Delayed poration after ultrashort electric pulses on Jurkat cell

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Abstract: Electrical properties of cancer cells have been studied widely to understand cancer. The increase in electrical conductivity of Jurkat cell suspension after ultrashort electric pulses has been considered as an important phenomenon of the interaction between pulsed electric field and cancer cells. Experimental evidence showed that such an interaction may result in delayed poration. In this paper an attempt has been made to find probability distribution function of poration after ultrashort electric pulses. The conductivity data collected from nanosecond pulse electroporation of Jurkat cancer cells is analysed to form a probability distribution function for pore formation after nanosecond electric field pulse. It has been found that the parameter in the function depends on pulse characteristics such as pulse magnitude, pulse width and number of pulses.

1 INTRODUCTION

Understanding cancer has been an objective of molecular and cellular biologists for many years. A variety of different techniques have been developed. One such technique is to use a pulsed electric field to control the function of biological cells via electroporation process. Electroporation is the term used to describe the appearance of pores in cell membrane due to an elevated transmembrane electric potential caused by an applied pulse electric field. Typically, it occurs with pulse width on the order of 0.1-10 ms and electric field on the order of kV/cm. It is found that such pulses promote transient membrane poration and cell survival. Various applications [1] in biology, biotechnology and medicine have been found to utilize such interaction.

In recent years, intensive research [2-3] has been carried out using ultra short electric pulses. As the pulse width decreases, it has been experimentally demonstrated that interaction with plasma membrane decreases while interaction with intracellular structure increases. Hypothesis has been made that the short pulse mainly interacts with the nuclear membrane. Modelling work has been carried out to understand the mechanisms [4]. The phenomenon raises the possibility that ultra short electric pulse could be used to induce apoptosis in mammalian cells by affecting intracellular structures [5].

Our earlier result on electrical conductivity of Jurkat cell suspension after ultrashort electric pulses indicated a gradual increase [6]. This may be related to the interaction between the pulses and nuclear membrane via a delayed poration [7]. In this paper conductivity data collected from nanosecond pulse electroporation of Jurkat cancer cells is analysed to form a probability distribution function for pore formation after nanosecond pulse electroporation.

2 EXPERIMENTAL RESULTS

The increase in electrical conductivity of Jurkat cell suspension has been reported [6]. The detailed cell preparation is crucial for the experiment. The Jurkat cell line used in the experiment is derived from human T-cell leukaemia. The cells were cultured in RPMI-1640 Medium and centrifuged three times and then washed with sucrose/glucose buffer. Finally, the cells were adjusted to a 5% concentration. Sufficient cell suspension was transferred to a gene pulser cuvette for pulsing. After pulsed electric field application, we transferred a small amount to the electrode chamber for conductivity measurement. The cell viability was checked occasionally to ensure that pulsed electric field did not induce significant cell death. As the conductivity is temperature sensitive the sample temperature was maintained at 25°C by using a thermostat. Figure 1 summaries the conductivity results obtained from Jurkat cell suspension after various electric pulses.

It can be seen that single 10ns and 150 kV/mm pulse have very minor effect on the suspension conductivity. Therefore this set of result will not be dealt with in the present paper. For the other results it has been noticed that magnitude, pulse duration and number of pulses seem to have significant influence on the conductivity. The change in conductivity may be represented by

$$\sigma = \sigma_0 + \Delta\sigma_{max} F(t) \qquad (1)$$

where σ_o is the conductivity of suspension before the application of electric pulse, $\Delta\sigma_{max}$ is the difference between stable conductivity after electric pulses and σ_o, F(t) the cumulative function reflecting the interaction between electric pulses and cells. The origin of conductivity is ions present in the cell suspension. The increase in conductivity can be attributed to the extra ions released from cells during poration. As there are

several millions of cells in the suspension, each cell may react differently to the pulsed electric field. We c an assume that the time of poration shows a continuous distribution. Therefore probability of the distribution function f(t) should also be continuous. The function f(t) is an important characteristic that allows one to understand the interaction between the cell and electric field.

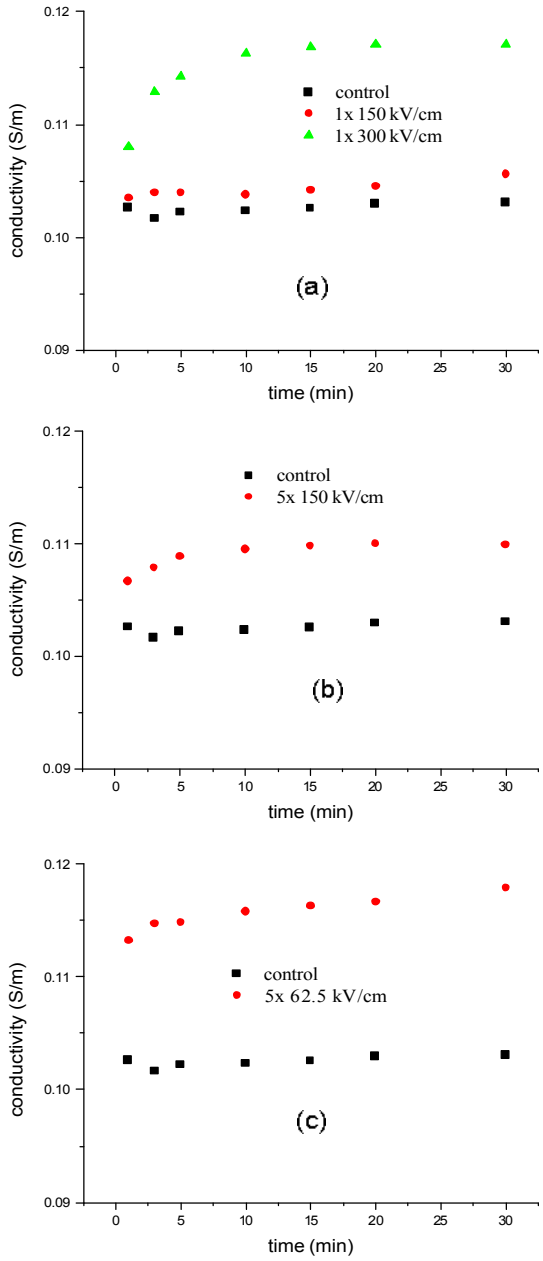

Fig.1: Conductivity in Jurkat cell suspensions reproduced from [6] with; a) single 150kV and 300kV 10ns pulses, b) 5 10ns 150kV pulses, b) 5 60ns 62.5kV pulses.

3 DETERMINATION OF PROBABILITY DISTRIBUTION FUNCTION f(t)

It has been recognised that the electroporation is typically followed by a process of pore sealing. Here we assume that a fixed amount of ions is released from a cell during its poration. To obtain function f(t), the following assumptions have been made:

(i) The total ions leak out from the cells are ΔN

(ii) The original number of ions in suspension is N_0

(iii) In terms of contribution to the conductivity no distinction among cells

Based on the observation on HL-60 cells after ultrashort electric pulse [7], the time of poration of each cell is different. The number of ions in the suspension at time t after pulses can be expressed by

$$N(t) = N_o + \Delta N \sum_{t=0}^{t} f(t)\Delta t \qquad (2)$$

It is clear that the increase in conductivity in suspension is cumulative effect. As the conductivity is proportional to the ions in the suspension, the more poration the more ions in the suspension and the higher the conductivity.

The cumulative distribution function F(t) is related to probability distribution function f(t) by

$$F(t) = \int_{-\infty}^{t} f(t)dt \qquad (3)$$

Various functions have been assumed for f(t), including normal distribution (Gaussian distribution) and exponential distribution, to get a better fit of conductivity data shown in Figure 1. It has been found that exponential distribution gives the best fit.

For a series of *r* points of data from *0* to *n* the data is differentiated using the method below where equation 4 shows the method used for most points of data and equations 5 and 6 show how the 2 end points are differentiated.

$$\frac{d\mathrm{S}(t_r)}{dt_r} = \frac{1}{2}\left[\frac{\mathrm{S}(t_r)-\mathrm{S}(t_{r-1})}{t_r - t_{r-1}} + \frac{\mathrm{S}(t_{r+1})-\mathrm{S}(t_r)}{t_{r+1}-t_r}\right] \qquad (4)$$

$$\frac{d\mathrm{S}(t_0)}{dt_0} = \frac{\mathrm{S}(t_1)-\mathrm{S}(t_0)}{t_1 - t_0} \qquad (5)$$

$$\frac{d\mathrm{S}(t_n)}{dt_n} = \frac{\mathrm{S}(t_n)-\mathrm{S}(t_{n-1})}{t_n - t_{n-1}} \qquad (6)$$

Time constant is derived by dividing the conductivity change after time t by the rate of change of conductivity at time t:

Time constant = (max conductivity-conductivity)/D-conductivity (7)

where D-conductivity is derived from data for conductivity differentiated using the three point numerical differentiation method shown above.

It has been found that a general form of probability distribution function is given by

$$f(t) = \frac{1}{\tau} e^{-\frac{t}{\tau}} \quad (8)$$

where τ is a time constant. The conductivity of the suspension is then given by

$$\sigma = \sigma_0 + \Delta\sigma_{max}(1 - e^{-\frac{t}{\tau}}) \quad (9)$$

There is a complicated relationship between the rate of conductivity increase and the pulse amplitude, duration and number. Increasing any of these decreases the time constant as shown in Table 1 below and also affects the final conductivity of the cell suspension.

Table 1 Time constant as a function of pulse amplitude, width and number

Pulse description	Time constant τ (min)
1x300kV/cm 10n	2.0282
5x150kV/cm 10n	1.7343
5x62.5kV/cm 60	1.3067

The delayed poration is believed to be a consequence of the interaction between electric pulses and intracellular structures.

4 DISCUSSION

The predicted data gives reasonably accurate interpretations of the experimentally found data. It can be seen that the time constant τ decreases for longer pulse high times, time constant may also be based on pulse amplitude.

The effects of ultra short pulses are thought to affect the intracellular membranes and structures rather than the cells membrane. This is because the charging times for internal structures are lower [8] where the charging time is given in equation 10.

$$t_c = (r_c + \frac{r_a}{2}) C_m \frac{D}{2} \quad (10)$$

Here τ_c is the charging time, ρ_c is the cytoplasm conductivity, ρ_a is the medium conductivity, C_m is the membrane capacitance and D is the diameter of the cell. If all other constants remain the same the charging time for the internal structures will be lower as D is lower, this however is an oversimplification as the internal structures will have different resistivities to the cytoplasm.

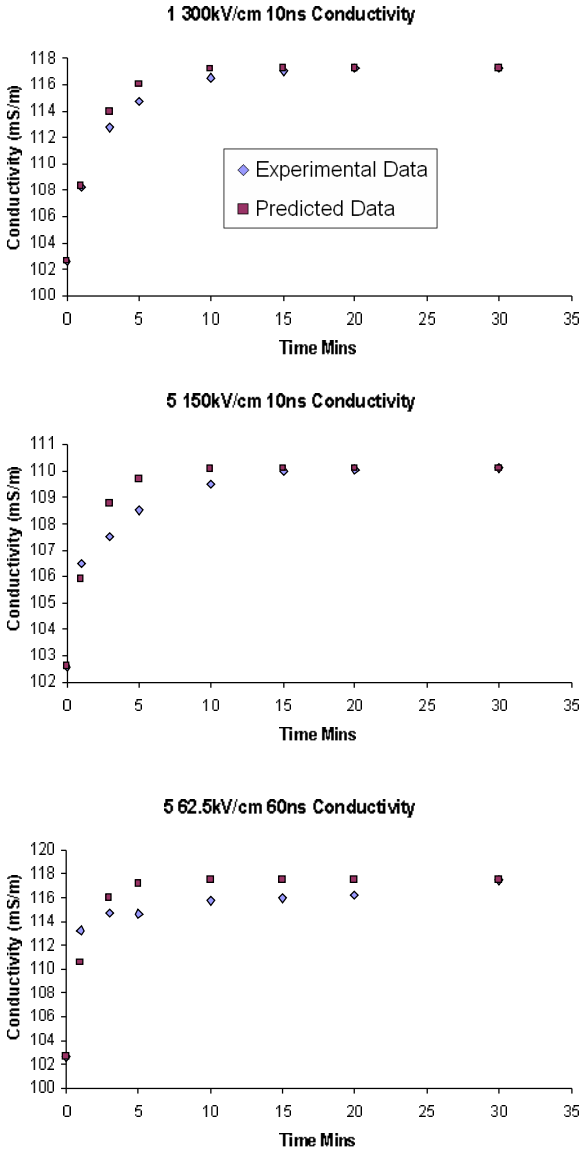

Fig.2: Conductivities of samples compared to statistically derived conductivities

For sufficiently short pulses of the orders of nano-seconds there will be insufficient time to charge the cells membrane however intracellular electroporation could still occur if the charging time is sufficient for the intracellular membranes. This effect is thought to trigger ion transfer within the cells and a rise in intracellular conductivity [6, 8] and further to this trigger nano-sized pore openings in the cell membrane. These effects are likely to cause an efflux of small ions, mostly Na^+ and K^+ through the cells membrane [6]

causing an increase in sample conductivity. Once this has happened the nano-sized pores in the cell are likely to close quickly causing no further change in suspension conductivity.

The rate at which this conductivity increase occurs could be related to the degree of intracellular electroporation. Longer pulses or a higher number of pulses causes larger pores to form in microsecond electroporation [9] if intracellular electroporation follows a similar mechanism the same could occur here causing a more rapid increase in intracellular conductivity and therefore the externally noticeable conductivity increase to occur faster. A further consideration is that with sufficiently long pulses electroporation will also begin to occur in the cells membrane. Similarly with multiple pulses the internals of the cell are more likely to be vulnerable to the repeated pulses [10] again causing internal electroporation to occur quicker and externally visible conductivity increases to occur more rapidly.

One possible explanation for the shape of the PDF could be related to the cell size, if cell size can be assumed to be distributed about a mean value D with a strong positive skew [11] and intracellular electroporation which causes this conductivity increase is based upon cell size for determining the charging time constants [8]; then the rate at which electroporation occurs within cells can be assumed to be distributed with a similar skew. This would cause conductivity to increase with a positive skew based upon the cell size distribution and the applied pulse characteristics. If this skew were strong enough then the distribution function could approximate to an exponential decay.

5 CONCLUSION

In this paper we have attempted to obtain a delayed electroporation distribution function after the ultrashort electric pulses. By analyzing the change in conductivity obtained from Jurkat cell suspension after ultrashort electric pulses it has been found that the delayed poration can be described by a probability of distribution function f(t) which takes a form of exponential decay. The time constant in the exponential decay function is related to the pulse characteristics such as pulse amplitude, pulse width and number of pulses applied. It may be possible to use the probability distribution function to evaluate the interaction between cells and the applied ultrashort pulse electric field.

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