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 the Safety rules and Rules for sterile work in cell culture laboratory. 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 biotechnology and in clinics for electrochemotherapy of tumors. Electroporation is also prerequisite for cell electrofusion, a promising method for production of monoclonal antibodies and cell vaccines.

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 or Propidium Iodide (PI) can be used to determine the effect of this parameter on the level of cell membrane permeabilization.

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

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 apply electric pulses with electric pulse generator Cliniporator™ (Igea, Italy) and you will use wire electrodes 5 mm apart.

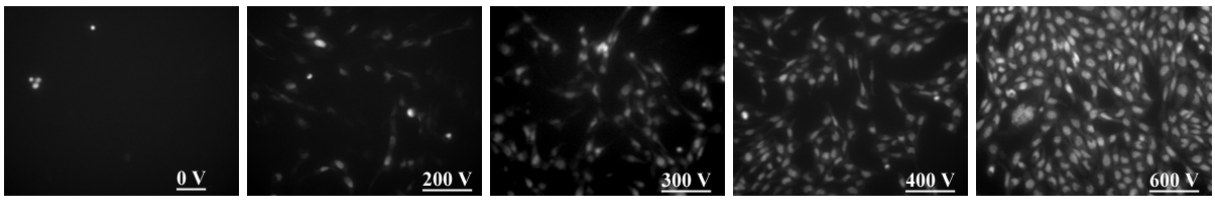

Figure 1: The sequence of the fluorescence images of attached cells obtained after cells were exposed to electric pulses with increasing pulse amplitude, according to the protocol described: from negative control (on the left) to maximum permeabilization (on the right). The images were obtained by fluorescence 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 to 5 minutes at room temperature than replace the buffer with 1 ml of fresh electroporation buffer. Electric pulse parameters are: 8 pulses, 100 µs duration and 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). 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:

Cemazar, M, Jarm T., Miklavcic D, Macek Lebar A., Ihan A., Kopitar N.A., Sersa G. Effect of electric field intensity on electropermeabilization and electrosensitivity of various tumor cell lines in vitro. *Electro and Magnetobiology* 17: 263-272, 1998

Puc M., Kotnik T., Mir L.M., Miklavcic D. Quantitative model of small molecules uptake after in vitro cell electropermeabilization. *Bioelectrochemistry* 60: 1 – 10, 2003

Rols M.P. Electropermeabilization, a physical method for the delivery of therapeutic molecules into cells. *Biochim. Biophys Acta* 1758: 423-428, 2006.

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

NOTES & RESULTS

voltage [V] (E [V/cm])	0 (0)	200 (400)	300 (600)	400 (800)	500 (1000)	800 (1600)
raw data [R.F.U.]						
permeabilization [%]						