L5 Triggering action potential and electroporation in excitable cells exposed to electric pulses

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

PREREQUISITES

Participants should be familiar with Laboratory safety (S1) and Electroporation hardware safety (S2). No other specific knowledge is required for this laboratory practice.

The aim of this laboratory practice is to observe triggering action potentials and electroporation in genetically engineered tet-on spiking human embryonic kidney (S-HEK) cells with the use of 100 µs electric pulses.

THEORETICAL BACKGROUND

Electric pulses have been used for triggering action potentials in excitable cells (electrostimulation) already for decades. However, when using higher electric fields, the cells' plasma membrane becomes permeabilized and additional ionic currents through pores/defects occur. These additional ionic currents affect cell excitability in a complex interplay between excitation and electroporation [1, 2, 3, 4].
To better understand the underlying mechanisms of electrostimulation and electroporation in excitable cells (nerves, muscles, cardiac), in vitro experimental work is of great importance. Genetically engineered S-HEK cells expressing a minimal complement of sodium and potassium channels ($Na_V1.5$ and $K_{ir}2.1$) needed for excitability are a simple and convenient excitable cell model for studying excitation and electroporation in vitro [5, 6].
The use of a fluorescent potentiometric dye ElectroFluor630 and fluorescence microscopy is an effective way to study responses in transmembrane voltage to electric pulses in excitable cells (action potentials and electroporation). Compared to classical electrophysiological methods such as patch clamp, these optical measurements are much easier and time efficient, as they do not require special technical skills. Also, high-voltage electric pulses do not interfere with the measurements (i.e. image acquisition).

EXPERIMENT

We will monitor the changes in transmembrane voltage (TMV) in excitable S-HEK cells (ATCC CRL-3479) using the fluorescent potentiometric dye ElectroFluor630 (Potentiometric Probes) under a fluorescence microscope and observe the triggering of action potentials and electroporation with 100 µs electric pulses of increasing amplitudes.

Protocol: S-HEK cells will be plated to Lab-Tek chambers (Thermo Fisher Scientific) 3 days before the experiment in concentration of 10^5 cells per well (Figure 1A). To prepare the cells for experiment, label the cells with 12 µM ElectroFluor630 in DMEM culture medium for 20 min in a refrigerator at 4°C (Figure 1B). Wash the cells three times with a Tyrode solution (2 mM KCl, 125 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 30 mM glucose, pH 7.3) and at the end, add 1.2 ml of low potassium Tyrode solution (0.5mM KCl, 126.5 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 10 mM HEPES, 30 mM glucose, pH 7.3) to cells.

Insert two parallel Pt/Ir wire electrodes with a 5 mm distance between them to the bottom of the Lab-Tek chamber (Figure 1C). Place the chamber under a fluorescence microscope (Thunder Imager Live Cell system for fluorescence microscopy, Leica Microsystems) and connect the electrodes to the pulse generator. Acquire a set of fluorescence images (635 nm excitation and 700 emission wavelength, ×40 objective) in time-lapse acquisition mode (Figure 1B), using sCMOS Leica fast camera and LAS X software: acquire 80 images, one image every 36 ms (around 2.8 s total duration of image acquisition). The first image acquisition represents control without pulse application. Further on, while recording, apply a single electric pulse of 100 µs and 63 V using a TTL signal from the microscope system that triggers the pulse generator and observe the fluorescence signal from the whole field of view.

Every two minutes, record a time-lapse in the same way as before but apply electric pulses of increasing voltage: 63, 75, 88, 100, 125, 150, 175, and 200 V (Figure 1D). The electric field to which the cells are exposed is estimated as the applied voltage-to-electrode-distance ratio (E ≈ 126, 150, 176, 200, 250, 300, 350, and 400 V/cm, respectively). At lower electric fields, the pulses will trigger action potentials, at higher electric fields, the pulses will cause electroporation which manifests as a prolonged depolarization.

The fluorescence signal can be further analyzed using a Matlab application: the acquired images are thresholded to extract the signal only from the membranes, the fluorescence signal is corrected for fading and the characteristic parameters of the signal are extracted (number of action potentials, amplitude, recovery to the baseline etc.), as described more in detail in [1, 2, 3].

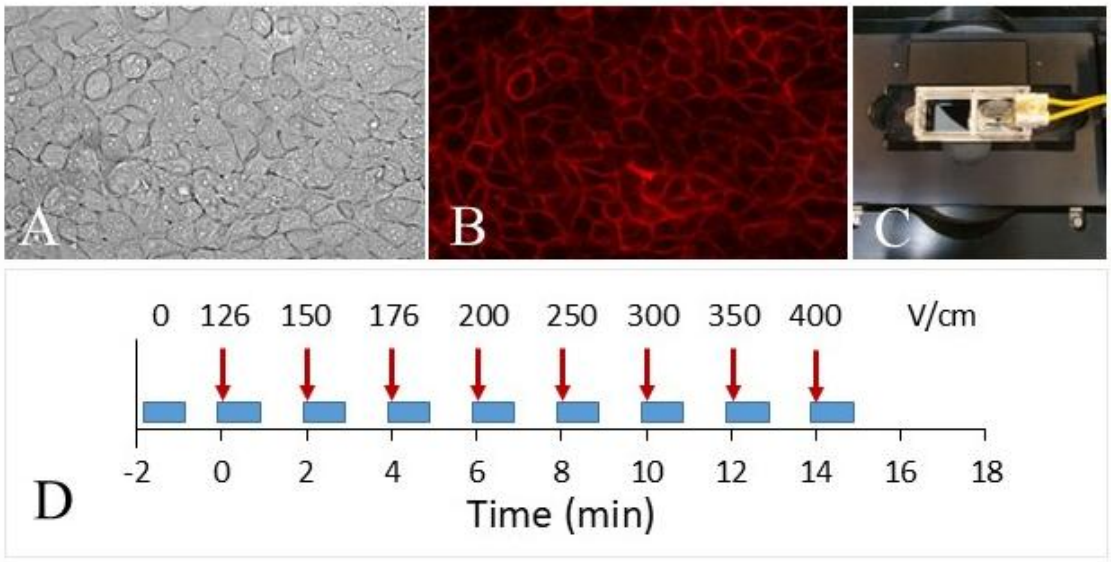

Figure 1: Monitoring triggering excitation and electroporation with 100 µs electric pulses optically with the use of the fluorescent potentiometric probe ElectroFluor630 in excitable S-HEK cells. A brightfield (A) and fluorescence image (B) of S-HEK cells. (C) Experimental chamber with the electrodes under the fluorescence microscope. (D) Experiment timeline: application of pulses of increasing electric field strength (red arrows) and time-lapse image acquisitions (blue tabs).

REFERENCES:

[1] Batista Napotnik T., Kos B., Jarm T., Miklavčič D., O'Connor R.P., Rems L. Genetically engineered HEK cells as a valuable tool for studying electroporation in excitable cells. *Sci Rep,* 14:720, 2024.

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[3] Batista Napotnik T., Kos B. & Rems L. Monitoring electroporation-induced changes in action potential generation in genetically engineered Tet-on spiking HEK cells. *J Visual Exp* (JoVE), 2024.

[4] Pakhomov A.G., Pakhomova O.N. The interplay of excitation and electroporation in nanosecond pulse stimulation. *Bioelectrochemistry,* 136:107598, 2020.

[5] McNamara H.M., Zhang H., Werley C.A., Cohen A.E. Optically controlled oscillators in an engineered bioelectric tissue. *Phys Rev* X, 6:031001, 2016.

[6] Tian H., Davis H.C., Wong-Campos J.D. et al. Video-based pooled screening yields improved far-red genetically encoded voltage indicators. *Nature Methods,* 20:1082-1094, 2023.

EXPECTED RESULTS

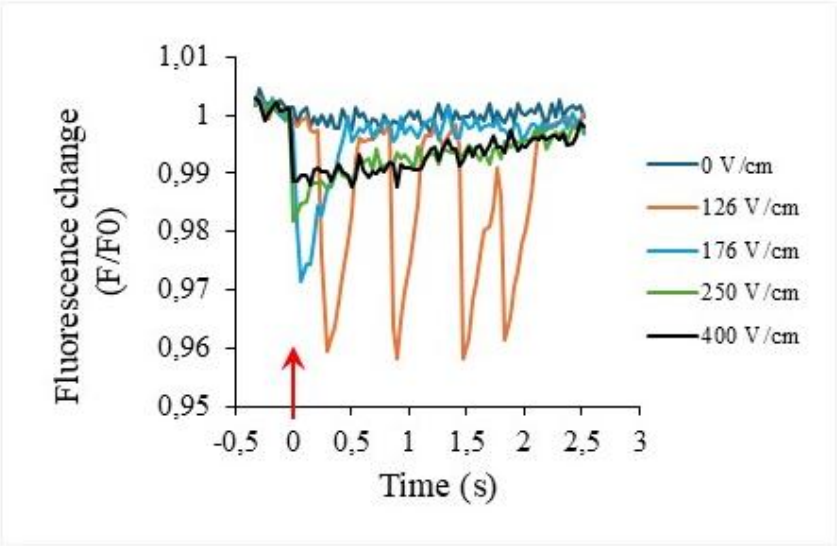

Figure 2: Excitable S-HEK cells exposed to single electric pulses of 100 µs and increasing electric fields E. With lower E (126, 176 V/cm), we trigger single or multiple action potentials - electrostimulation. With higher E (250 V/cm), action potentials are longer due to electroporation, eventually culminating in prolonged depolarization without returning to the baseline transmembrane voltage during 2.8 s of monitoring (400 V/cm).

NOTES & RESULTS