

Design and Construction of a Programmable Electroporation system for Biological Applications

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Abstract-Studies into electroporation have grown rapidly in biotechnology and medicine in recent years. This paper presents the design and construction of a low cost programmable electroporation system for biological applications. The system consists of a control module, a pulse generation circuit and a high voltage switch using a power MOSFET. The programmable electroporation has been designed, developed and tested. Using a standard commercial electroporation cuvette, it is possible to generate electric fields of 100 to 1000V/cm with programmed pulse lengths of 10 μ sec to 20msec. The system was evaluated with Hela cells and propidium dye to evaluate transfection rates under a variety of electroporation conditions. Initial results showed that the electroporation system achieved a peak cell transfection efficiency of 48.74% at 600V/cm with pulse lengths of 10 ms.

Keywords - Electroporation, biological applications, Hela cell

I. INTRODUCTION

The use of electroporation systems in biotechnology and medicine has lead to new methods of cancer treatment, gene therapy, drug delivery [1-3]. The main purpose of electroporation is to apply electric field to open pores in the cell membrane and facilitate the delivery of foreign materials inside the cell, or kill the cell completely. It has been used to insert genes and dyes into mammalian cells. Thus the electroporation system is an important mechanism for cell therapy, genetic therapy and drug delivery [4, 5].

Electroporation typically uses high voltage pulses of microsecond to millisecond duration. Optimum electroporation parameters will vary depending upon cell type and purpose. For example, electric field strengths of 1000V/cm and 100 μ sec pulses are used for drug delivery and low voltage and longer pulses, such as 200 V/cm, 20-50 msec are used for gene therapy. It is therefore desirable to be able to control voltage levels, duty cycles and pulse durations. Commercial systems with this level of functionality are expensive and most these use rectangular pulse shapes which have been shown to be the most effective at achieving poration [5]. For example, the commercial electroporation ECM 830 made by BTX Harvard Apparatus [6] can gives two

mode operations. Firstly, High voltage mode is ranged of 30 volt to 3000 volt and the pulse length of 10 μ sec to 600 μ sec (1 μ s resolution). The second mode is low voltage which gives output voltage of 5volt to 500Volt and pulse length of 10msec to 999msec (1ms resolution). This paper describes a simple programmable electronic circuit that can vary pulse duration, frequency and number of pulses. It also includes power metal-oxide-semiconductor field-effect transistors (MOSET) to switch the high voltage (up to hundred of Volts) and generate the pulses. The system is possible to generate electric fields of 100 to 1000V/cm with programmed pulse lengths of 10 μ sec to 20msec (1 μ s resolution). Such an electroporation system presents a very cost effective system that can be used in the biological applications described above.

II. ELETROPORATION SYSTEM DESIGN

Fig. 1 shows the optimum pulse width and electric field for a range of biological applications. For applications involving cell poration optimum field strength is in the range of 1 to 2 KV/cm. For drug delivery applications the optimum pulse width should be around 10⁻⁵ sec and for gene therapy 10⁻³ sec [7]. It is clear a range of electric field and pulse width are required. The system presented here is able to adjust the pulse length from 100 μ sec to 10 msec and the system can support several of electric field from 100 V/cm to 1000 V/cm.

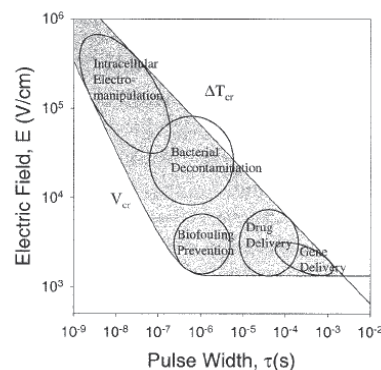


Figure 1 Range of electric field and pulse width for biological applications [7]

Cell poration is shown diagrammatically in Fig. 2. A cell membrane is a lipid bilayer of cell membrane which can be opened as shown by a short pulsed electric field to form pores.

To determine the electric filed (E), is simply given by equation (1).

$$E = \frac{V}{d} \quad (1)$$

Where V is an applied voltage
 d is a distance between two electrodes

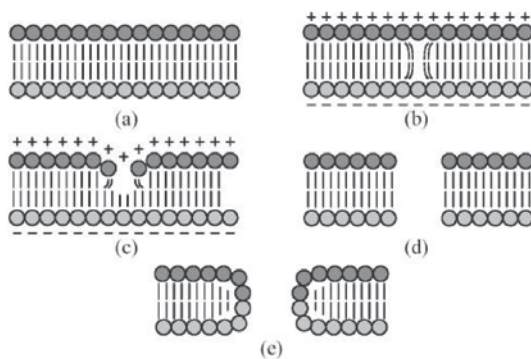


Figure 2 Process of pore formation (a) normal cell membrane, (b) a cell excited short electrical pulse resulting in irregular molecular structure (c) the membrane being method (d) the cell with a temporary hydrophobic pore and (e) the cell with a membrane restructuring [7]

The block diagram of the electroporation system is shown in Fig. 3. It includes five stages comprising a pulse generator programming, an inverter/driver, a high power switch, a high voltage power supply and a load (electroporation cuvette). To control the parameters of the pulses and the number of pulse it was decided to use

a microcontroller. The microcontroller produces square wave pulses and it can control pulse width between 10 μ sec and 20 msec. In addition, the user can program any sequence of pulses. The criteria for choosing the microcontroller are a fast clock frequency which would allow a high rate of instruction per second and a sufficient number of input/output pins. The PIC16F84 microcontroller by Microchip Technology Inc. was selected [8]. The PIC 16F84 has a clock frequency of 4MHz, ability to execute a 200 nsec instruction cycle and an offers an instruction set consisting of 35 single word instructions. The MPLAB IDE v7.5 software and MPLAB ICD2 in circuit debugger was used to initially program the microcontroller.

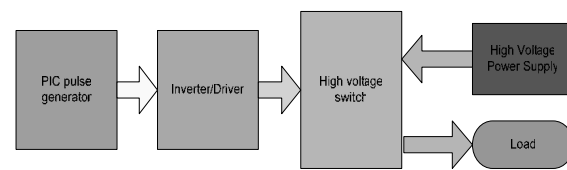


Figure 3 Block diagram of electroporation system

In use, the electroporation parameters are input by the user through two push button switches. The microcontroller monitors these inputs push button and updates the parameters on an LCD screen for display. In operation the microcontroller generates square wave pulse at port RB0. The square wave pulses have amplitude of 5 V which is connected to the transistor driver. The entire circuit is shown in Fig. 4. Thus the square wave pulse can be precisely defined by the user. The pulse generator can output either a single short pulse or a number of multiple pulses.

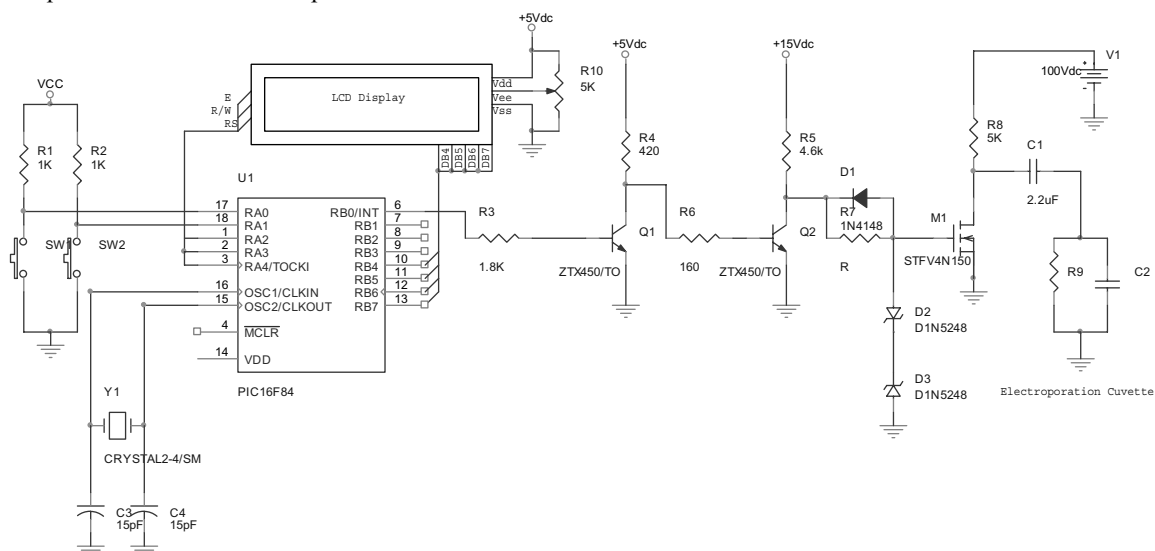


Figure 4 the schematic of an electroporation system

The Inverter and driver are designed using transistors (ZTX450). The function of the inverter circuit is to invert the 5 volt signal from the pulse generator. The function of transistor driver circuit is to convert the inverter circuit output to 15 volt. The driver circuit is used to switch MOSFET which control the high voltage. The idea MOSFET for this research would able to survive a drain source breakdown voltage of 1500 V and it has a drain current of 4 A. Furthermore, the high power MOSFET must be able to turn on and turn off quickly and have a low on-state resistance. Thus, the MOSFET (STFV4N150) is used in the circuit. In addition, the MOSFET has an on static drain source resistance 5 Ω and up to 7 Ω in the maximum. The STFV4N150 has an input capacitance of 1300 pF that must be charged and discharged in order to turn on and turn off. The MOSFET will rapidly charge and discharge when the square wave pulse from the driver circuit become to the gate of the MOSFET. It can provide a pulse width of a few micro-second or a continuous dc supply. In addition, the high voltage supply current will pass through a 5 k Ω resistor which acts as a current limit to protect the MOSFET by damping the voltage during the turn on time. When the 15 volt source is applied, the high voltage supply is switched on and used to charge a 2.2 μ F capacitor. The output of the capacitor is then discharged across a 1 mm standard commercial cuvette with electrical fields of between 1 V/cm to 1KV/cm.

The gate of MOSFET should not exceed ± 18 V which keeps the gate safe between ranges of ± 18 V. The MOSFET is protected by two zener diodes (1N5248). The diode (1N4148) is chosen because of its fast reverse recovery time of 4ns. The function of diode is to allow current bypass the resistor so that the MOSFET turn off fast. The MOSFET is turn off state, the capacitor (2.2 μ F) will be charged and storage an energy high voltage. When the MOSFET is switched on, the capacitor produces negative pulse voltage across the load.

III. METHOD AND EXPERIMENT ELECTROPORATION CIRCUIT

This section describes the performance of the circuit. The practical output of the electroporation system is also compared simulation of prediction of voltage output. In order to understand the performance of the electroporation circuit, it was simulated in order to determine efficiency and voltage output. Simulation performed in PSpice Design manager program version 9.2.1. The circuit was evaluated with various input voltages in the range input voltage of 10Vdc and 100Vdc. The simulation results are given in table 1 where they are compared with measured values. The efficiency (η) of the electroporation circuit is given by equation (2)

$$\eta = \frac{V_{\text{output}}}{V_{\text{Input}}} \times 100\% \quad (2)$$

Voltage Input (Vdc)	Simulation (Vdc)	Measured (Vdc)	Efficiency (η) of simulation (%)	Efficiency (η) of measured (%)
10	9.97	9.4	99.7	94.0
20	19.94	18.6	99.7	93.0
30	29.92	27.6	99.7	92.0
40	39.89	38	99.7	95.0
50	49.86	46.8	99.7	93.6
60	59.83	56	99.7	93.3
70	69.80	64	99.7	91.4
80	79.78	70.4	99.7	88.0
90	87.75	76.8	99.7	85.3
100	99.72	84.0	97.5	84.0

Table 1 the efficiency of simulation and measured of electroporation circuit

It can be seen that the simulated efficiency is consistently about 99.7%, whereas the measured efficiency depends on the input voltage. The efficiency of the circuit falls with measured output voltage as shown in Fig.5.

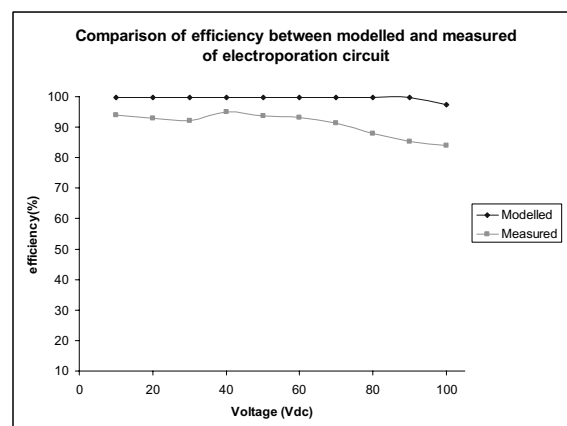


Figure 5 comparisons between simulated and measured

IV. THE OUTPUT WAVE FORM

This section presents the output waveform from the electroporation circuit where connected to the load. The output signal of electroporation circuit at 10 V and 100 V is shown in Fig. 6 and Fig. 7, respectively. A Tektronix TDS2002 Oscilloscope (60 MHz, 1GS/S) was used to capture the output waveform.

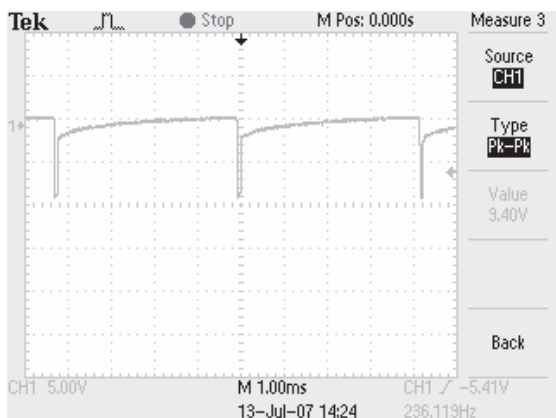


Figure 6 Output wave form of measured: Output voltage 9.4V. Input voltage 10 V. Vertical scale: 5V/division. Horizontal: 1 ms/division



Fig 7 Output wave form of measured: Output voltage-102 Volts. Input voltage 100 V. Vertical scale: 20V/division Horizontal: 100μs/division

V. EXPERIMENT PROCEDURE

The performance of the electroporation system was evaluated by determining the transfection rate of Human cervical cancer (Hela cells). These were mixed propidium iodide in an electroporation cuvette. A haemocytometer (Hausser Scientific, Horsham, PA) and a fluorescent microscope (Axiovert 200 ZEISS) were used to observe the transfection rate. Hela cells are one of the most well know cell line and can be rapidly grown in suspension. Hela cells were grown in (10 ml) suspension, and then incubated at 37 °C. Electroporation was performed at room temperature, 25°C, by introducing 0.3 ml of the HeLa cell suspension into a 1 mm gap cuvette (BTX, Holliston, MA, USA). Then 10μg/ml of the viability stain propidium iodide was added into the electroporation cuvette. Cuvettes were used only one time per test and pulse lengths were applied using the electroporation circuit. The pulse lengths of 5 ms and 10 ms, and field strengths in the range from 100 V/cm to 1000 V/cm were applied. After electroporation, cells were incubated at 37 °C. To determine the successful transfection cells, the

haemocytometer and fluorescent microscope were used to count the number of Hela cells demonstrating dye uptake. The percentage of uptake dye into the Hela cells was determined and is shown in Fig.8. Therefore, the circuit can be used to achieve electroporation with Hela cells. This experiment then identified the optimum parameters for the pulse length and electric field for cell transfection as discuss as in the next section.

VI. DISCUSSION OF RESULTS

A. The effect of electric pulses length on transfection rate

Fig. 8 shows the effect of pulse length on cell transfection rate. A pulse length of 10 ms rate was found to have a transfection rate approximately 10% better than of 5 ms pulses. Therefore, cell transfection rate does depend upon the electric pulse length.

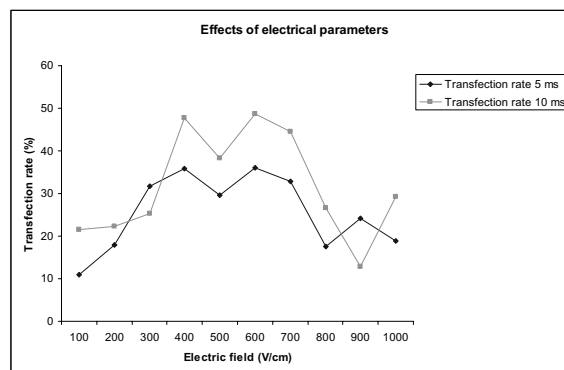


Figure 8 Effects of electric field parameters

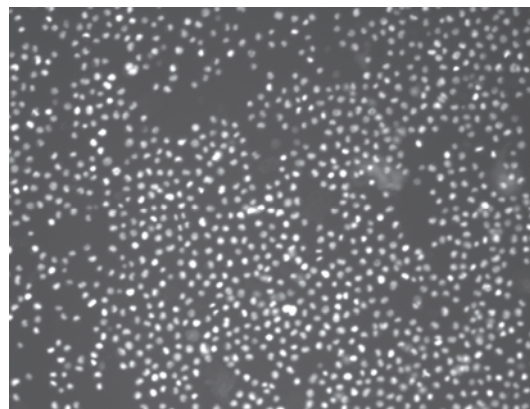


Figure 9 HeLa cell before electroporation

B. The effect of electric field on the transfection rate

The optimal electric field strength for electroporation may vary depending on the cell type. For Hela cells, electric fields in the range of 400V/cm to 700V/cm yield the highest transfection rates as shown in Fig.8. Peak cell transfection rate was 48.74 % at 600V/cm, pulse length 10ms. Transfection rates considerably decrease in electric fields above 700V/cm. This induces cell death

and reduces the overall transfection rate. Fig. 9 shows HeLa cells before electroporation and Fig. 10 shows the successful of transfection HeLa cells after electroporation.



Figure 10 the result of transfection HeLa cells (0.5 μ l) after electroporation (electric field strength 600 V/cm, pulse width length 10 ms)

VII. CONCLUSION

The programmable electroporation system has been designed, developed and tested. The simulated voltage output from the electroporation circuit was comparable with the measured results. The system was used to determine the effects of pulse length and electric field upon electroporation. These were explored with HeLa cells and propidium dye. For HeLa cells, the optimal electric field was in the ranges of 400V/cm to 700V/cm. A pulse length of 10 ms was found to be preferable to 5 ms, and when combined with a field strength of 600V/cm a peak transfection rate of 48.7% was achieved. Therefore, the system can potentially be used in biological applications such as gene therapy and drug delivery

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