Electropermeabilization detection with propidium iodide in plated cells L3

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Duration of the experiments: 60 min
Max. number of participants: 4
Location: Cell Culture Laboratory 2
Level: basic

PREREQUISITES

Participants should be familiar with Laboratory safety (S1). No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

When the cell is exposed to the external electric field of sufficient amplitude and duration, its membrane is electroporated and becomes permeabilized for the molecules that otherwise cannot pass cell membrane. Increasing amplitude of electric field increases the level of cell membrane permeabilization and the number of cells that are permeabilized (Figure 1). During the state of cell membrane permeability the molecules in cell surroundings (*i.e.* drugs, fluorescent dyes) can enter the cytoplasm by diffusion which is dependent on the concentration gradient. After some time cell membrane reseals and we obtain the cell with entrapped molecules. Electroporation is nowadays widely used in medicine, e.g. electrochemotherapy, non-thermal irreversible electroporation as a method of tissue ablation, gene therapy, DNA vaccination and transdermal drug delivery, as well as in biotechnology and food processing.

The electroporation efficiency can be monitored by incorporation of fluorescent dyes into the cell and is strongly affected by the amplitude of electric field. For this purpose different fluorescent dyes that cannot enter intact viable cell, such as Lucifer Yellow, Yo-Pro-1, Propidium Iodide (PI) can be used to determine the effect of different parameters of electric pulses on the level of cell membrane permeabilization. When PI enters the cell it binds to double stranded DNA and/or RNA by intercalating between basepairs with little or no sequence preference. When PI is bound, the fluorescence excitation and emission maxima are shifted which is known as the Stoke's shift. The excitation maximum is 535 nm and the emission maximum is 617 nm. Bound PI is highly fluorescent (20-fold higher than unbound PI) and the fluorescence can be measured.

In the experiment, Gemini X2 electroporator (Hardvard apparatus BTX, USA) will be used. It can produce square and exponential pulses. During the experiment current will be monitored with an oscilloscope and a current probe.

The aim of this laboratory practice is the demonstration of the relationship between cell membrane electropermeabilization and electric pulse amplitude using fluorescent dye and spectrofluorometer.

EXPERIMENT

We will detect electropermeabilization spectrofluorometrically using fluorescent dye PI. The effect of the pulse amplitude on the degree of cell membrane permeabilization will be determined for a train of eight 100 µs rectangular pulses delivered with the repetition frequency 1 Hz. The number of fluorescent cells that are consequence of efficient electroporation increases with increasing pulse amplitude is presented in Figure 1.

Protocol: You will use Chinese hamster ovary cells (CHO), plated previous day in 24 well plate in concentration 2.5 x 10^5 cells per well. Cells are attached to the culture dish surface. Immediately before electric pulses are applied replace the growth medium with electroporation buffer containing 0.15 mM PI. As electroporation buffer you will use isotonic 10 mM K_2HPO_4/KH_2PO_4 containing 1 mM $MgCl_2$ and 250 mM sucrose with pH 7.4. You will use wire electrodes 4 mm apart. Electric field between wire electrodes can be approximated by E=U/d, where U is the applied voltage and d is the distance between the electrodes.

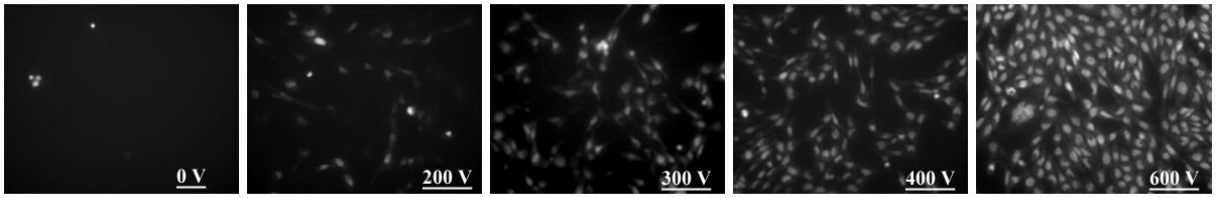

Figure 1: The sequence of the fluorescence images of attached cells obtained after cells were exposed to electric pulses with increasing pulse amplitude, according to the protocol described: from negative control (on the left) to maximum permeabilization (on the right). The images were obtained by fluorescence microscopy under 20 × objective magnifications.

Remove the 24 well plate from the incubator and replace the growth medium with electroporation buffer containing PI (300 µl/well). Apply electric pulses and leave the cells for 3 minutes at room temperature than replace the buffer with 1 ml of fresh electroporation buffer to stop the influx of PI into the cells. Electric pulse parameters are: 8 pulses, 100 µs duration and pulse repetition frequency 1 Hz, while pulse amplitude increases gradually. Increase the pulse amplitude from 0 V (negative control) to 200 V, 300 V, 400 V, 500 V and 800 V (positive control). 800 V was previously determined as a suitable positive control for these pulse parameters, since it permeabilizes all the cells. You will determine the fluorescence intensity for different pulse amplitudes spectrofluorometrically (Tecan, Infinite 200). Set the appropriate excitation (535 nm) and emission (617 nm) wavelengths for PI. Calculate the percentage of permeabilized cells for a given pulse amplitude from the data obtained. The negative control represents no permeabilization while the positive control represents the highest possible permeabilization.

FURTHER READING:

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Sixou S, Teissie J. Exogenous uptake and release of molecules by electroloaded cells: a digitized videomicroscopy study. *Bioelectrochemistry and Bioenergetics* 31: 237-257, 1993.

Marjanovič I, Kandušer M, Miklavčič D, Manček Keber M, Pavlin M. Comparison of flow cytometry, fluorescence microscopy and spectrofluorometry for analysis of gene electrotransfer efficiency. J. Membrane Biol. 247: 1259-1267, 2014.

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NOTES & RESULTS

voltage [V]	0	200	300	400	600	800
Electric field [kV/cm]	0	0.50	0.75	1.00	1.5	2.00
raw data [R.F.U.]						
permeabilization [%]						

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