Electropermeabilization detection of attached cells with propidium iodide 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 aseptic 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, flourescent dyes) can enter the cytoplasm by diffusion which is depandent on the concentration gradient. After some time cell membrane reseals and we obtain the cell with entraped molecules. Electroporation is nowadays widely used in biotechnology and in clinics for electrochemotherapy of tumours. 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 flourescent dyes into the cell and is strongly affected by the amplitude of electric field. For this purpose different fluorescence dyes impermeant for intact viable cell, such as Lucifer Yellow or Propidium Iodide can be used to determine the effect of this parameter on the level of cell membrane permeabilization.

The aim of this practical exercise is the demonstration of the relationsheep between cell membrane electropermeabilization and electric pulse amplitude.

EXPERIMENT

We will detect electropermeabilization spectrofluorometricly using fluorescence dye propidium iodide. 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 multiwell microplate in concentration 2.5 x 10^5 cells per well. Cells are attached to the culture dish surface. Imediately before electric pulses are applied replace the growth medium with electroporation buffer containing 0.15 mM propidium iodide. As electroporation buffer you will use isosmolar 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.

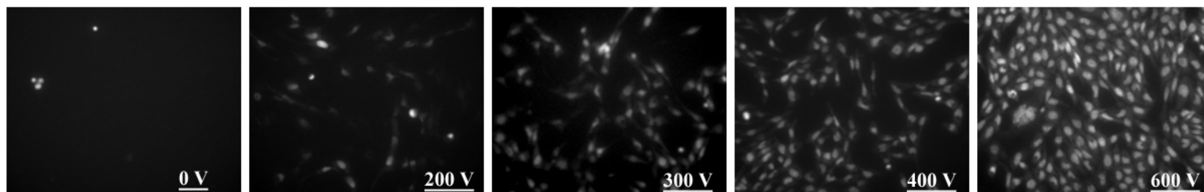

Figure 1: Sequence fluorescence images of attached cells obtained after cell exposed to electric pulses with increasing pulse amplitude according to the protocol described: from negative control (on the left) to maximum permeabilization (on the right). Images obtained by fluorescence microscopy at objetive magnification 20x.

Remove the multiwell plate from the incubator and in first four wells replace the growth medium with electroporation buffer containing propidium iodide (300 µl/well). Apply electric pulses and leave the cells for 3 to 5 minutes at room temperature than replace the medium with 1 ml of fresh electroporation medium. Electric pulse parameters are: 8 pulses, duration 100 µs and repetition frequency 1 Hz. The pulse amplitude is increased from 0 V for control treatment, to 200 V, 300 V, 400 V and 500 V. The 0 V represents negative control as cells were not exposed to electric pulses, and the florescence is a consequence of dead cells presented in the cell culture. For positive control you will expose cells to 800 V as at this pulse amplitude all cells are permeabilized. You will determine fluorescence intensity for different pulse amplitude in specrofuorimeter Tecan , Infinite 200. Excitation λ for propidium iodide is 535 nm and emission λ is 617 nm. From the data obtained you will calculate the percentage of permeabilized cells. Negative control represents 0 % permeabilization while positive control represents 100 % 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 (1800)
raw data [R.F.U.]						
permeabilization [%]						

NOTES & RESULTS