Monitoring cell membrane depolarization due to electroporation using fluorescent plasma membrane potential indicator L13

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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.

BACKGROUND

All cells maintain an electric potential difference across their plasma membranes, which results from the differences in membrane permeabilities for potassium, sodium, calcium, and chloride ions. 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, and the membrane is considered hyperpolarized. 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. When cells are electroporated, their transmembrane voltage changes and the membrane remains depolarized for several minutes after pulse exposure. As membrane depolarization acts as a biological signal, factors that influence prolonged depolarization upon electroporation can have an important influence on the biological outcome of electroporation.

The aim of this laboratory practice is to monitor the time course of transmembrane voltage changes after exposure to conventional electroporation pulses, and to determine the influence of pulse amplitude and ambient temperature on the extent and longevity of membrane depolarization.

EXPERIMENT

We will monitor the time course of membrane potential changes using the plasma membrane potential indicator (PMPI) fluorescent dye of the FLIPR Membrane Potential Assays Kit (Molecular Devices). PMPI consists of a two-part system which includes a fluorescent anionic voltage-sensor and a quencher. When the interior of the cell has a relatively negative charge the anion dye remains on the extracellular side, where the quencher prevents fluorescence excitation. During depolarization the voltage sensor translocates to the intracellular side, which increases the fluorescence intensity inside the cells. This translocation is reversible, which makes it possible to monitor membrane depolarization and repolarization as the cells recover and return to their baseline level. The fluorescence intensity is linearly proportional to the change in the membrane potential difference, making this method comparable to patch-clamp measurements upon dye calibration.

Protocol: U87-MG human glioblastoma cells will be plated in Nunc Lab-Tek II chambered coverglass three to four days before the experiments. On the day of the experiments, the cells will be stained 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. The cells will be exposed to a single 100 μs pulse of selected amplitude, delivered by BTX Gemini pulse generator through a pair of Pt-Ir wire electrodes. Time lapse images of the cells will be acquired before and after pulse application. We will compare the response of the cells at room temperature and at 37°C, to observe how cell recovery depends on the temperature. The cells will be imaged on inverted microscope Leica DMi8 with LED8 illumination source controlled by the LasX software (all Leica Microsystems). The membrane potential dye will be excited with green LED (555/28 nm) and its florescence will be passed through DFT51010 filter and detected with the Leica DFC9000 Gt camera.

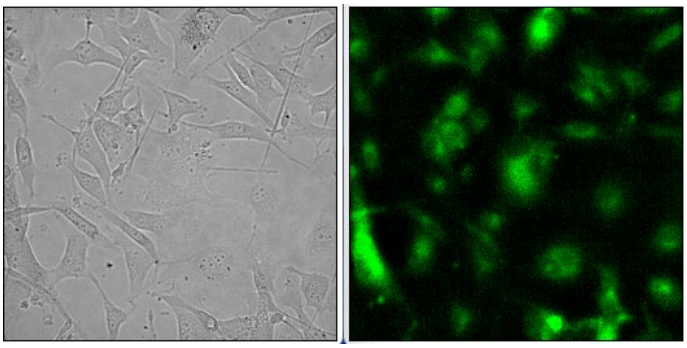

Figure 1: Brightfield image (*left*) and fluorescence (*right*) of U87-MG cells stained with FLIPR membrane potential assay.

FURTHER READING:

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NOTES & RESULS