L10

Monitoring cell membrane depolarization due to electroporation using fluorescent plasma membrane potential indicator

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Duration of the experiments: 90 min
Max. number of participants: 4
Location: Cell Culture Laboratory 3
Level: Advanced

PREREQUISITES

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

The aim of this laboratory practice is to monitor the time course of transmembrane voltage changes after exposure to electroporation pulses, and to determine the influence of cell-type dependent response, based on the ion channel expression profile.

THEORTICAL BACKGROUND

All cells maintain an electric potential difference across their plasma membranes. This potential difference is called the resting transmembrane voltage (or resting potential) and is maintained by a system of ion channels and pumps. By convention the resting transmembrane voltage is negative, meaning that the cell interior is electrically more negative compared to its exterior [1]. The value of the resting transmembrane voltage changes dynamically with the cell cycle and has an important biological function by controlling the activity of various membrane proteins [2]. When cells are electroporated, their transmembrane voltage changes and the membrane remains depolarized for several minutes after pulse exposure [3-5]. As membrane depolarization acts as a biological signal [2], factors that influence prolonged depolarization upon electroporation can have an important influence on the biological outcome of electroporation.

EXPERIMENT

We will monitor the time course of transmembrane voltage changes using the FLIPR Membrane Potential (FMP) dye [6]. The FMP dye consists of two parts: a fluorescent anionic voltage-sensor dye and a quencher molecule. When the interior of the cell has a relatively negative charge (is negative with respect to its surroundings) the voltage sensor remains on the extracellular side, where the quencher absorbs its fluorescence and consequently minimizes its signal. During membrane depolarization the voltage sensor translocates with positive ions to the intracellular side, which increases the fluorescence intensity inside the cell. This translocation is reversible, meaning that the voltage sensor can return to the outside of the cell, which makes it possible to monitor membrane depolarization and repolarization as the cell recovers and returns to its resting transmembrane voltage. The fluorescence intensity is linearly proportional to the change in the transmembrane voltage, making this method comparable to patch-clamp measurements upon dye calibration.

Protocol: U-87 MG human glioblastoma cells and Chinese hamster ovary (CHO-K1) cells will be plated in Nunc Lab-Tek II chambered coverglass three to four days before the experiment. On the day of the experiment, we will stain the cells for 30 min at 37°C with the Component A of the FLIPR Membrane Potential Assay Red (Molecular Devices, #R7291), diluted in Live Cell Imaging Solution (Invitrogen,

#A14291DJ). Afterwards, the cells will be placed on the microscope stage. Time lapse images of the cells will be acquired before and after the exposure to a single 100 ms pulse of selected amplitude, delivered by a pulse generator B10 HV-LV (Leroy Biotech, France) through a pair of Pt-Ir wire electrodes. We will compare the response of the two cell lines at 37°C, to observe how transmembrane voltage recovery can be influenced by different expression of ion channels (low expression in CHO-K1 cells and high expression in U-87 MG cells). Inverted microscope Leica DMi8 with LED8 illumination source controlled by the LasX software (all from Leica Microsystems) will be used to image cells. The dye will be excited with green LED (555/28 nm), its florescence will pass through DFT51010 filter, and changes in transmembrane voltage will be detected with the Leica DFC9000 Gt camera.

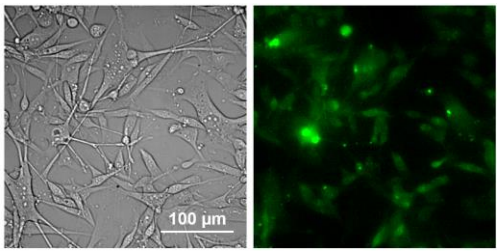

Figure 1: Brightfield image (*left*) and fluorescence (*right*) of U-87 MG cells stained with FMP dye.

REFERENCES:

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NOTES & RESULS