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Boosting membrane transmissibility with an impulse electric field

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Conductivity of cells in PEF based on electroporation is specified by electrical membrane properties which are largely dependent on its geometric structure. Basic geometrical properties of a cell and a membrane are formulated in the article. They are defined as objects of mathematical modeling with a goal to theoretically ground the methodical modes of electroporation, dealing with pulse conductometry of cells.

A probabilistic model of a cell conductivity is proposed for a membrane electroporation in PEF with increasing tension. Exponential characteristic of a cell conductivity growth is constructed assuming the increase in elektropor caliber until a membrane disruption occurs when critical field strength is reached. The developed model allows theoretically ground and compute field parameters, providing safe and critical modes of electroporation while performing pulse conductometry of living animal cells.

Keywords: *electroporation, membrane, electropore, mathematical model, conductivity, mechanical component.*

1. Introduction. Electroporation is a universal method of temporary increase of membrane cell permeability by imposing of external PEF certain strength. At such physical influence on cell in a membrane make and then evolves an electropores of various calibers owing to strong electric interaction of PEF with charges of a membrane and cytoplasm ions [1 - 3]. On process and result of membrane interaction with PEF, i.e. the electroporation, a great influence are rendered by heterogeneity biological membrane structure which has both own natural pores, and various protein inclusions that results in heterogeneity of electro physical properties. It as result is reflected in the cellular conductivity being at the same time the indicator and the integrated characteristic of electroporation process as whole [4]. To consider when modeling all features of natural membrane structure it is impossible but if to apply a probability approach to electropores formation, possibility to construct model of cell conductivity on the basis of membrane electroporation is opened

Physical and mathematical model of cell conductivity, constructed on the basis of electropores formation probability in a membrane under the influence of pulse electric field (PEF) is presented. The model assumes that in a membrane an electropores of different calibers which distribution submits to Gauss's normal law are formed. Using the integral equation for total current through membrane electropore, the equation for its conductivity, including function of electropore formation probability (conductivity integral) is received. The general view of electropore formation probability function is received by solution of differential equation. Substitution of the solution of this equation to conductivity integral gave the general view of conductivity function connecting it with electropore caliber. Comparison of the constructed probability electroporation model with experimental data on mice oocyte conductivity showed that the main reason for exponential increase of cell conductivity in increasing PEF strength is similar nature of conductivity increase with electropore caliber up to membrane breakdown.

2. Task formulation. Electroporation modeling according to this approach is expedient for beginning with consideration of integrated characteristic of electropore formation, – the free energy considering mechanical and electric contribution of forces at electroporation [2]. The electropore is thus approximated by the round cylinder of radius r and the height h equal to membrane thickness, fig. 1.

Free energy of electropore represents generally the sum:

$$\Delta E = \Delta E_m + \Delta E_e + E_o \tag{1}$$

where ΔE_m – mechanical component, ΔE_e – electric component, E_o – any constant. The mechanical component of energy of a electropore is defined by expression [2, 5, 6]:

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

where E_p – linear density energy of pore edge, E_{mw} – surface density energy of interaction a membrane-water.

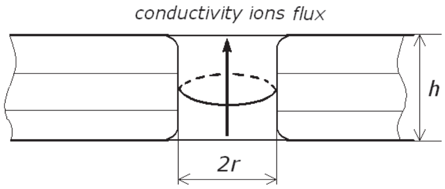


Fig. 1 Electropore approximation by the cylinder on a membrane surface

It is known that the mechanical breakdown of flat membrane is reached, if one or several electropore exceeds critical radius $r > r_c$, where $r_c = E_p / E_{mw}$ [2, 6, 7]. Considering that cell radius on 4 - 5 orders exceeds electropore radius [2, 5], in a pore vicinity it is possible to neglect curvature of membrane surface and consider as it flat.

The electric component of energy (1) represents electropore as the condenser with some conductivity of leak [8,9]:

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

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 division, connected with resistance distribution on the brink (to solution) and within a pore. This function writes as follows [2]:

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

where G_p and G_s – conductivity within a pore and solution at entrance in a pore respectively. The effect of voltage division has following physical explanation. In solution about an entrance in a pore there is a non-uniform electric field. The related voltage failure is estimated by introduction of modeling resistor with the resistance R_s which value is set by formula [8]:

$$R_s \approx 1/2G_s r \tag{5}$$

Respectively internal resistance of a pore R_p is estimated through its conductivity G_p [2]:

$$R_p = h/(\pi r^2 G_p) \tag{6}$$

These resistance R_s and R_p are included consistently, forming a voltage divider, as leads to TMP reduction at the expense of falling on R_s. Considering these resistance, a current through an electropore, according to the Ohm's law, it is possible to write down, as:

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

For calculation of total current through a surface of all membrane, which electroporated by PEF force, we will enter concept of probability formation density of electropore $n(r,t)$, as the function depending on radius (caliber) of electropore and time. The combination of various physical forces (1) operates process of electropore evolution therefore in a membrane there are electropores of different caliber [9]. Distribution on calibers can be described by density function $p(r)$. It is supposed that the quantity of electropores $w(t)$ changes in time owing to their rising and reparation of that having radius $r_{\min}$. From these assumptions follows that distribution on radiuses and time is two independent functions therefore it is possible to write down that total density of formation probability an electropore is function of their product:

$$n(r,t) = p(r)\,w(t) \tag{8}$$

Having defined density of electropore formation probability, it is possible to write down the equation for total current through membrane, using [2,5]:

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

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

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

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

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

3. **Creation of physical and mathematical electroporation probability model.** For definition of general view of probability density electropore formation function $n(r,t)$ it is possible to use the differential equation of change speed of this, deduced on the basis of the equation [2,5,6]:

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

where D_p – an effective constant of diffusion for a pore, radius r [9]. The value ΔE taken in the form of (1), in the equation (11) has crucial importance as $\left(\partial \Delta E / \partial r\right)_U$ there is the effective force changing of electropore caliber.

Further, volume conductivity of solution is function of concentration and mobility of the ions being in it [10]:

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

where z_i – charge, α_i – mobility and C_i – concentration i – ion's, $e = 1{,}6 \cdot 10^{-19}$ K (electron charge). Unlike solution volume conductivity in a pore is reduced [2]:

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

where: μ_i^o – standard chemical potential i – ion's within pore, H_i – a hindrance factor to ion movement in a pore – Renkin's function [11]:

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

Substituting function (14) in the equation (13), we receive expression for conductivity within a pore:

$$G_p = \sum_{i=1}^{n} (z_i\, e)^2 \alpha_i\, C_i \left[1 - 4{,}1 \left(\frac{r_i}{r} \right) \right] \times$$
$$\times \exp\left(\frac{\mu_i^o}{kT} \right) + \overline{O} \left[\left(\frac{r_i}{r} \right)^2 \right] \qquad (15)$$

Further we will consistently substitute in the equation for an electric component of energy (3) written down above expression (4), (12) and (13):

$$E_e = -\pi(\varepsilon_w - \varepsilon_l) U^2 h^{-2} \times$$
$$\Delta \times \int_{r_{\min}}^{r} \left[\frac{2h\, G_s}{2h\, G_s + \pi\, r\, G_p(r)} \right]^2 r\, dr =$$
$$= -4\pi(\varepsilon_w - \varepsilon_l) U^2 \times$$
$$\times \ln \left(\frac{r + \dfrac{2h}{\pi b_i} - 4{,}1 r_i}{r_{\min} + \dfrac{2h}{\pi b_i} - 4{,}1 r_i} \right)^2 \left[1 - \left(\frac{4h}{\pi b_i} - 8{,}2 r_i \right) \right], \qquad (16)$$

where $\qquad b_i := \exp\left(\mu_i^o / kT \right).$

Coming back to density of electropore formation probability, we will notice that at reversible electroporation of membrane in PEF the quantity of being formed electropores have an order ~10^4-10^5 [1, 2, 12]. Therefore it is possible to argue safely that distribution of electropore on calibers at any moment of their evolution submits to Gauss's law. Therefore, it is possible to enter the corresponding probability density for $p(r)$:

$$n(r,t) = p(r)\, w(t) = \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{r^2}{2} \right) w(t)\,. \qquad (17)$$

Substituting ratios (1), (2), (16), (17) in the equation (11), we receive the differential equation with being divided variables:

$$\frac{\partial n}{\partial t} = A(r) w(t)\,, \qquad (18)$$

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

$$A(r) = D_p \left\langle \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{r^2}{2} \right) (r^2 - 1) + \right.$$
$$+ \frac{1}{kT} \left\{ \frac{-r}{\sqrt{2\pi}} \exp\left(-\frac{r^2}{2} \right) \times \right.$$
$$\times 2\pi \left(\begin{array}{l} (E_p - E_{mw} r) - 2(\varepsilon_w - \varepsilon_l) U^2 \times \\ \times \left[2h + \pi\, r \sum_{i=1}^{n} \left[1 - 4{,}1 \left(\dfrac{r_i}{r} \right) \right] b_i \right]^{-2} r \end{array} \right) +$$
$$+ \frac{1}{\sqrt{2\pi}} \exp\left(-\frac{r^2}{2} \right) \cdot \left[-2\pi E_{mw} - 4\pi\left(\varepsilon_w - \varepsilon_l \right) U^2 \times \right.$$
$$\times \left(-2\pi\, r \left(2h + \pi\, r \sum_{i=1}^{n} \left[1 - 4{,}1 \left(\frac{r_i}{r} \right) \right] b_i \right)^{-3} \sum_{i=1}^{n} b_i + \right.$$

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

The solution of the equation (18) looks like:

$$w(t)=\exp\left[A(r)\,t\right] \qquad (20)$$

Thus, function $n(r,t)$ from expression (17) with use of the decision (20) will become:

$$n(r,t)=\frac{1}{\sqrt{2\pi}}\exp\left[A(r)\,t-\frac{r^2}{2}\right] \qquad (21)$$

For receiving a final type of function of the total membrane conductivity, we will substitute the received probability (21) to the conductivity integral (10), having consistently considered expressions (5), (6), (12), (13), (16) then we will carry out integration:

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

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

$$=2\pi h\exp\left(A(r)-\frac{r^2}{2}\right)\exp\left(\frac{2\pi E_p D_p}{kT}t\right)\times$$

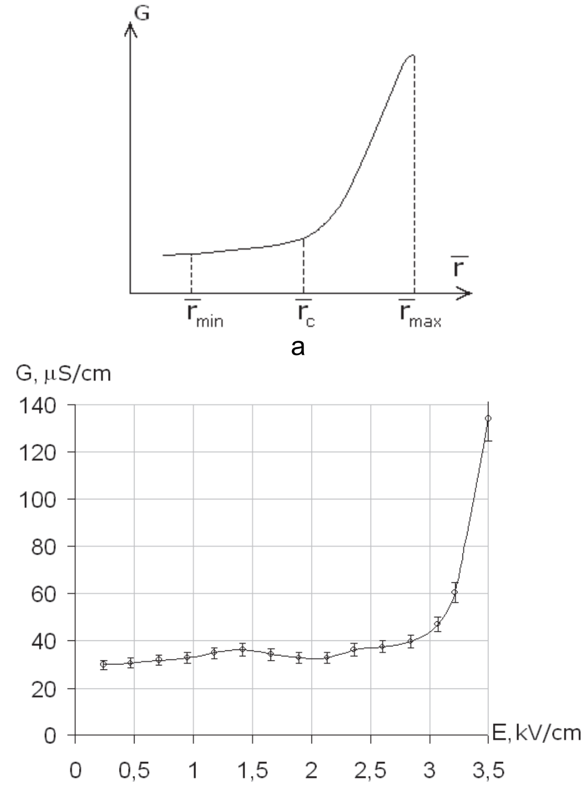
$$\times\sum_{i=1}^{n}(z_i e)^2\alpha_i C_i+\frac{1}{t}\exp\left(A(r)-\frac{r^2}{2}\right)\times$$

$$\times\frac{\dfrac{1}{\pi}-\left(\dfrac{2{,}05}{h}+8{,}2\pi\right)\displaystyle\sum_{i=1}^{n}(z_i e)^2\alpha_i C_i r_i b_i}{2\sqrt{\displaystyle\sum_{i=1}^{n}(z_i e)^2\alpha_i C_i(2h-4{,}1\pi)}}\times$$

$$\times\left[\frac{\ln\left|r-\displaystyle\sum_{i=1}^{n}(z_i e)^2\alpha_i C_i(2h-4{,}1\pi)\right|}{\ln\left|r+\displaystyle\sum_{i=1}^{n}(z_i e)^2\alpha_i C_i(2h-4{,}1\pi)\right|}-\right.$$

$$\left.-\frac{\ln\left|r_{\min}-\displaystyle\sum_{i=1}^{n}(z_i e)^2\alpha_i C_i(2h-4{,}1\pi)\right|}{\ln\left|r_{\min}+\displaystyle\sum_{i=1}^{n}(z_i e)^2\alpha_i C_i(2h-4{,}1\pi)\right|}\right]. \qquad (22)$$

Analyzing formula (22) received on the basis of probability approach, it is possible to notice that dependence of membrane conductivity on electropore caliber has exponential character. If to assume that electropores caliber is the mean (most credible) value at given PEF strength, then in according to formula (22) we will get the mean value of conductivity at this mean caliber in the moment of time t. Then with increase of PEF strength mean electropores caliber and conductivity both will increase also, and formula (22) can be examined as function, describing this process which is shown on the fig.2a. Such supposition is legitimate, as the mean electropores size practically linearly depends on the field strength in the mode of reversible electroporation, and exponentially – at irreversible [5, 6, 13 -15]. Moreover, for theoretical function (22) restriction on the right in the value $r_{\max}=2r_c$ [1, 2] is entered as electropore radius cannot infinitely increase but only to membrane breakdown. Temporary dependence of conductivity in this case can be neglected, since time of electropore reparation (in a mode of reversible electroporation) much less chosen period of PEF strength change. Function (22) qualitatively coincides with data of our experiments on measurement of cells conductivity in PEF with increasing intensity [4], fig.2(b).



a

b

Fig. 2: a) theoretical dependence of membrane conductivity on mean electropore radius (probability model); b) experimental dependence of mice oocyte from PEF strength

The minimum electropore radius and conductivity are defined by membrane properties and physical and chemical solution factors, and also small PEF strength. With PEF strength increase the electropore radius also increase slowly, reaching critical, and above a certain field strength (individually for cell with all solution factors) at $r > r_c$ and further to $r_{\max}$ a membrane is irreversible breakdown. It correspond to critical PEF strength of irreversible breakdown which is defined in a point of the maximum conductivity curvature [4, 16], fig. 2b. The interval $r_{\min} < r < r_c$, fig. 2a, corresponds to phase of reversible membrane electroporation with various extents of its damage and a reparation.

Thus, it is possible to argue that the main reason for exponential increase of cell conductivity in PEF with increasing strength (in experiment) is similar nature of conductivity increase with electropore caliber up to membrane breakdown.

4. Conclusion. The proposed probabilistic model of cell transmissibility for electroporation in PEF has an exponential nature of a membrane penetration, depending on pore sizes, with adequacy in relation to experimental data. It has been shown that electric pore radius increases with growth in field intensity, reaching its critical value and followed by a membrane disruption.

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Анотація

Підвищення проникності мембрани клітини при дії імпульсного електричного поля

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Провідність клітини в імпульсному електромагнітному полі (ІЕП), заснована на явищі електропорації, і визначається електричними властивостями мембрани, що багато в чому залежать від її геометричної структури. У статті сформульовані основні геометричні властивості клітини і мембрани, як об'єктів математичного моделювання з метою теоретичного обґрунтування методичних режимів електропорації імпульсной кондуктометрії клітин в середовищах.

Запропоновано імовірнісна модель провідності клітини при електропорації мембрани в ІЕП зростаючої напруженості, побудована експоненціальна характеристика зростання провідноості клітини з зростанням середнього калібру електропор, до розриву мембрани при перевищенні критичної напруженості поля. Побудована модель дозволяє теоретично обгрунтувати і рорахувати апаратні параметри поля, що забезпечують безпечні і критичні режими електропорації при імпульсній кондуктометрії живих клітин тварин.

Ключові слова: *електропорація, мембрана, електропори, математична модель, провідність, механічна складова.*

Аннотация

Повышение проницаемости мембраны клетки при воздействии импульсного электрического поля

Ю.Е. Мегель, В.А. Шигимага, С.Н. Коваленко, Т.Б. Беликова

Проводимость клетки в импульсном электромагнитном поле (ИЭП), основанная на явлении электропорации, определяется электрическими свойствами мембраны, во многом зависящими от ее геометрической структуры. В статье сформулированы основные геометрические свойства клетки и мембраны, как объектов математического моделирования с целью теоретического обоснования методических режимов электропорации при импульсной кондуктометрии клеток в средах.

Предложена вероятностная модель проводимости клетки при электропорации мембраны в ИЭП возрастающей напряженности, построена экспоненциальная характеристика роста проводимости клетки с ростом среднего калибра электропор, вплоть до разрыва мембраны при превышении критической напряженности поля. Построенная модель позволяет теоретически обосновать и рассчитать аппаратные параметры поля, обеспечивающие безопасные и критические режимы электропорации при импульсной кондуктометрии живых клеток животных.

Ключевые слова: *электропорация, мембрана, электропоры, математическая модель, проводимость, механическая составляющая.*

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