Part 2. Biology and biotechnology

UDC 51-73:577.352.4:537.311

BIOPHYSICAL MODEL OF BIOLOGICAL CELL CONDUCTIVITY BASED ON THE MEMBRANE ELECTROPORATION PROBABILITY

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Summary. The membrane electroporation of a biological cell was well known as a convenient, multipurpose and universal way of temporarily increasing its permeability in a pulsed electric field (PEF) with certain parameters. The process and result of the membrane interaction with the PEF is greatly influenced by its heterogeneous biological structure, which has both native pores of various sizes and various protein inclusions. This leads to heterogeneity of the electrophysical properties. All this ultimately affects the cellular conduction in the PEF, which is both an indicator and an integral characteristic of the electroporation process of the membrane as a whole. This process can be modelled, considering the physical properties of the membrane and the cells, as conductors of the pulsed current. However, to consider in modelling all the features of the native structure of the membrane pores, as well as newly formed electropores as a result of interaction with the external PEF is impossible. However, if we apply a probabilistic approach to the formation of electropores, it becomes possible to construct an adequate model of electroporation.

In this article is presented the developed biophysical (BP) model of cell conductivity, constructed on the basis of the electropores formation probability in a membrane under the influence of a pulse electric field (PEF). The model assumes that in membrane are formed electropores of different calibers, the distribution of which submits to normal Gauss's law. The integral for the total conductivity of the electroporated membrane is obtained using the integral equation for the total current through the electropore membrane and the equation for its conductivity, including the formation of the electropore probability function. The general view of the electropore formation probability function is received by solution of Fokker-Planck's differential equation. Substitution of this equation solution to conductivity integral gave the general view of the conductivity function connecting it with electropore caliber. A comparison of the constructed probability electroporation BP model with experimental data on mice oocyte conductivity showed that the main reason for exponential increase of cell conductivity in increasing electrical field strength is similar nature of conductivity increase with increasing electropore caliber up to membrane breakdown. The constructed probability BP model of cell conductivity at membrane electroporation in increasing PEF agrees with the experimental data.

Keywords: pulse electric field, increasing strength, electropore caliber, cell membrane, Gauss's law, conductivity integral

Introduction. The pulsed electric field (PEF) is widely used in the latest biotechnologies for electromanipulation with cells, which underlies modern methods of cellular, genetic engineering and biomedicine (Miklavčič, 2017; Rems and Miklavčič, 2016; Yarmush et al., 2014; Shigimaga, 2014, 2015). The main method of electromanipulation is electroporation — an adequate effect on the transport function of the cell membrane by temporarily increasing its permeability due to the formation of an electropores in the PEF. The PEF is formed by special equipment in a liquid medium with cells (Dermol-Cerne and Miklavčič, 2018; Hoiles, Krishnamurthy and Cornell, 2018; Shigimaga, 2013a,b, 2017). From the point of view on physical impact, the events occurring in the membrane during and after application of the external field initially develop on the basis of strong electrical interaction with the membrane, and then, probably, on the basis of the pressure. In addition, this pressure arises as a result of electrodiffusion under the field forces (Miklavčič, 2017; Chang et al., 1991; Smith and Weaver, 2012). However, despite the generalizing models of this process based on artificial membranes are modeled, the mechanism of the biological membrane electroporation is still not completely clear. It is supposed that the following fact is established: an external PEF, affecting the cell, changes the electrochemical potential on both sides of the membrane, and as a result it disrupts the phospholipid double layer. (Gurtovenko and Lyulina, 2014; Fernández, Risk and Vernier, 2018; Neu and Neu, 2009; Luitel, Schroeter and Powell, 2007; Mahnič-Kalamiza, Miklavčič and Vorobiev, 2014; Kotnik et al., 2012). This leads to the separation and

accumulation of charges on the membrane between the cytoplasm and the external medium. It is expressed in an increase in the transmembrane potential (TMP). Thereby it induces a temporary instability in the polarized lipid bilayer (Fernández, Risk and Vernier, 2018; Fuertes et al., 2011; Towhidi et al., 2008; Kotnik, Pucihar and Miklavčič, 2010; Kotnik et al., 2012; Morshed, Shams and Mussivand, 2013; Polak et al., 2014). Consequently, the unstable state of the membrane starts up a gradual change in its shape to a more energetic advantageous shape by forming perforating channels of different sizes. They are realized by nanoscale pores across the membrane (Böckmann et al., 2008; Kotnik, Pucihar and Miklavčič, 2010; Kotnik et al., 2012; Wang et al., 2010). This phenomenon is defined by the term 'electroporation'. Thus, the main process that characterizes electroporation as a biophysical phenomenon is the formation of electropores in the membrane.

In view of the complexity of the biological systems structure at any level, including the cellular system, an attempt to unambiguously describe them within the bounds of one or even several BP models to explain the effects of membrane electroporation in the PEF is doomed to failure in advance (Shigimaga, 2014). Great number of such models were made (Son et al., 2014; Neu and Neu, 2009; Miklavčič and Towhidi, 2010; Mahnič-Kalamiza, Miklavčič and Vorobiev, 2014; Kotnik et al., 2012; Miklavčič, 2012; Polak et al., 2014; Shigimaga and Megel', 2012; Shigimaga, 2013a,b; Morshed, Shams and Mussivand, 2013; Smith and Weaver, 2012) and all of them in a varying degree have described the effects of PEF on the membrane of a real biological cell. To model the electroporation of membranes in the PEF of simple biological cells, as an example are usually preferred the denuclearized erythrocytes. They are very simple in structure, a huge number of researches is devoted to them, and therefore artificial bilipid membranes (BLM) are predictable by physical properties or abstract spherical cells (Hoiles, Krishnamurthy and Cornell, 2018; Smith and Weaver, 2012; Hoiles et al., 2014; Polak et al., 2014; Pavlin et al., 2008; Miklavčič, 2012, 2017; Morshed, Shams and Mussivand, 2013). Reproductive cells of animals and, especially, multicellular embryos, which are much more complex in structure and composition, are very difficult as bioobjects, for BP modeling of electrical conductivity. Therefore, the modeling of the conductive properties of these bioobjects is practically unknown, except several recent works (Miklavčič, 2017; Shigimaga, 2013a,b, 2014; Shigimaga et al., 2017).

The application of the electroporation method requires the justification of certain electrical treatment regimes in the PEF of living biological cells so that they keep their functional for further use, in particular, in animal reproduction biotechnology. The value parameters of these regimes are determined, first of all, by the electrical characteristics of the membrane, the cell, and the liquid medium. These characteristics can be obtained experimentally during measurements of the conductivity of a biological cell using the method and equipment of pulsed conductometry in a variable PEF (Smolyaninova, Shigimaga, and Kolesnikova, 2009; Smolyaninova et al., 2014; Kolesnikova, Shigimaga and Smolyaninova, 2013; Shigimaga, Levkin and Megel, 2011; Shigimaga and Megel', 2011a,b, 2012; Shigimaga, 2013a,b, 2014, 2017). The variable field strength provides a different degree of the membrane electroporation and therefore makes it possible to justify and calculate all the necessary modes of exposure on the varying of the cell rely conductivity. This allows not only to realize well-known applications but also to develop new applications of electroporation in the biotechnology of animal reproduction and biomedicine within the framework of a single hardware -methodical process of pulsed conductometry (Shigimaga, 2015). On the other hand, the justification of the PEF parameters during the process of pulsed conductometry requires a comprehension of the physical mechanism of the electropores formation (which are available in many calibers) with increasing field strength. Disclosure of this mechanism is a bit of possible with the help of the BP modeling of the membrane electroporation process which was considered below, and using the known physical laws of the electric current flow through it and also various mathematical methods and approaches.

Material and methods. Electroporation BP modeling should be started with consideration of integrated characteristic of electropore formation — the free energy describing mechanical and electric contribution of forces at electroporation (den Otter, 2009; Chang et al., 1991; Böckmann et al., 2008). The electropore is thus approximated by the round cylinder of radius (*r*) and the height (*h*) equal to membrane thickness (Fig. 1).

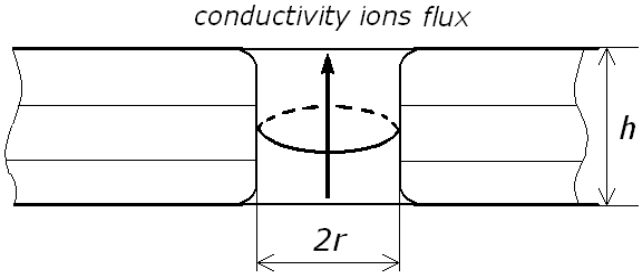

Figure 1. Electropore approximation by the cylinder on a membrane surface

Free electropore energy represents generally by the sum:

$$\Delta E = \Delta E_m + \Delta E_e + E_o \quad (1)$$

where ΔE_m — mechanical component, ΔE_e — electric component, E_o — constant.

The mechanical component of electropore energy is defined by the formula (Böckmann et al. 2008):

$$\Delta E_m = \pi\left(2 E_p r - E_{mw} r^2\right) \quad (2)$$

where E_p — linear energy density of pore edge, E_{mw} — surface energy density of interaction at membrane-water interface.

It is known that mechanical breakdown of flat membrane is reached, when one or several electropores exceeds critical radius $r > r_c$, where $r_c = E_p / E_{mw}$ (Chang et al., 1991; Böckmann et al., 2008). Considering that cell radius exceeds electropore radius by 4–5 orders of magnitude, it is possible to neglect curvature of membrane surface in a pore vicinity and consider it as flat surface.

The electric component of energy (1) represents electropore as the condenser with some conductivity leak (Chang et al., 1991):

$$\Delta E_e = -\frac{\pi(\varepsilon_w - \varepsilon_l)U^2}{h^2}\int_{r_{min}}^{r} g^2 r\, dr \qquad (3)$$

where ε_w and ε_l — dielectric permeability of water and membrane lipids respectively, U — transmembrane potential (TMP), $g(r)$ — the function considering effect of external voltage distribution, connected with resistance distribution on the boundary (with solution) and within a pore. This function can be written as follows (Chang et al., 1991):

$$g(r) = \left[\frac{2hG_s}{2hG_s + \pi r G_p(r)}\right] \qquad (4)$$

where G_p and G_s — conductivity within a pore and solution at pore entrance respectively.

The effect of voltage distribution has following physical explanation. There is a non-uniform electric field in the solution around pore entrance. The related voltage fall is estimated by introduction of resistor with the resistance R_s which value is set by formula (Chang et al., 1991):

$$R_s \approx \frac{1}{2G_s r} \qquad (5)$$

Respectively, the internal resistance of a pore R_p is estimated through its conductivity G_p (Chang et al., 1991):

$$R_p = \frac{h}{\pi r^2 G_p} \qquad (6)$$

These resistances R_s and R_p are included in series, forming a voltage divider, leading to TMP reduction by the value of R_s. Considering these resistances, it is possible to write down a current through an electropore, according to the Ohm's law as:

$$I_p = \frac{U}{R_s(r) + R_p(r)} \qquad (7)$$

For calculation of the total current through a surface of the membrane, which electroporated by PEF force, we will introduce the concept of probability formation density of electropore $n(r,t)$, as a function depending on radius (caliber) of electropore and time. The process of the electropore evolution represents a combination of various physical forces (1), therefore electropores of different radii are present in a membrane (Talele and Gaynor, 2010). Radii distribution can be described by density function $p(r)$. It is supposed that the number of electropores $w(t)$ changes in time due to their appearance and reparation having radius r_{min}. From these assumptions it follows that radii and time distribution are two independent functions. Therefore, it is possible to write down a total density of an electropore formation probability as a function of their product (Shigimaga, 2014):

$$n(r,t) = p(r)w(t) \qquad (8)$$

Having defined electropore formation probability density, it is possible to write down the equation for total current through membrane, using (Chang et al., 1991):

$$I(r,t) = U\int_{r_{min}}^{r}\left[\frac{p(r)w(t)}{R_s(r) + R_p(r)}\right]dr \qquad (9)$$

From equation (9), using ratios (5) and (6), the equation for total conductivity of electropoated membrane becomes (conductivity integral):

$$G(r,t) = \int_{r_{min}}^{r}\left[\frac{p(r)w(t)}{R_s(r) + R_p(r)}\right]dr = \\ = 2\pi\int_{r_{min}}^{r}\left[\frac{G_s G_p r^2}{2hG_s + \pi r G_p}p(r)w(t)\right]dr \qquad (10)$$

Thus, the solution of the equation (10), including finding of function type $n(r,t)$, will results in probability BP model of cell conductivity.

Results. For defining the general view of electropore formation probability density function $n(r,t)$ it is possible to use the differential equation, deduced on the basis of the Fokker-Planck's equation (Kamenshchikov, 2014; Risken, 1996):

$$\frac{\partial n}{\partial t} = D_p\left[\frac{\partial^2 n}{\partial r^2} + \frac{1}{kT}\frac{\partial}{\partial r}\left(n\frac{\partial \Delta E}{\partial r}\right)\right] \qquad (11)$$

where D_p — an effective diffusion constant for a pore of radius *r*.

The value ΔE was taken in the form of (1), and in the equation (11) has crucial importance as $(\partial \Delta E/\partial r)_U$ is the effective force changing of electropore radius.

Further, the volume conductivity of solution is a function of concentration and mobility of the ions (Chang et al., 1991):

$$G_s = \sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i \qquad (12)$$

where z_i — charge, α_i — mobility and C_i — concentration of ions, $e = 1{,}6 \cdot 10^{-19}$ K (electron charge).

It is assumed that the transport of ions through the membrane occurs by passing them through a pore which is large enough to contain hydrated ions, for example Na^+ and Cl^-. However, the presence of ions in small pores requires considering the effect that inhibits their

movement (Smith and Weaver, 2012; Ziegler and Vernier, 2008; Chang et al., 1991).

Thus, opposite to the solution, the volume conductivity in a pore is reduced:

$$G_p = \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i H_i \exp\left(\frac{\mu_i^o}{kT}\right) \qquad (13)$$

where μ_i^o — standard chemical potential of the i-th ion within pore, $\mu_i^o = \frac{(z_i e)^2}{\varepsilon_l} P\left(\frac{\varepsilon_l}{\varepsilon_w}\right)$ moreover, the value has a maximum value of 0.25, H_i – a hindrance factor of ion movement in a pore — the Renkin function (Chang et al., 1991):

$$H_i = H(r, r_i) = \left[1 - \left(\frac{r_i}{r}\right)\right]^2 \left[1 - 2{,}1\left(\frac{r_i}{r}\right) + 2{,}09\left(\frac{r_i}{r}\right)^3 - 0{,}95\left(\frac{r_i}{r}\right)^5\right] \qquad (14)$$

Simplifying further cumbersome calculations with the expression (14), by the way of restricting the summands of summits by a small value, we will assume that the Renkin function can be written in the form:

$$H(r, r_i) = 1 - 4{,}1\left(\frac{r_i}{r}\right) + \overline{O}\left[\left(\frac{r_i}{r}\right)^2\right] \qquad (15)$$

Substituting function (15) in the equation (13), we receive the expression for conductivity within a pore:

$$G_p = \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \left\{1 - 4{,}1\left(\frac{r_i}{r}\right) + \overline{O}\left[\left(\frac{r_i}{r}\right)^2\right]\right\} \exp\left(\frac{\mu_i^o}{kT}\right) = \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] \exp\left(\frac{\mu_i^o}{kT}\right) + \overline{O}\left[\left(\frac{r_i}{r}\right)^2\right] \qquad (16)$$

Substituting (4), (12) and (13) in equation (3) we will get the electric component of the energy:

$$\Delta E_e = -\frac{\pi(\varepsilon_w - \varepsilon_l)U^2}{h^2} \int_{r_{min}}^{r} \left[\frac{2h G_s}{2h G_s + \pi r G_p(r)}\right]^2 r\, dr = -\frac{\pi(\varepsilon_w - \varepsilon_l)U^2}{h^2} \int_{r_{min}}^{r} \left[\frac{2h \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i}{2h \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i + \pi r \sum_{i=1}^{n} (z_i e)^2 \alpha_i C_i \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i}\right]^2 r dr =$$

$$= -\frac{\pi(\varepsilon_w - \varepsilon_l)U^2}{h^2} \int_{r_{min}}^{r} \left[\frac{2h}{2h + \pi r \sum_{i=1}^{n} \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i}\right]^2 r dr \qquad (17)$$

Taking the constants behind the integral sign (17) and introducing the symbol:

$$K = -4\pi(\varepsilon_w - \varepsilon_l)U^2 \qquad (18)$$

we obtain an integral with a variable upper limit, which is calculated as follows:

$$K \int_{r_{min}}^{r} \left[\frac{1}{2h + \pi r \sum_{i=1}^{n} \left[1 - 4{,}1\left(\frac{r_i}{r}\right)\right] b_i}\right]^2 r dr =$$

$$= K \int_{r_{min}}^{r} \left[\frac{r}{\sum_{i=1}^{n} \frac{4h^2}{(\pi b_i)^2} + \sum_{i=1}^{n} \left[16{,}8(r_i)^2 - 16{,}4\left(\frac{h r_i}{\pi b_i}\right)\right] + r^2 + \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right) r}\right]^2 dr =$$

$$= K \int_{r_{min}}^{r} \frac{\frac{1}{2} d(r^2) + d\left[r \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right)\right] - d\left[r \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right)\right]}{r^2 + r \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right) + \sum_{i=1}^{n} \left[\left(\frac{2h}{\pi b_i}\right)^2 + 16{,}8(r_i)^2 - 16{,}4\left(\frac{r_i}{\pi b_i}\right)\right]} =$$

$$= K \left[ln\left(\frac{r + \sum_{i=1}^{n} \left(\frac{2h}{\pi b_i} - 4{,}1 r_i\right)}{r_{min} + \sum_{i=1}^{n} \left(\frac{2h}{\pi b_i} - 4{,}1 r_i\right)}\right)^2 - \sum_{i=1}^{n} \left(\frac{4h}{\pi b_i} - 8{,}2 r_i\right) ln\left(\frac{r + \sum_{i=1}^{n} \left(\frac{2h}{\pi b_i} - 4{,}1 r_i\right)}{r_{min} + \sum_{i=1}^{n} \left(\frac{2h}{\pi b_i} - 4{,}1 r_i\right)}\right)^2\right] \qquad (19)$$

Considering expression (19), we obtain the electric component of the energy formation of the electric pore:

$$\Delta E_e = -4\pi(\varepsilon_w - \varepsilon_l)U^2 ln\left(\frac{r+\frac{2h}{\pi b_i}-4{,}1r_i}{r_{min}+\frac{2h}{\pi b_i}-4{,}1r_i}\right)^2 \cdot \left[1-\left(\frac{4h}{\pi b_i}-8{,}2r_i\right)\right] \quad (20)$$

where $b_i := exp\left(\frac{\mu_i^o}{kT}\right)$.

Considering electropore formation probability density again, we will notice that at reversible electroporation of membrane in PEF the number of formed electropores is of an order ~ 10^4–10^5 (Krassowska and Filev, 2007). Therefore, it is possible to argue safely that the radii distribution of electropore at any moment of their evolution follows the Gauss's law. Therefore, it is possible to introduce the corresponding probability density for $p(r)$:

$$n(r,t) = p(r)w(t) = \frac{1}{\sqrt{2\pi}}exp\left(-\frac{r^2}{2}\right)w(t) \quad (21)$$

where $p(r) = \frac{1}{\sqrt{2\pi}}exp\left(-\frac{r^2}{2}\right)$ — density by Gauss's law.

Substituting ratios (1), (2), (20), (21) in the equation (11), we receive the differential equation with divided variables:

$$\frac{\partial n}{\partial t} = A(r)w(t) \quad (22)$$

where $A(r)$ — designation of the function part depending on pore radius:

$$A(r) = D_p\left\langle \frac{1}{\sqrt{2\pi}}exp\left(-\frac{r^2}{2}\right)\left(r^2-1\right)+\frac{1}{kT}\left\{\frac{-r}{\sqrt{2\pi}}exp\left(-\frac{r^2}{2}\right)\cdot\right.\right.$$

$$\cdot 2\pi\left(\left(E_p - E_{mw}r\right)-2(\varepsilon_w-\varepsilon_l)U^2\left[\frac{1}{2h+\pi r\sum_{i=1}^{n}\left[1-4{,}1\left(\frac{r_i}{r}\right)\right]b_i}\right]^2 r\right)+$$

$$+\frac{1}{\sqrt{2\pi}}exp\left(-\frac{r^2}{2}\right)\cdot\left[-2\pi E_{mw}-4\pi(\varepsilon_w-\varepsilon_l)U^2\cdot\left(\frac{-2\pi r}{\left(2h+\pi r\sum_{i=1}^{n}\left[1-4{,}1\left(\frac{r_i}{r}\right)\right]b_i\right)^3}\sum_{i=1}^{n}b_i+\right.\right.$$

$$\left.\left.\left.+\left[\frac{1}{2h+\pi r\sum_{i=1}^{n}\left[1-4{,}1\left(\frac{r_i}{r}\right)\right]b_i}\right]^2\right)\right]\right\}\Bigg\rangle \quad (23)$$

The solution of the equation (22) looks like:

$$w(t) = exp\left[A(r)t\right] \quad (24)$$

Thus, the function $n(r,t)$ from expression (21) combined with the solution of (24) will become:

$$n(r,t) = \frac{1}{\sqrt{2\pi}}exp\left[A(r)t-\frac{r^2}{2}\right] \quad (25)$$

To get the total membrane conductivity final function type, we will substitute the obtained probability (25) to the conductivity integral (10), consistently considering expressions (5), (6), (12), (13), (20).

Afterward the integration is performed:

$$G(r,t) = \int_{r_{min}}^{r}\left[\frac{\frac{1}{\sqrt{2\pi}}exp\left[A(r)t-\frac{r^2}{2}\right]}{\frac{1}{2G_s r}+\frac{h}{\pi r^2 G_p}}\right]dr = \sqrt{2\pi}\int_{r_{min}}^{r}\left[\frac{G_s G_p\, exp\left[A(r)t-\frac{r^2}{2}\right]r^2}{\pi r G_p + 2G_s h}\right]dr =$$

$$= \sqrt{2\pi}\int_{r_{min}}^{r} \frac{\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i \cdot \sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i \left[1-4{,}1\left(\frac{r_i}{r}\right)\right] b_i \left\{exp\left[A(r)t-\frac{r^2}{2}\right]r^2\right\}}{\pi r \sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i \left[1-4{,}1\left(\frac{r_i}{r}\right)\right] b_i + 2h\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i} dr =$$

$$= \sqrt{2\pi}\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i b_i \int_{r_{min}}^{r} \frac{\sum_{i=1}^{n}\left[1-4{,}1\left(\frac{r_i}{r}\right)\right]\cdot\left\{exp\left[A(r)t-\frac{r^2}{2}\right]r^2\right\}}{\pi r \sum_{i=1}^{n}\left[1-4{,}1\left(\frac{r_i}{r}\right)\right] b_i + 2h} dr =$$

$$= 2\pi h\left(exp\left(A(r)-\frac{r^2}{2}\right)exp\left(\frac{2\pi E_p D_p}{kT}t\right)\right)\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i +$$

$$+\left(\exp\left(A(r)-\frac{r^2}{2}\right)\right)\frac{1}{t}\left(\frac{\frac{1}{\pi}-\frac{2{,}05}{h}\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i r_i b_i - 8{,}2\pi\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i r_i b_i}{2\sqrt{\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i (2h-4{,}1\pi)}}\right)\cdot$$

$$\cdot\left[\frac{ln\left|r-\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i (2h-4{,}1\pi)\right|}{ln\left|r+\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i (2h-4{,}1\pi)\right|}-\frac{ln\left|r_{min}-\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i (2h-4{,}1\pi)\right|}{ln\left|r_{min}+\sum_{i=1}^{n}(z_i e)^2 \alpha_i C_i (2h-4{,}1\pi)\right|}\right] \quad (26)$$

In the formula (26) the following estimate was used:

$$exp\left[A(r)-\frac{r^2}{2}\right] \le exp\left(-\frac{2\pi E_p D_p}{kT}\right) \quad (27)$$

The resulting formula (26) can be considerably simplified by replacing complex algebraic expressions, that do not contain variables, by some constants K_i, where i = 1, 2, 3, 4, to the following form:

$$G(r,t) = exp\left(A(r)-\frac{r^2}{2}\right)\left\{exp\left(\frac{2\pi E_p D_p}{kT}t\right)\cdot K_1 + \frac{K_2}{2t\sqrt{K_3}}\cdot\left[\frac{ln|r-K_3|}{ln|r+K_3|}-K_4\right]\right\} \quad (28)$$

Discussion. To find the characteristic parameters that determine the state of the membrane and its integrity, it is possible to make several theoretical models based on the approximation of the experimental dependencies on different functions. These parameters are chosen due to the technological necessity to influence the cells by the PEF for the implementation of electrofusion, stimulation, electrical breakdown, etc. (Smolyaninova, Shigimaga, and Kolesnikova, 2009; Smolyaninova et al., 2014; Saulis et al., 2013; Kotnik et al., 2012; Gowrishankar, Smith and Weaver, 2013; Krassowska and Filev, 2007; Miklavčič, 2012; Kolesnikova, Shigimaga and Smolyaninova, 2013; Shigimaga, Levkin and Megel, 2011; Shigimaga and Megel', 2011a,b, 2012; Shigimaga, 2014, 2015). However, it should be specially noted that in known models, the conductivity of cells, as a natural indicator of the membrane electroporation process, is rarely used as a parameter (Rems et al., 2016; Suzuki et al., 2011; Morshed, Shams and Mussivand, 2013; Schmeer et al., 2004; Dehez et al., 2014; Pucihar et al., 2011; Gowrishankar, Smith and Weaver, 2013), and the continuous effect on the membrane and the cell of the PEF increasing intensity is hardly considered at all, except a small number of works by Prof. D. Miklavčič, as well as our recent work about BP modeling (Kramar, Miklavčič and Maček Lebar, 2007; Kramar et al., 2012; Pucihar et al., 2011; Shigimaga, 2013a,b, 2014; Shigimaga et al., 2017). It is interesting to note that the probabilistic or statistical approaches have not been used recently in BP modeling. Nevertheless, in the productivity of one of these approaches we can convince, for example, the alternative electroporation model which has been proposed in the work (Golberg and Rubinsky, 2010). However, this work is rather of an applied nature, whereas it considers only irreversible electroporation in connection with the application of this effect in the clinical practice of biomedicine for the lysis of certain cells in the tissue. The advantages of using continuous influence on the cell by IEP of increasing intensity become into the open in the context of the above-findings of BP conductivity modelling based on the electroporation representation of the membrane, as a probabilistic process which is represented by the formula (28).

Analyzing the formula (28) derived on the basis of the probability approach, it is possible to notice that dependence of membrane conductivity on electropore radius has as exponential character. If assumed that electropores radius is the mean value at given PEF strength, then according to equation (28) we will get the mean value of conductivity at mean radius $\bar{r}$ at the

moment of time *t*. With the increase of PEF strength, the mean electropores radius $\bar{r}$ and conductivity will also increase, and equation (28) can be considered as a function, describing this process (Fig. 2).

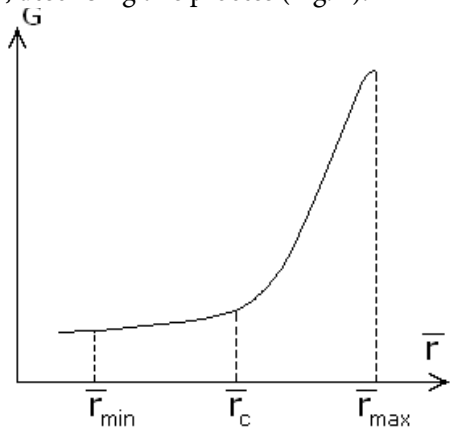

Figure. 2. Theoretical dependence of membrane conductivity on mean electropore radius (probability BP model)

Such suggestion is legitimate, as the mean electropore size depends almost linearly on the field strength in the reversible electroporation mode, and exponentially — in irreversible (Golberg and Rubinsky, 2010; Pavlin et al., 2008; Kotnik et al., 2012). Additionally, the theoretical function (28) is limited by the value $r_{max} = 2r_c$ as electropore radius cannot infinitely increase but only to membrane breakdown (Shigimaga, 2014). Time dependence of conductivity in this case can be neglected, since time of electropore reparation (in reversible electroporation mode) is much less than the selected period of PEF strength variation. Function (28) qualitatively describes our experimental data on cells conductivity in PEF with increasing intensity (Fig. 3).

The minimum radius of the electroporator and the conductivity are defined by the membrane properties and the physicochemical properties of the solution, and also small PEF strength. With PEF strength increases the radius of the electroporator also slowly increases, reaching a critical radius. Above a certain field strength (individual for cells with all the solution factors), the membrane reaches r_{max} and undergoes an irreversible breakdown. This corresponds to the critical PEF strength for irreversible breakdown, which is defined at a point of the maximum conductivity curvature (Shigimaga, 2014, 2015) (Fig. 3).

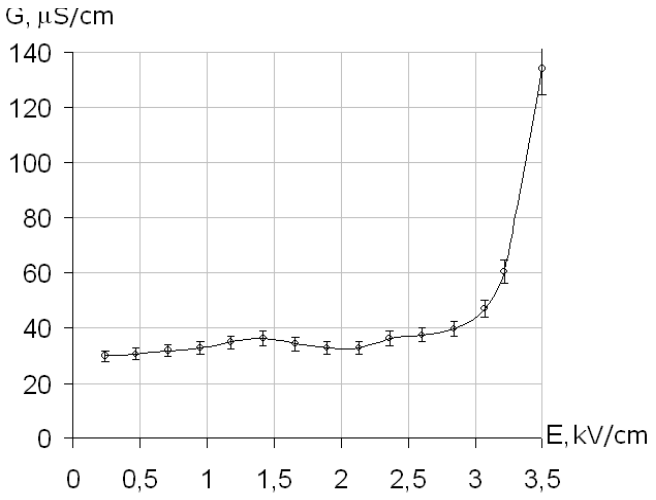

Figure. 3. Experimental dependence of mice oocyte condutivity from PEF strength

The $r_{min} < r < r_c$ interval (Fig. 2), corresponds to reversible membrane electroporation phase with various extents of damage and reparation.

Thus, it is possible to suggest that the main reason for exponential increase in the conductivity of cells in PEF with increasing strength (in experiment) is similar in nature to an conductivity increase with electropore radius up to membrane breakdown. In addition, the constructed probability BP model of cell conductivity at membrane electroporation in increasing PEF agrees with the experimental data.

Conclusions. A probabilistic BP model is made on the basis of the physical characteristics of the cell conductivity during the membrane electroporation in the PEF of increasing intensity. The exponential characteristic of the cell conductivity rise with the average radius increase of the electropores (up to the rupture of the membrane in the case of increase of the critical field intensity) is obtained based on the BP model. It is adequate to the experimental data. The constructed BP model allows theoretical justification and calculation of the PEF parameters. The variable field intensity provides a different degree of the membrane electroporation and therefore makes it possible to justify and calculate all of its necessary safe and critical regimes for pulsed conductometry of living animal cells according to their varying conductivity. Within the scope of an integrated hardware-methodical process of pulsed conductometry it is possible not only to realize the known applications, but to develop new applications of electroporation in the biotechnology of animal reproduction and biomedicine. It is prospective approach.

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