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

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

PREREQUISITES

Participants should be familiar with Laboratory safety (S1) and Electroporation hardware safety (S2). The basic knowledge of handling with cells is required for this laboratory practice.

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

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 electroporation [1]. 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 [2].
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 [3, 4, 5, 6].

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 (calcium uptake), where they bind to the dye and change its excitation and emission spectrum (Figure 1) [7].

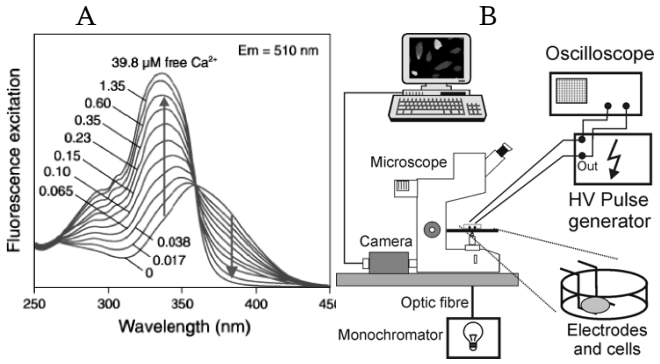

Figure 1: (A) Fluorescence excitation spectra of Fura-2 for different concentrations of Ca^{2+} (image from *https://www.thermofisher.com/si/en/home/references/molecular-probes-the-handbook/indicators-for-ca2-mg2-zn2-and-other-metal-ions/fluorescent-ca2-indicators-excited-with-uv-light.html*). (B) Schematic of the experiment.

Protocol: The experiments will be performed on H9c2 rat cardiac myoblast cell line grown in Lab-Tek chambers (TermoFisher Scientific) in Dulbecco's Modified Eagle Medium DMEM supplemented with 10% fetal bovine serum, L-glutamine (all three from Sigma-Aldrich) and antibiotics. Plate 8×104 cells on cover glass of Lab-Tek chamber and keep them for 24 hours in the incubator. Before experiments, replace the culture medium with fresh medium containing 2 µM Fura-2AM (TermoFisher Scientific). After 25-30 minutes of incubation at 37°C wash the excess dye and leave 1.5 ml of culture medium in the chamber.
Place the chamber under a fluorescence microscope (Thunder Imager Live Cell system for fluorescence microscopy, Leica Microsystems) and use ×63 objective. Insert two parallel Pt/Ir wire electrodes with a 5 mm distance between them to the bottom of the chamber. Acquire a pair of two images (with 340, 380 nm excitation wavelength) each second for one minute in time-lapse acquisition mode, using sCMOS Leica fast camera and LASX software. Apply the electric pulse at the 5th s of recording: using a BetaTech device, deliver one electric pulse of 100 µs with voltages varying from 150 to 300 V. In LASX software, observe how the ratio image (R = F340/F380) changes in time in each cell [3, 4].
Wait for 5 minutes (resealing and recovery) 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.

REFERENCES:

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EXPECTED RESULTS

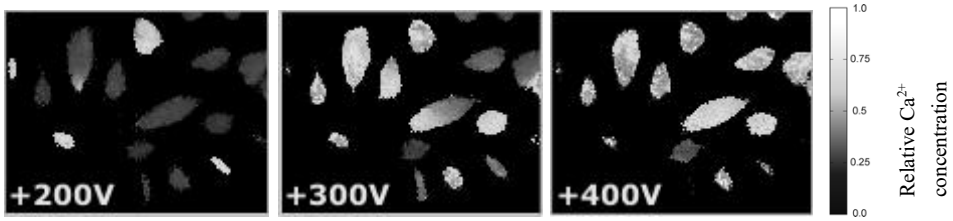

Figure 2: Cells stained with Fura-2AM and exposed to electric pulse with increasing amplitude. With increasing electric field, more cells are permeabilized, Ca^{2+} concentration in cells is higher and recovery of basal internal calcium takes longer time. Additional effects of cell size, shape and orientation to electroporation extent will be observed and discussed.

NOTES & RESULTS