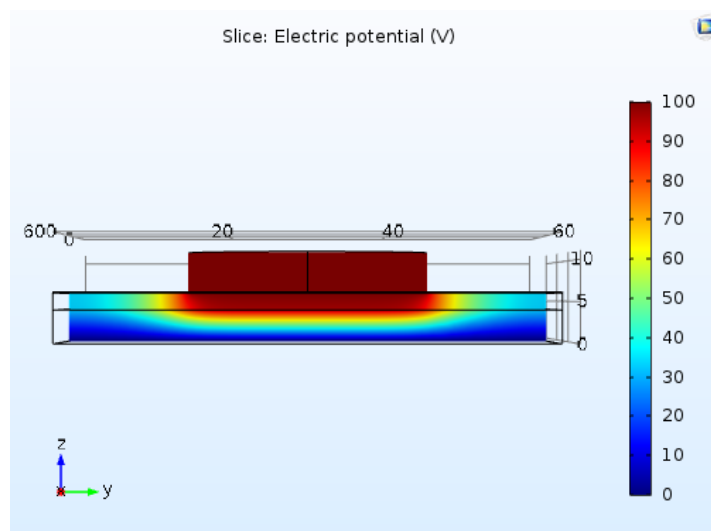


Fig. 5. Skin electroporation

At the Fig.5 we can see claret rectangle that is an electrode. Skin is presented by three layers, sizes of which of them were taken from literature data.

3. Electroporator

The electroporator is designed to generate electrical pulses for the purpose of a temporary opening (up to 30 seconds) in the cell membranes of pores and the introduction into the cell of genetic material, medicinal or other substances. At the same time, membrane rupture does not occur, which reduces the traumatism of cells and increases their viability.

The purpose of the electroporator is to apply a pulse of voltage across the electrodes. The resulting voltage across each cell, creates pores in the cell membranes, allowing external molecules to flow into the cell.

The principle of the action of the electroporator is based on the effect of the creation of pores in the bilayer lipid membrane of cells under the action of an electric field to introduce macromolecules (for example, nucleic acids) into cells of bacteria, mammals or plants. The device is controlled by a microprocessor, the output parameters are displayed on the liquid crystal display.

The principle of operation of known electroporators is based on the accumulation of charge on the capacitor followed by discharge of the capacitor through a biological load (cell, tissue, organ).

By setting the voltage to be applied, an electric field of known voltage-per-mm can be applied. The field is needed only for a brief period of time, so an electroporator applies only a short pulse. At the Fig.3 there is a simple electrical circuit of electroporator.

One of the reasons for the success of the electroporation as a method of choice for gene transfer and studies of membrane behavior in electric fields is the conceptual simplicity of the experimental devices used to induce electroporation. They consist of two electrodes embedded in the cell suspension. The electrodes are connected to a high voltage pulse generator which allows to control the voltage and duration of the electric pulse. The gene DNA or any other water-soluble substance, which should be transferred into cells, is in the medium. The pulse application results in formation of pores in the cell membrane. This leads to exchange of molecules between the medium and the cytoplasm by diffusion, electroosmosis or other mechanisms. The membranes resealed after the pulse and the substance of interest is entrapped inside the cells. [4]

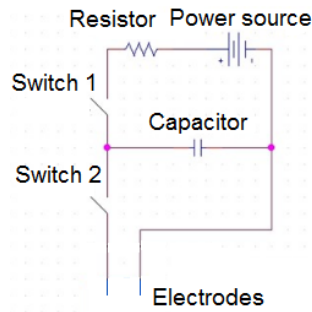


Fig. 6. Simple electrical circuit of electroporator [8]

First of all electroporator needs to have voltage source (power source). Depends from needs it can be different size of source. Resistor as a voltage divider, that will control current. Capacitor that will accumulate and give the pulse. Time constant in the RC circuit (3):

$$\tau = RC. \quad (3)$$

The application of an external electric field leads to currents of ions, which accumulate at the membrane surfaces and give rise to induced surface potentials on both sides of the membrane. This results in creation of a voltage V across the membrane. The basic relationship which is mostly used to estimate this transmembrane voltage induced by a rectangular pulse of field strength E is [4]

$$V = V_m [1 - \exp(-\tau/t_p)], \quad (4)$$

where V_m is the maximal value of the transmembrane voltage (usually it is about 300-500 mV), τ is the duration of the pulse and t_p is the characteristic polarization time [4].

In the most part of cases we assume that all cells are spherical. In this case, for spherical membrane with radius R , voltage V_m and the charging time constant t_p are given by

$$V_m = 1.5ER \cos \delta, \quad (5)$$

$$t_p = RC_m(r_i + 0.5r_o), \quad (6)$$

where δ is the angle between E and the radius vector, C_m is the membrane capacitance, r_i and r_o are resistivities inside and outside the cell, and the membrane conductance was neglected [4].

For different needs, uses different characteristics, the main of them is at the table1.

Table 1 Characteristics of electroporators [6-8]

Electroporator characteristics	for cells	for surgery	for cosmetology
mains voltage, V	220		
Set voltage, V	10- 3000	2000-3000	10-110
Pulse length, msec	0.05- 100	0.07-0.09	0.08-20
Type of pulse	Square/exponential decay		
Frequency, Hz	50	(250- 500) ^{10³}	50

For nowadays there are a lot of different electroporators at global market. All of them are created by some foreign companies, such as: Bio-Rad Laboratories (USA), Eppendorf (USA), BTX (USA), Thermo Scientific (USA), Harvard Apparatus (USA), HT Company (UK). And such apparatus costs correspondingly expensive (table2).

Table 2. Costs of electroporators [7-8]

For cells			
Electroporator	MicroPulser Electroporator from Bio-Rad, USA	BTX™ E CM™ 830 Series Electropo rator System, USA	Eppendorf Multiporator , USA
Price, €	8200	10000	8900
For beauty			
Electroporator	Young- in 49E, China	Max-E48 Electropo rator - MAX- E48, USA	PULSE for Electropor ation, USA
Price, €	260	500	2300

Unfortunately, electroporators that were created for treating cancer do not have wide information about themselves. Mostly they still using in experiments, to find the best parameters for treating. That is why universities or other organization keeps it in secret and it does not produced widely on global market.

There is needs to create own electroporator that will be cheaper and will have wide area of applying such as treating akne and scars after hard akne form, rejuvenation, fighting with pain syndrome, help at problems with joints and others. This will help many people with different problems.

Conclusions. Without a doubt, the methods of electroporation and electroporation therapy require further development, research and improvement.

The method of electroporation is widely used both in genetic engineering and in medicine. Also, electroporation is the safest new method of treating in any case, that makes it interesting for most part of scientists. In each area there are its own devices are created - electroporators that meet the necessary requirements. If to create unique apparatus for electroporation it will be one of the devises that will be using widely.

There are a lot of different electroporators at the global market. All of them are from foreign countries. The needs are to create own Ukrainian electroporator that will be cheaper, and do the same work as other electroporators or even better. No one used electroporator for treating amputating limbs yet. In such case future apparatus will created for treating skin of different areas. The main goal is to create unique apparatus for treating different problems, and that will be lightweight, portable, easy for home use.

Conflict of interest statement: The authors state that there are no conflicts of interest regarding the publication of this article.

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Unique	phenomenon of destabilization of a cell membrane under the action of a pulse, which leads	-

DOI:LCC - № RL1-803 Treating Skin with Use of ElectroporationOleksandra Skrynska1, Julia Antonova-Rafi 1, Igor Khudetsky 11 Igor Sikorsky Kyiv Polytechnic InstituteAddress for Correspondence: Student, National Technical University of Ukraine "Igor Sikorsky Kyiv Polytechnic Institute"E-mail: sasha-aevum@ukr.netAbstract. Since the opening of the electroporation method, E. Neuman was passed a lot of time. This method allows penetrating inside the cell of high-molecular compounds, by increasing the permeability of the