A Control System of Cell Electroporation induced by Double Nanosecond Pulsed Electric Field

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Abstract. A feedback linearization control system, which is used to study and analyze cell electroporation induced by double nanosecond pulsed electric field, is proposed in this paper. Then the effects of the control system which controls the process of electroporation induced by unipolar or bipolar double pulse are investigated to achieve controllability of electroporation. The simulation results show that the output of the control system, namely, the transmembrane potential can reach a stable value of 0.78 (-0.78)V, the radius increases linearly from 0.86nm to 0.88nm and the pore density increases linearly to 2.072×10^8m^-2 (2.077×10^8m^-2) when the input of the control system, the expected transmembrane potential is set to 0.79 (-0.79) V, and these results are in well agreement with the simulation result obtained by computing the related equations of electroporation, demonstrating that the control system developed in study can well regulate cell electroporation under the action of double nanosecond pulsed electric field. Overall, the control system can help the researcher to obtain the corresponding output electroporation parameters by directly inputting the expected transmembrane potential.

1. Introduction
Electroporation is a phenomenon in which a pulsed electric field is applied to create transient pores in the cell membrane to facilitate the entry of large molecules into the cell. In recent years, this technology has a good application prospect and has been successfully applied in gene transfer [1], drug delivery [2], and cancer treatment [3].

The effectiveness of electroporation is closely related to the parameters of the pulsed electric field. It is difficult to establish the relationship between the parameters of pulses and biological effects due to the nonlinear characteristics of the electroporation model, which seriously restricts the application of this technology. Therefore, it is urgent to establish an effective control system to regulate the process of electroporation to realize the wide application of this technology.

Relevant studies on electroporation control system have also been carried out. Yang et al[4] proposed a data-driven feedforward control system based on the assumption that transmembrane potential is constant. Ma Ou et al[5] proposed a linearized feedback control system for the electroporation model. This system assumes that all state variables are measurable, so the system has some limitations. Jiang Wen et al[6] proposed a feedback control system to regulate the radius of pores based on a double pulse. In literature [7], a linear model predictive controller is used to regulate the electroporation process. Yi Jingang et al[8] designed a nonlinear model predictive controller based on multiple pulses. However, the above control systems are all based on electroporation under the action of millisecond/microsecond pulse. At present, nanosecond pulse is of great interest to researchers due to its unique targeted apoptotic effect[9], so researchers are eager to establish a control system for cell electroporation under the action
of nanosecond pulse to control cell apoptosis.

Therefore, a feedback linearization control system based on electroporation model for a spherical cell induced by nanosecond pulse was developed in this paper. This system realizes effective regulation of electroporation through controlling the transmembrane potential caused by nanosecond pulse.

2. Numerical model

2.1 The model of electroporation

Under the action of the pulsed electric field, there is a potential difference between the inside and outside of the cell membrane, which is named the transmembrane potential. Some pores will be formed in the surface of membrane induced by transmembrane potential. These pores will expand, and reseal when the transmembrane potential disappears. This section will model this process.

In this paper, the cell is equivalent to the circuit model as shown in literature [10] to calculate the transmembrane potential. Therefore, the transmembrane potential \( V_m \) can be expressed by an ordinary differential equation as shown in equation (1):

\[
\frac{dV_m}{dt} = \frac{V_0}{CR_S} - \frac{I_p}{C} \left( \frac{1}{R_S} + \frac{1}{R} \right) V_m
\]

(1)

\( I_p = \sum_{n=1}^{K} \frac{V_m}{h/(\pi g r_n^2) + 1/(2gr_n)} \)

(2)

Then the pore density is used to represent that the number of pores which are formed in the surface of membrane. Krassowska et al. [11] proposed an ordinary differential equation to describe the change of pore density in the process of electroporation. The pore density \( N \) is shown as follows:

\[
\frac{dN}{dt} = \alpha e^{(v_m/v_p)} \left( 1 - \frac{N}{N_0 e^{(v_m/v_p)}} \right)
\]

(3)

It is assumed that the radius of pore created by equation (3) is \( r_m \), and the radius changed to minimize the energy \( w_m \) of the lipid bilayer. If there are \( K \) pores with radius \( r_n \) \((n=1,2,\ldots,K)\) at a certain moment, the change of radius is determined by the energy of the lipid bilayer. The formula is as follows:

\[
\frac{dr_n}{dt} = -\frac{D}{kT} \frac{\partial w_m}{\partial r_n}, \quad n = 1, 2, \ldots, K
\]

(4)

\[
w_m = \sum_{n=1}^{K} w_{nl} \left( \frac{r_n}{r_m} \right)^4 + 2\pi w_{nl} r_n - 2\pi \sigma_1 - \frac{2\sigma_1 - \sigma_0}{1 - A_p/A} r_n^2 - \int_{r_n}^{r_m} \frac{F_{max}}{(1 + r_h/(r + r_f))} V_m^2 dr
\]

(5)

In the above equations, \( V_m \) is the transmembrane potential, \( N \) is the pore density, \( r_n \) is the radius of the \( n \)th pore, \( A_p = \sum_{n=1}^{K} \pi r_n^2 \) is the sum of the areas of all the large pores and other parameters are constants. Details of the above equations, its application, the value of parameters and the dynamical aspects of electroporation have been analyzed in literature [10], and will not be discussed here.

2.2 The control system

Some researchers find that after the action of the first pulse with high amplitude, the number of pores on the cell membrane does not change under the action of the second pulse with low amplitude, the radii of all pores are the similar and the change law of radius is consistent. Therefore, the corresponding control system can be designed to control parameters of electroporation on the assumption that the number of pores is constant and the radius of each pore is consistent.

Therefore, the pore density \( N \), the radius of pores \( R \) and transmembrane potential \( V_m \) are taken as the control variables. According to the above equations, the process of electroporation can be expressed by the following state space expression:
\[
\dot{X} = F(X) + G(X)U \\
Y = H(X)
\]

\[
X = [x_1, x_2, x_3]^T = [N, R, V_m]^T
\]

\[
F(X) = \begin{bmatrix}
    f_1(X) \\
    f_2(X) \\
    f_3(X)
\end{bmatrix} = 
\begin{bmatrix}
    \frac{d}{dx_1}\left(\frac{4w_n r^4}{x^2} + \frac{F_{\text{max}}(x_2 + r_j)}{r_k + x_2 + r_j} \right) - 2\pi w_{ed} + 2\pi x_2 (2\sigma_1 - \frac{2\sigma_1 - \sigma_0}{A - K \pi x_2^2}) \\
    \frac{2gK \pi x_2^2 x_3}{C (\pi x_2 + 2h)} - \frac{1}{R_s + 1} x_3 \\
    \frac{1}{CR_i}
\end{bmatrix}
\]

\[
G(X) = \begin{bmatrix}
    0 \\
    0 \\
    \frac{1}{CR_i}
\end{bmatrix}^T
\]

\[
U = \text{the control input designed according to the desired control objectives and system performance.}
\]

\[
\text{The output function } H(x) \text{ varies with the output. In equations (8), } K \text{ is the number of pores.}
\]

According to the above equations, electroporation is a highly nonlinear multi-dimensional dynamic system with only one control input, so it is difficult to control three state variables with one input. Adding additional control inputs means that the process of electroporation needs to be redefined, which is difficult and beyond the scope of this paper. Besides the radii of pores and pore density cannot be measured by relevant instruments, but the transmembrane potential can be obtained by relevant instruments. Moreover, both the radii of pores and pore density are related to transmembrane potential, so they can be controlled to control the change of transmembrane potential. Therefore, the state variable \( x_3 \) (i.e., transmembrane potential \( V_m \)) is selected as the control target.

Therefore, the output equation of equation (6) becomes \( Y = H(X) = x_3 \).

According to equation (6), the control quantity can be obtained

\[
U = G(X)^{-1}\left(\dot{x}_3 - f_3(X)\right)
\]

Using a linear servo controller to track the state variable \( x_3 \), then

\[
\dot{x}_3 = K_p (x_{ed} - Y) + K_d (\dot{x}_{ed} - \dot{Y})
\]

Where \( x_{ed} \) is the input, the expected value of the output which is the stable value of the state variable \( x_3 \) in this paper, and \( Y \) is the output. The block diagram of the feedback control system is shown in figure 1. In figure 1, “system” represents the process of electroporation, which is described by equation (8). Consequently, under the action of the control system, the output \( Y \) will follow the input \( x_{ed} \).

![Figure 1. Block diagram of the control system.](image-url)
3. Simulation results and discussion

3.1 Simulation results of the double pulse and bipolar pulse

The dynamic model of electroporation and control system proposed in this paper are realized by Matlab. Because the control system proposed in this paper is only applicable to electroporation under the action of a unipolar pulse or a bipolar pulse. So in order to prove the effect of the proposed feedback control system, the results without control and the results with feedback control are compared and discussed.

The parameters of the double pulse and the bipolar pulse are shown in figure 2. Under the action of a and b, the variation curve of the parameters of electroporation with time is shown in figure 3.

As we can see, the curves of all parameters in these cases are similar. The number of pores (figure 3. (a)) increases to 615 at the end of the first pulse and then stabilizes at 615. The transmembrane potential (figure 3. (b)) follows the pulse (figure 2), and then stabilizes at 0.79V and -0.79V in the second pulse, respectively. The mean radius (figure 3. (c)) increases slowly to 0.86nm in the first pulse, and remain unchanged during the time interval. Finally, it rises to 0.88nm in the second pulse with time. The pore density (figure 3. (d)) fluctuates sharply during the first pulse under the influence of the pore generation, and then stabilizes at $2.076\times10^8$ and $2.071\times10^8$, respectively.

According to the above simulation results, the input of the control system is set to 0.79V and -0.79V, respectively. The initial state of control system is set to $X(0) = [2.076\times10^8 0.86\times10^{-9} 0]^T$ and $X(0) = [2.071\times10^8 0.86\times10^{-9} 0]^T$, respectively. And the value of $K$ is set to 615. In addition, $K_p$ is $10^{11}$, $K_d$ is 1. The result is shown in figure 4.

![Figure 2. The parameters of unipolar and bipolar double pulse](image)

![Figure 3. The curve of each parameter with time induced by unipolar pulse (bipolar pulse)](image)

![Figure 4. The variation trend of each parameter under the action of control system](image)

In Figure 4 (b), (c), (d), the solid line and the dashed lines represent the result of each parameter
between 20ns and 30ns induced by unipolar pulse/bipolar pulse respectively. Figure 4. (a) shows the curve of the input, the expected transmembrane potential. Moreover, it can be seen from figure 4. (b) that the transmembrane potential reaches a stable value of 0.78 (-0.78) V in an instant. During this process, the radius (figure 4. (c)) increases from 0.86nm to 0.88nm with time. The pore density (figure 4. (d)) also increases linearly with time to 2.072×10^8 m⁻² (2.077×10^8 m⁻²).

The above results demonstrate that the control system can make the output follow the input, and the control system can only control the transmembrane potential to make the change law of all states follow the change law of the real situation. In addition, the values of all states are similar to the simulation results, indicating that the control system can simulate the process of electroporation. Therefore, obtaining the required electroporation parameters by constantly modulating pulse parameters can be avoided. The variation of other parameters with time can be obtained by inputting a desired transmembrane potential into the control system and setting corresponding initial values.

Although the control system established in this paper only controls the change of transmembrane potential, it still has good control effects. However, the control system still needs to know the initial value of each state variable and the number of pores through simulation to realize the control. And these values cannot be measured in real-time experiments, so there are still many challenges in the implementation of the control system. Moreover, the control system designed in this paper is only suitable for the electroporation under the action of unipolar pulses/bipolar pulse mentioned in this paper. So further research is needed for the control of parameter electroporation under the action of other pulses.

4. Conclusion
In this paper, the equivalent circuit model of cell, the pore density equation and the change equation of radius are combined to analyze the process of electroporation caused by the pulsed electric field. Based on this, a feedback linear control system is proposed. The control system controls the variation of pore density and radius by controlling the transmembrane potential. The simulation results show that the control system has good control effects which means the control system can help the researcher to input the expected transmembrane potential to obtain the corresponding changes of the electroporation parameters.

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References
[1] Spanggaard, I., Dahlstroem, K., Laessoee, L. et al. Gene therapy for patients with advanced solid tumors: a phase I study using gene electrotransfer to muscle with the integrin inhibitor plasmid AMEP[J]. Acta Oncologica, 2017, 56(7):909-916.
[2] Kotnik, T., Frey, W., Sack, M. et al. Electroporation-based applications in biotechnology[J]. Trends in Biotechnology, 2015, 33(8):480-488.
[3] Forjanic, T., Markele, B., Marcan, M. et al. Electroporation induced stress response and its effect on gene electrotransfer efficacy: in vivo imaging and numerical modelling[J]. IEEE Transactions on Biomedical Engineering, 2019, 68(9): 2671-2683.
[4] Yang, R., Tarn, T.J., Zhang, M. Data-Driven Feedforward Control for Electroporation Mediated Gene Delivery in Gene Therapy[J]. IEEE Transactions on Control Systems Technology, 2010, 18(4):935-943.
[5] Ma, O., Zhang, M. Dynamics Modeling and Control of Electroporation-Mediated Gene Delivery[J]. IEEE Transactions on Automation Science and Engineering, 2009, 6(2):228-238.
[6] Jiang, Wen., Zhao, X., Dynamics and control of the two-pulse protocol in electroporation: numerical exploration. Math Biosci. 2011;232(1):24-30.
[7] Ge, P., Yi, J., Li, J., and Lin, H. 2010. “Model predictive control of an electroporation process”. In Proc. ASME Dyn. Syst. Control Conf. Paper #DSCC2010-4243
[8] Yi, J., Li, J., Lin, H. Spherical Modeling and Nonlinear Model Predictive Control of Electroporation[C] ASME 2011 Dynamic Systems and Control Conference and Bath/ASME Symposium on Fluid Power and Motion Control. 2011.

[9] He, L., Xiao, D., Feng, J., Yao, C & Tang, L. Induction of apoptosis of liver cancer cells by nanosecond pulsed electric fields (nsPEFs). Med. Oncol. 34 (2017).

[10] Smith, KC., Neu, JC., Krassowska, W. Model of creation and evolution of stable electropores for DNA delivery. Biophys J. 2004; 86(5): 2813-2826.

[11] Neu, JC., Krassowska, W. Asymptotic model of electroporation[J]. Physical review E,1999,59(3):3471.