PERIODICO di MINERALOGIA Volume 89, No. 1, 2020

Thermal effects during electroporation: theoretical and experimental considerations

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Abstract

Electroporation is based on interaction of a cell with an external electric field. This phenomenon is accompanied by side effects, like temperature rise, that could affect the experimental results. This paper contains some theoretical considerations and experimental results regarding thermal effects of electric field during electroporation with exponential pulses and bipolar electric pulses.

Keywords: Electroporation, thermal effects, bipolar electric pulses

Introduction

Cells exposure to strong electric field is leading to dramatic changes in transport processes across the cell membrane [1, 2]. The exact mechanism of these changes is not well known but is generally agreed that an elevated transmembrane potential creates pores in cell membrane [3, 4].

A direct relation between the electrical field and the induced transmembrane potential was established for a spherical cell [2-4]:

$$V = 1.5 * R * E * \cos\theta \quad (1)$$

where R = cell's radius, E = the applied electric field and θ the angle between E and the site on the cell membrane where V is determined.

Cells permeabilization using electric fields has already a lot of practical applications, for example drug delivery [5] or direct gene transfer into a large variety of cells and tissues [6].

The most important parameters characterizing an electroporation experiment are the electric field (shape and amplitude) and the application time. Other significant parameters, especially for electromediated gene transfer, are represented by electroporation buffer, cell or tissue type.

During electroporation, a consequence of the electric field application is the solution heating. The temperature rise could be a very important parameter, affecting the obtained results from an electroporation experiment. Nevertheless, this side effect was usually neglected, only few records about it being available.

It is well known that the electric field strength necessary for membrane poration is lower at higher temperatures [7]. On the other hand, a heat shock could be beneficial for genetic transformation purposes [8]. Other recognized effects of temperature rise are enthalpy effects [9, 10], solvent expansion and shock wave [9, 10]. A more sophisticated study of

thermal effects has been conducted by Pliquett [11], who found, in certain conditions, much larger solution heating than expected.

In this paper, some theoretical aspects and experimental results on thermal effects during electroporation are considered.

Materials and Methods

Electroporation experiments

Electroporation was carried out using electroporation cuvettes, having planar Al (aluminum) electrodes, 2 mm aperture (BioRAD, USA). The reason for choosing them was an anomalous heating observed during electroporation experiments using Al electrodes cuvettes [11].

Two types of electric pulses, one exponential and the other as an succession of bipolar electric pulses, are used for our experiments. The applied voltage varied in the range 0-1000V for the exponential pulses and, 0-600V for bipolar electric pulses.

Electroporation buffers

In order to observe the effect of ionic strength on thermal effects during electroporation, two different solutions were considered. First solution we used was 100 mM PBS (phosphate buffered saline) and the other one a non ionic solution (glycerol 15%).

Temperature recording

The temperature measurements have been performed using a very thin cooper – constantan thermocouple, placed at equal distance between the Al electrodes, into the electroporation buffer. The reference temperature for thermocouple was kept constant at 25 ^{0}C. The temperature recording started 20 ms after electrical discharge and only the maximum obtained temperatures were recorded.

Mathematical considerations

The electrical energy delivered by the electric pulse can be calculated as follows:

$$W = \int_0^T U(t) * I(T) * dt \quad (2)$$

Usually, exponential pulses are obtained discharging a capacitor (previously charged at U voltage). In this case, the electrical energy, neglecting other effects, will be:

$$W = \frac{1}{2} C * U^2 \quad (3)$$

where C is the capacity (F).

Considering only an ohmic heating, the temperature shift can be now easily obtained:

$$\Delta T = \frac{C * U^2}{2m * c} \quad (4)$$

where m is the mass and c the specific heat.

For a single square pulse, the temperature shift is given by:

$$\Delta T = \frac{U^2 * t}{m * c * R} \quad (5)$$

where t is the application time of the electric field (pulse width) and R the electric resistance.

The relationships 3-5 are obtained assuming only an ohmic heating and minor modifications of electroporation buffer resistance R during electroporation. Also, only the heat dissipation in the electroporation bulk was considered.

Results and Discussions

Exponential discharge

The simplest way to evaluate the heat generated by an exponential discharge is to consider only the ohmic heating of the solution. Assuming a specific heat of water (c= 4180 J/kg) as equal with the specific heat of our solutions, for 500 μl buffer amount, the relation between voltage and temperature will be:

$$\Delta T = 7.1*10^{-6} *U^2 \quad (6)$$

where U is the applied voltage.

For U = 1000 V, the maximum of temperature rise will be 7.1 ^{0}C. Of course, the thermal dissipation on Al electrodes was neglected. It is known that Al has a very good thermal conductivity and, for electroporation experiments, the contact surface with the electroporation buffer is very large, so the measured temperature could be lower than the estimated one. The results representing the temperature shift after electropulsing using exponential pulses having different amplitudes and non ionic buffer are showed in Figure 1. Indeed, the measured temperature was no more than 6.5 ^{0}C. This value is much closed to the estimated one, which means a uniform heating between the electrodes and no temperature gradients present.

A different situation was obtained using PBS as electroporation buffer. Although the relation (6) is no related to the chemical composition of the buffer or the ionic strength, a very large temperature shift, up to 23 ^{0}C, was obtained (Figure 1). This effect suggests another heating mechanism, located to the liquid-electrode interfaces rather than a purely ohmic heating. The same situation, using Al electrodes, was obtained by Pliquett [11], who observed large differences, about 30 ^{0}C, between the measured value and the value calculated from power consumption for a 4 mm aperture cuvette. Their explanation involves a thin oxide layer formation on the Al surface. The heat generated on this interface will be dissipated into electroporation bulk slower, so the maximum temperature will be achieved in few seconds after electroporation.

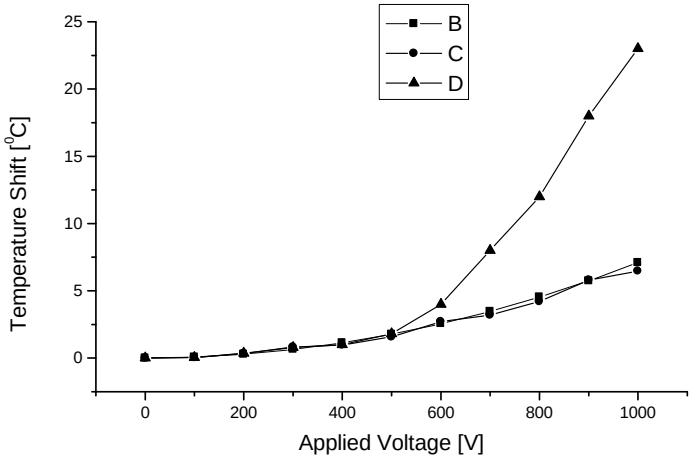

Figure 1. Temperature shift vs applied voltage. B - theoretical assumption, C -measured temperature for non ionic buffer, D- measured temperature for PBS buffer. All records are for exponential pulses.

Bipolar electric pulses

Unlike the unipolar electric pulses, the bipolar electric pulses produced less heat. The temperature shift was less than 2 ^{0}C (data not shown), both for non ionic and PBS buffer. The succession of bipolar electric pulses consists of very short (8 μsec) electric pulses, with opposite polarity, separated by a 10 μsec width. The total length of pulses train was fixed at 3.6 ms. The electric field will act effectiveness only 1.6 ms. Moreover, the bipolar electric pulses can be considered as alternative currents. Following the thin oxide layer formation at electrode interface, a large capacitance and resistance of this layer could be accepted. This capacitor has low impedance at high frequencies, so the entire voltage will be applied only to the bulk solution.

Conclusions

In some situation, the thermal effects during electroporation can not be neglected. Temperature rise of more than 30 ^{0}C are obtained in specific conditions. Prolonged exposure of cells to high temperatures decrease cell viability and this will be not an advantage for cell electrotransformation efficiency. This effect is strictly related to Al electrodes and oxidizing capacity of electroporation buffer. In order to avoid this drawback, use of bipolar electric pulses for electroporation could be preferred.

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