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Electroporation-Induced Cell Lysis in SWLA-2 Hybridomas

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***Abstract*—This paper describes experimental results involving the percentage cell lysis in SWLA-2 murine hybridomas produced by square wave electric field pulses of 100, 200, and 300 V across a 1 mm gap width in a standard cuvette. Pulse lengths were of 0.2 and 0.6 ms duration; 1, 2, or 3 pulses were applied with 100 ms time interval between pulses. Cells were cultured and separate samples examined at 48 hours to determine cell mortality. Nearly 90% cell mortality was produced by applying 3 pulses at of 0.6 ms duration at 300 V.**

INTRODUCTION

In recent decades, electroporation technology has proven useful for a wide range of applications. In particular, *in vivo* delivery of molecules and genetic materials to eukaryotic cells has been carried out using electropermeabilization techniques with field strengths of less than lethal magnitudes [1]. Investigations of the role of amplitude, number, as well as duration of unipolar rectangular pulses have been provided in the comprehensive studies of [2-7]. These papers were largely oriented towards discovering the most important parameters related to successful permeabilization, rather than cell lysing. The studies described in this paper, using varied numbers of DC pulses of varying voltages, were performed to gain an understanding of the parameters related to lysing of eukaryotic cells.

I. MATERIALS AND METHODS

A. Cell line

The cell line used in this experiment was the SWLA-2 murine hybridoma (HB12560), purchased from ATCC [8]. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) with L-glutamine (0.8 mM), sodium pyruvate (1 mM), non-essential amino acids (0.1 mM), Hepes buffer (20 mM), and 10% fetal calf serum. The cells were grown at 37°C in 5% CO_2.

B Preparation

The hybridomas used in these experiments were prepared as follows prior to the experiments. The total number of cells was obtained by counting them in Neubauer's hemocytometer using Trypan blue stain 0.2%. Cells were centrifuged at 130 × g for 10 min. The supernatant was discarded and the pellet was re-suspended at 3 x 10^5 cells/ml.

C. Electrical Equipment and Cuvettes

Square waves of 0.2 and 0.6 ms, with voltages of 100 V, 200 V, and 300 V were applied by an ECM 830 Square Wave Electroporation System from BTX (a division of Genetronics, Inc., San Diego, CA). The number of pulses applied to the cells was also varied—one, two, or three pulses were applied. The time period between the pulses was 1 second. Eppendorf electroporation cuvettes with a 1 mm gap width and 100 µl volume were used to expose cells to the electric field. The voltage from the electroporation system was applied across the 1 mm gap width of the cuvette. As noted in [7], the voltage-to-distance ratio (VDR) of the voltage delivered to the electrodes divided by the distance between them is a reasonable estimate of the electric field between the plates.

D. Determination of percentage of viable cells

The cells were inoculated into a 24-well plate (100 µl cells/well) containing 2 ml of culture media. Each experiment was then repeated on a subsequent sample (cuvette) and added into an adjacent well on the plate. The number of viable cells was determined by counting the number of visible cells in three pre-determined areas of the well. The cells were counted at 48 hours post exposure to the electric fields. The cell numbers were then averaged for each well. The cell counts were relative to control cells that had not been exposed to electric fields. Each experiment (consisting of two readings for each data point) was repeated on two separate days. Thus, four readings were ultimately averaged to obtain the final data shown here.

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II. Results and Discussion

TABLE I
PERCENT CELL LYSIS AS A FUNCTION OF PULSE PARAMETERS

Number of pulses	Pulse duration	100 V	200 V	300 V[a]
1	0.2 ms	0.74	0.52	0.33
2	"	0.51	0.37	0.27
3	"	0.47	0.31	0.24
1	0.6 ms	0.50	0.33	0.20
2	"	0.43	0.23	0.16
3	"	0.33	0.19	0.13

Results for 0.2 and 0.6 ms pulse lengths are shown in Table 1, and illustrated Figs. 1 and 2.

Fig. 1. Normalized fractional survival rate for SWLA cell line (0.2 msec. pulse width)

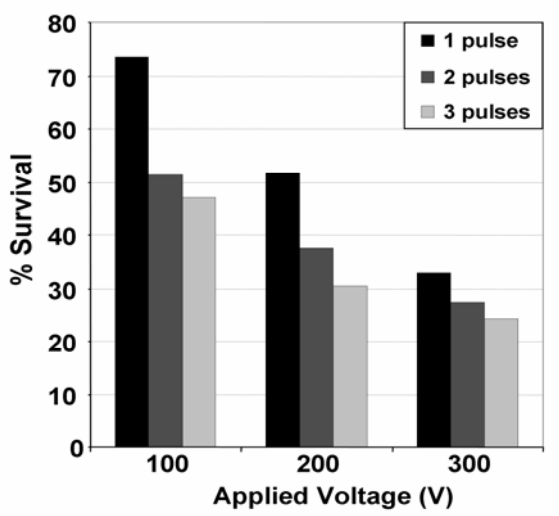
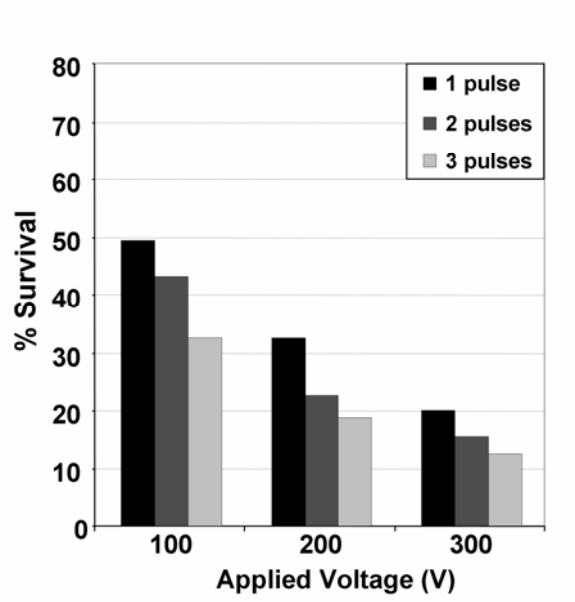


Fig. 2. Normalized fractional survival rate for SWLA cell line (0.6 msec. pulse width)



These results reveal a 90% lethal estimate electromagnetic field strength, $VDR_{90\%,}$ of approximately 3,000 V/cm when three 0.6 ms pulses were supplied. The value of $VDR_{90\%,}$ is somewhat higher than that reported by [7] for DC3F cells, a line of spontaneously transformed Chinese hamster fibroblasts. Kotnik *et al* reported a $VDR_{90\%,}$ of approximately 1100 V/cm, but 8 square wave pulses of 1 ms each were applied to attain this lower value.

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