Cells Electroporation using sine waves: a frequency-dependent phenomenon

Electroporation des cellules avec des ondes sinusoïdales : un phenomène dépendant de la fréquence

***Tomás García-Sánchez*[1] *and Lluis M. Mir*[2]**

[1] *Department of Information and Communication Technologies, Universitat Pompeu Fabra, Barcelona, Spain. tomas.garcia-sanchez@outlook.com*

[2] *Vectorology and Anticancer Therapies, UMR 8203, CNRS, Univ. Paris-Sud, Gustave Roussy, Université Paris-Saclay, 94805 Villejuif, France,*

Mots clés (en français et en anglais) : Electroporation, radiofréquence, champ électrique. Electroporation, radiofrequency, electric field

Résumé/Abstract

L'électroporation de la membrane cellulaire causée par des ondes sinusoïdales a été étudiée. Des rafales uniques de différentes durées et intensités ont été appliquées aux cellules. Des impulsions à onde carrée présentant des caractéristiques équivalentes ont également été appliquées à des fins de comparaison. L'efficacité de l'électroperméabilisation de la membrane cellulaire a été étudiée à la fois in vitro et in vivo. En accord avec la théorie, les résultats montrent une nette dépendance du degré de perméabilisation de la membrane cellulaire en fonction de la fréquence des ondes. Les résultats obtenus montrent la capacité des signaux sinusoïdaux à provoquer l'électroporation des cellules et comment, en modifiant la fréquence du champ électrique oscillant appliqué, il est possible de moduler avec précision l'ampleur de la perméabilisation causée à la membrane cellulaire.

In this study cell electroporation using AC sine waves was studied. Single bursts of different durations and intensities were used. Also square wave electric pulses with the same characteristics were applied for the comparison. The efficacy of the electropermeabilization of the cell membrane was studied both *in vitro* and *in vivo*. In agreement with theory, results show a clear frequency dependence in the extent of the permeabilization of cell membrane. The results obtained show the ability of sine signals to perform electroporation and how by modifying the frequency of the oscillating electric field applied it is possible to accurately modulate the extent of permeabilization performed to the cell membrane.

1 Introduction

The interaction of the cell membrane with high intensity pulsed electric fields is routinely used to provoke the transient permeabilization of the cell membrane in a phenomenon termed electroporation. This technique has traditionally used short square direct current (DC) pulses. On the contrary, although theoretically described, few experimental reports have deeply studied the ability of alternating current (AC) signals to cause cell permeabilization [1]. According to Schwann equation, the induced transmembrane potential (ITP) by an oscillating electric field varies with frequency and it is negligible for frequencies above 1 MHz [2]. If the ITP reaches a given threshold, the cell membrane is electropermeabilized.

Nowadays, one of the main applications of electroporation is Electrochemotherapy (ECT)[3] . In this technique, reversible electroporation is applied to tumour tissue in order to facilitate the penetration of chemotherapeutic agents into cancerous cells. ECT represents a clinical option for the treatment of patients with superficial tumours with very successful results [4].

2 Methods

In order to correctly assess the frequency dependence of membrane permeabilization, it is necessary to concentrate the energy delivered to cells in a narrow spectral band. For that purpose, in the present study we used single sinusoidal bursts of different frequencies to study the frequency-dependent response of the cells in vitro. Afterwards, we applied the same concept to study the efficiency of ECT treatment in a model of subcutaneous tumour in mice.

3 Results

Results show how sinusoidal waves are suitable for performing electroporation of both cells and living tissues. The results demonstrate the expected frequency dependence and how by only modifying the frequency of the applied signal it is possible to control the extent of permeabilization. The electroporation efficiency shows a low pass filter frequency dependence with a loss of efficacy for increasing frequencies.

Additionally, the comparison with square waves allowed us to assess the equivalence between both types of wave forms. These results show that there exists a relation in terms of Root Mean Square (RMS) values.

4 Conclusion

Sine waves exposure represents an interesting and effective alternative for the electropermeabilization of biological tissues. The application of the energy to cell membrane in a narrow spectral band can help to better understand the still poorly known mechanism of membrane electroporation. Further research is necessary to explore the possible limitations or improvements of this type of electric field exposure

Our data suggests that depending on the electric field AC frequency it is possible to modulate the amount of permeabilization performed to cells. By only varying the frequency of the signal applied, instead of amplitude, duration or number of pulses we could control the extent of permeabilization performed in the biological sample exposed.

Références bibliographiques

[1] S. Katsuki, et al. “Biological Effects of Narrow Band Pulsed Electric Fields,” IEEE Trans. Dielectr. Electr. Insul., vol. 14, no. 3, pp. 663–668, 2007.

[2] C. Grosse and H. P. Schwan, “Cellular membrane potentials induced by alternating fields,” Biophys. J., vol. 63, no. 6, pp. 1632–1642, 1992.

[3] L.M. Mir, S. Orlowski, J. Belehradek Jr., C. Paoletti, Electrochemotherapy potentiation of antitumour effect of bleomycin by local electric pulses, Eur. J. Cancer 27 (1991) 68–72.

[4] Bertino, G., Sersa, G., De Terlizzi, F., Occhini, A., Plaschke, C. C., Groselj, A., … Benazzo, M. (2016). European Research on Electrochemotherapy in Head and Neck Cancer (EURECA) project: Results of the treatment of skin cancer. European Journal of Cancer, 63, 41–52. https://doi.org/10.1016/j.ejca.2016.05.001