Monitoring cell membrane electroporation with ratiometric fluorescent dye Fura-2AM L2

Gorazd Pucihar
University of Ljubljana, Faculty of Electrical Engineering

Duration of the experiments: 60 min
Max. number of participants: 4
Location: Cell Culture Laboratory 1
Level: Advanced

PREREQUISITES

Participants should be familiar with the Safety rules for handling with chemicals and Rules for sterile work in cell culture. The basic knowledge of handling with cells is required for this laboratory practice.

THEORETICAL BACKGROUND

Exposure of a biological cell to a sufficiently strong external electric field leads to a detectable increase of membrane permeability, a phenomenon termed electropermeabilization. Because it is assumed that an increased permeability is related to the occurrence of hydrophilic pores in the membrane, the phenomenon is often termed electroporation. Provided that the parameters of the electric field (amplitude, duration, number of pulses, frequency) are moderate, the increased permeability is reversible, and cells recover within a few minutes after the exposure. During the state of high permeability the molecules for which the membrane is otherwise impermeable (e.g. drugs, DNA) can be transported across the membrane. Electroporation is nowadays used in biochemistry, molecular biology, and different fields of medicine. It has already become an established method in oncology for electrochemotherapy of tumors, and holds great promises in gene therapy.

The efficiency of electroporation is influenced by the parameters of the electric field, cell size and geometry, and physiological characteristics of the medium surrounding the cell. Different fluorescent dyes (e.g. Propidium Iodide, Lucifer Yellow, Fura-2, Fura-3,…) can be employed to investigate the influence of these parameters on electroporation and the same dyes can be used to monitor electroporation.

The aim of this laboratory practice is to monitor electroporation with fluorescent dye and to determine the effect of cell size, shape and orientation on the efficiency of electroporation.

EXPERIMENT

We will monitor cell membrane electroporation using a fluorescent calcium sensitive indicator Fura-2AM. Calcium ions, present in the extracellular medium, do not readily cross an intact (nonporated) cell membrane and the intracellular Ca^{2+} concentration is low. Once the membrane becomes permeable due to electroporation, Ca^{2+} ions enter the cells, where they bind to the dye and change its excitation and emission spectrum (Figure 1).

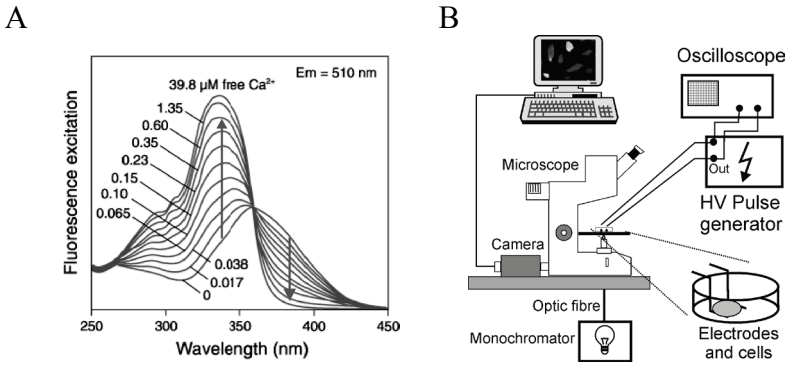

Figure 1. (A) Fluorescence excitation spectra of Fura-2 for different concentrations of Ca^{2+} (image from *http://probes.invitrogen.com/handbook/figures/0554.html*). (B) Schematic of the experiment.

Protocol: The experiments will be performed on Chinese hamster ovary cells (CHO) grown in Lab-Tek chambers (Nunc, Germany) in culture medium HAM-F12 supplemented with 8% fetal calf serum, L-glutamine (all three from Sigma-Aldrich, Steinheim, Germany) and antibiotics. Plate 2×10^4 cells on cover glass of Lab-Tek chamber and keep them for 20 hours in the incubator. Before experiments, replace the culture medium with fresh medium containing 1.5 µM Fura-2 AM (Invitrogen, Eugene, Oregon, USA). After 25-30 minutes of incubation at 37°C wash the excess dye and leave 1 ml of culture medium in the chamber.

Place the chamber under a fluorescence microscope (Zeiss AxioVert 200, Germany) and use ×40 objective. Position two parallel Pt/Ir wire electrodes with a 4 mm distance between them to the bottom of the chamber . Acquire the images using a cooled CCD camera (VisiCam 1280, Visitron, Germany) and MetaFluor 7.1.1 software (Molecular Devices, Downingtown, PA, USA).

Using a Cliniporator™ device, deliver one electric pulse of 100 µs with voltages varying from 150 to 300 V. Immediately after the pulse, acquire two fluorescence images of cells at 540 nm, one after excitation with 345 nm and the other after excitation with 385 nm. Divide these two images in MetaFluor to obtain the ratio image ($R = F_{340}/F_{380}$). Wait for 5 minutes and apply pulse with a higher amplitude. After each pulse, determine which cells are being electroporated (Figure 2). Observe, which cells become electroporated at lower and which at higher pulse amplitudes.

Figure 2. Cells stained with Fura-2AM and exposed to electric pulse with increasing amplitude.

FURTHER READING:

Neumann, E., S. Kakorin, and K. Toensing. Fundamentals of electroporative delivery of drugs and genes. *Bioelectrochem. Bioenerg.* 48:3-16, 1999.

Teissié, J., and M. P. Rols. An experimental evaluation of the critical potential difference inducing cell membrane electropermeabilization. *Biophys. J.* 65:409-413, 1993.

Grynkiewicz, G., M. Poenie, and R.Y. Tsien. A new generation of Ca^{2+} indicators with greatly improved fluorescence properties. *J. Biol. Chem.* 260:3440-3450, 1985.

Teruel M.N., and T. Meyer. Electroporation-induced formation of individual calcium entry sites in the cell body and processes of adherent cells. *Biophys. J.* 73:1785-1796, 1997.

Valič B., Golzio M., Pavlin M., Schatz A., Faurie C., Gabriel B., Teissié J., Rols M.P., and Miklavčič D. Effect of electric field induced transmembrane potential on spheroidal cells: theory and experiment. *Eur. Biophys. J.* 32: 519-528, 2003.

Towhidi L., Kotnik T., Pucihar G., Firoozabadi S.M.P., Mozdarani H., Miklavčič D. Variability of the minimal transmembrane voltage resulting in detectable membrane electroporation. *Electromagn. Biol. Med.* 27: 372-385, 2008.

NOTES & RESULTS