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Electroporator for *in vitro* cell permeabilization

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The use of high voltage electric pulse technology, electroporation, in cell biology, biotechnology and medicine has attracted an enormous interest. Electroporation is a transient phenomenon that increases the permeability of cell plasma membrane. In the state of high permeability, the plasma membrane allows small and large molecules to be introduced into the cytoplasm, although the cell plasma membrane represents a considerable barrier for them in its normal state. The effectiveness of electroporation depends on many parameters that can be divided into the parameters of the electric field and the parameters that define the state of cells and their surrounding, i.e. temperature, osmotic pressure, etc. In this article, we present a prototype electroporator ***GT-1*** *for in vitro electropermeabilization that we have developed. Our electroporator offers a vast flexibility of parameters and can generate high and low voltage pulses, of which the latter ones are used for electrophoretic transfer of charged molecules through permeabilized cell plasma membrane.*

Key words: electroporation - instrumentation - methods; cell membrane permeabilization

Introduction

Viability of a cell depends on the integrity of its plasma membrane. The plasma membrane prevents the exchange of substances between intracellular and extracellular spaces. Exposing the cell to the electric field can increase the permeability of the cell plasma membrane. The increased permeability of the plasma membrane allows small and large molecules to be introduced into the cytoplasm. This phenomenon is transient and is termed electropermeabilization and often also referred to as electroporation. The electroporation is widely used in different medical and biological applications *i.e.* electrochemotherapy, transdermal drug delivery and gene transfection. The effectiveness of all these applications depends on many parameters that can be divided into the parameters of the electric field and the parameters that define the state of cell and its surrounding, *i.e.* temperature, osmotic pressure, conductivity of cytoplasm and extracellular fluids etc. With respect to this, optimal parameters of electroporation have to be used to achieve best efficiency of the method.[1]

For *in vitro* experiments, where most commonly used electrodes are parallel plates with a 2 mm inner distance, the threshold voltages typically range from 120 V to 300 V[1,2], with

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the pulse durations from several microseconds to several milliseconds.[1,3] Beside this, it is often necessary to deliver more then one pulse to increase efficiency of permeabilization. In that case, pulses must be delivered in a certain period requiring repetition frequencies from 1 Hz to several hundred Hz.

All these demands are fulfilled by special devices, often referred to as electroporators. Nowadays, there are a lot of commercially available electroporators that are designed for *in vitro* experiments. The problem of most of these electroporators is that the flexibility of the parameters of electric pulses is not sufficient, especially if we want to study the effects of different pulse parameters on the cell permeabilization, survival or average uptake of different molecules.

In this paper, we present the design of electroporator for *in vitro* cell plasma membrane permeabilization. The device is operated by an internal computer that allows the user to choose the parameters of electric field. The computer drives the pulse generator that is composed of a digital pulse generator generating the signal, high voltage amplifier amplifying the signal, and current amplifier that provides the signal with sufficient energy to prevent the voltage amplitude from dropping during the pulse delivery. Beside this, the last version of the developed electroporator also includes a special unit that generates low voltage pulses usually used for the electrophoretic transfer of the charged molecules through the permeabilized cell plasma membrane.

System design

Figure 1 shows the basic system design of the electroporator. It consists of a computer, pulse generator, voltage amplifier, current amplifier and low voltage pulse generator. Besides this, the device comprises two high voltage power supplies and several low voltage devices that are necessary for normal operation but are not drawn on the figure.

The internal computer of the device is in the first place used for selecting pulse parameters. Therefore, the computer includes a user interface composed of a display and a keyboard. The second task of the computer is to control pulse generation after activation has

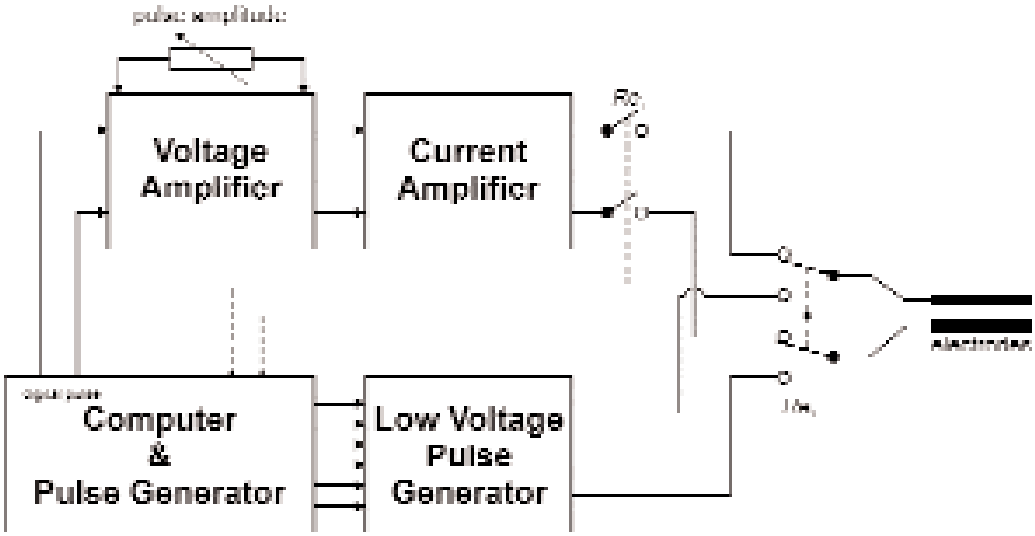

Figure 1. Block diagram of *in vitro* electroporator **GT-1**.

been triggered. All pulse parameters, except pulse amplitude, are then transferred to the pulse generator. This subunit generates digital signal that is then amplified in the voltage amplifier to the value that we set by external potentiometer. The amplified signal is then intensified by the energy from the current amplifier because we have to fulfill the energy requirement as defined by the load between electrodes.[4] At this point, the generation of the high voltage signal that is used for permeabilization of cell plasma membrane is finished.

The low voltage pulse generator, which we have constructed just recently, is used for the electrophoretic transfer of charged molecules through permeabilized cell plasma membrane. The structure of this subunit, controlled by the internal computer, is similar to that of the DC power supply. This version allows to change the amplitude in 10 V steps in a range from 0 V to 50 V.

Performance and experimental results

The developed *in vitro* electroporator, which is still a prototype, has been used in our laboratory for more than two years. During that time, we found and repaired some deficiencies in design and we also made several other improvements that reflect on greater flexibility of the parameters. The current prototype **GT-1** allows the user to set high and low voltage pulses in a range that is given in Table 1. The pulse repetition frequency f is calculated by using the following equation:

$$f = \frac{1}{T \cdot (1 + D_R)}, \qquad (1)$$

where T is pulse duration and D_R is value of the parameter Delay/Pulse ratio.

Furthermore, to demonstrate the performance of the device we designed an experiment where we measured voltage and current on the output (Fig. 2). In the experiment, we exposed 50 µl of pure SMEM, which was placed between 2mm plate electrodes, to five consecutive 100 µs pulses of 500 V at a repetition frequency of 2.5 kHz (D_R = 3).

Table 1. Output parameters of the developed in vitro electroporator

	Parameter	Value		
		Min.	Max.	Increment
HIGH VOLTAGE PULSES	Pulse duration	5µs	5000µs	1µs & 50µs
	Delay/Pulse ratio*	3	65535	1
	Pulse amplitude	25V	500V	linear
	Number of pulses	1	128	1
	Pulse current		30A	
LOW VOLTAGE PULSES	Pulse duration	5ms	9999ms	1ms
	Delay duration	0ms	9999ms	1ms
	Pulse amplitude	0V	50V	10V
	Number of pulses	1	1000	1
	Pulse current		1A	

* - parameter defines pulse repetition frequency that is calculated by the equation (1).

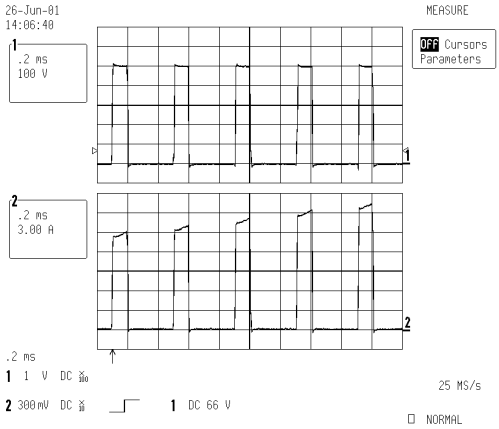

Figure 2. Performance of electroporator loaded with 50 µl drop of SMEM that was placed between 2 mm plate electrodes. Electroporator was programmed to generate five consecutive 100 µs pulses of 500 V and repetition frequency of 2.5 kHz (D_R = 3). Top trace (signal 1) presents voltage and bottom trace (signal 2) presents current flowing through the system. The measurements were performed using LeCroy LT9310C digital oscilloscope, a LeCroy AP015 current probe, and a Tektronix P5100 1:100 voltage probe. The voltage was 500 V, while current increased from 14 A to 19 A.

It is evident from the Figure 2 that the instrument was able to deliver all the pulses without any distortion. Even more, it could easily increase the current between two consecutive pulses that is usually necessary due to the polarization of the sample between the electrodes.

To compare the performance of the prototype **GT-1** with that of Jouan GHT 1287B, we evaluated cell permeabilization, using a bleomycin method[5], with both devices. The results of experiments that are shown on Figure 3 are comparable. T-test showed no statistically significant difference between both experiments.

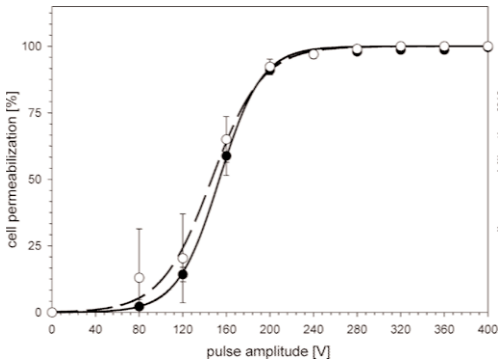

Figure 3. Comparison of cell permeabilization as a function of pulse amplitude obtained with electropulsator Jouan GHT 1287B (●), and *in vitro* electroporator **GT-1** (○).

Besides the performance experiments that we carried out, our colleagues already published the results of the experiments using the developed electroporator.[6,7] The two experiments were performed to study the influence of the parameters of electroporation (medium conductivity [6] and pulse repetition frequency [7]) on the cell permeabilization, survival and average uptake.

Conclusions

The comparison of the developed prototype **GT-1** with the commercially available device Jouan showed that **GT-1** fulfils all demands of the *in vitro* investigations. Furthermore, it offers a vast flexibility of the parameters and has the ability to generate high and low voltage pulses, where low voltage pulses can be used for the electrophoretic transfer of charged molecules through permeabilized cell plasma membrane.

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