

L15 Measurements of the induced transmembrane voltage with fluorescent dye ElectroFluor630

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Duration of the experiments: 60 min

Max. number of participants: 3

Location: Cell Culture Laboratory

Level: Basic

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 measure the induced transmembrane voltage ITV on a spherical cell by means of a fluorescent potentiometric dye ElectroFluor630.

THEORETICAL BACKGROUND

When a biological cell is exposed to an external electric field an induced transmembrane voltage (ITV) forms across its membrane. This transmembrane voltage represents the difference in electric potentials on the two sides of the cell membrane, i.e., in the cytosol and the extracellular medium close to the membrane. The amplitude of the ITV is proportional to the amplitude of the applied electric field, and with a sufficiently strong field, this leads to an increase in membrane permeability – electroporation [1]. Increased permeability is detected in the regions of the cell membrane where the ITV exceeds a sufficiently high value, in the range of 250–1000 mV, depending on the cell type and the sensitivity of the detection method [2]. Correlation between ITV and electroporation has been described using different theoretical models [3]. The ITV varies with the position on the cell membrane and is influenced by cell geometry and physiological characteristics of the medium surrounding the cell [1, 3]. It is therefore important to determine the distribution of the ITV across the cell membrane. For simple geometric shapes the ITV can be calculated analytically (e.g. for a spherical cell, using Schwan's equation). For irregular cell shapes, experimental and numerical methods are the only feasible approach to determine the ITV [1].

EXPERIMENT

Potentiometric fluorescent dyes allow observing spatiotemporal profile of the ITV along the membrane and measuring its value [1, 2, 4, 5]. ElectroFluor630 (Di-4-ANEQ(F) PTEA, Potentiometric Probes, USA) is a fast potentiometric fluorescent dye, which becomes fluorescent when it binds to the cell membrane. Its relative change in fluorescence intensity being proportional to the change in ITV [6]. The dye reacts to the change in the ITV by changing the intramolecular charge distribution that produces corresponding changes in the spectral profile or intensity of the dye's fluorescence.

Protocol: The experiments follow the protocols in [7, 8, 9] and are performed on Chinese hamster ovary cells (CHO-K1) grown in Lab-Tek chambers (Thermo Fisher Scientific) in culture medium HAM-F12 supplemented with 10% fetal bovine serum, L-glutamine (all three from Merck) and antibiotics. Trypsinize the cells, seed them to the Lab-Tek chambers (7x 10⁴ cells per well) and incubate for two hours in the CO₂ incubator at 37°C. Cells will attach to the cover glass of a Lab-Tek chamber but still retain their spherical shape. Carefully replace the culture medium with 1 ml of fresh HAM-F12 medium containing 12 µM ElectroFluor630 dye and place for 20 min in a refrigerator at ~ 4 °C. After washing add 1 ml of potassium

phosphate buffer KPB, consisting of 250 mM sucrose, 10 mM phosphate (K₂HPO₄/KH₂PO₄), and 1 mM MgCl₂, pH 7.4, in the chamber. Position two parallel Pt/Ir wire electrodes, with a 5 mm distance between them, to the bottom of the chamber. Place the chamber under a fluorescence microscope (Thunder Imager Live Cell system for fluorescence microscopy, Leica Microsystems, Germany) and use ×63 objective. Set a single 60 V, 50 ms pulse on the programmable square wave pulse generator. Use a voltage divider to further decrease the voltage on the electrodes to 50 V. This will result in an electric field of ~100 V/cm in between the electrodes. The pulse must be synchronized with the image acquisition. Set the excitation wavelength to 635 nm and use DFT51010 filter to detect fluorescence (emission 700 nm).

Find the cells of interest. Acquire the control fluorescence image and subsequently the image during a pulse application, using the LAS X software (Leica). Apply four pulses. For each pulse, acquire a pair of images, one immediately before (control image) and one during the pulse (pulse image) (Figures 1A and B).

To *qualitatively* display the ITV on the cell membrane, convert the acquired 12-bit images to 8-bit images in LAX software. For each pulse, obtain the difference image by subtracting (on a pixel-by-pixel basis) the control image from the pulse image. (Figure 1C).

To *quantitatively* determine the ITV, open the acquired, unprocessed fluorescence images in ImageJ/Fiji. Determine the region of interest (ROI) at the site of the membrane (start at the top – cathode side, freehand line, save ROI) and measure the fluorescence intensities along this region for the control and pulse image (Multi Plot). Transfer the values (arc distance and fluorescence intensity) to a pre-prepared spreadsheet. Measure background fluorescence and cell diameter. Determine the absolute fluorescence changes (ΔF) by subtracting the pixel values of each control image F_{Ci} from its corresponding pulse image F_{Pi} :

$$\Delta F = F_{Pi} - F_{Ci}$$

For each pair of images determine the relative change in the fluorescence (ΔF_{rel}) by subtracting background ($\Delta F/(F_{Ci} - F_{bckg})$):

$$\Delta F_{rel} = \frac{\Delta F}{F_{Ci} - F_{bckg}}$$

The relative change in the dye fluorescence with respect to the membrane voltage is approximately -15%/100 mV. From this you can determine the experimentally measured induced transmembrane voltage (ITV_{exp}):

$$ITV_{exp} = \Delta F_{rel} \frac{-100 \text{ mV}}{15\%}$$

Average the relative changes calculated for all four acquired pairs of images to reduce noise. Compare the measured voltage with the analytical expression for the ITV_{Schwan} on a spherical cell (Schwan equation):

$$ITV_{Schwan} = \frac{3}{2} E R_{cell} \cos \theta$$

(R is cell radius, E is electric field strength and θ is the angle between the direction of the electric field and the vector normal to the membrane surface).

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EXPECTED RESULTS

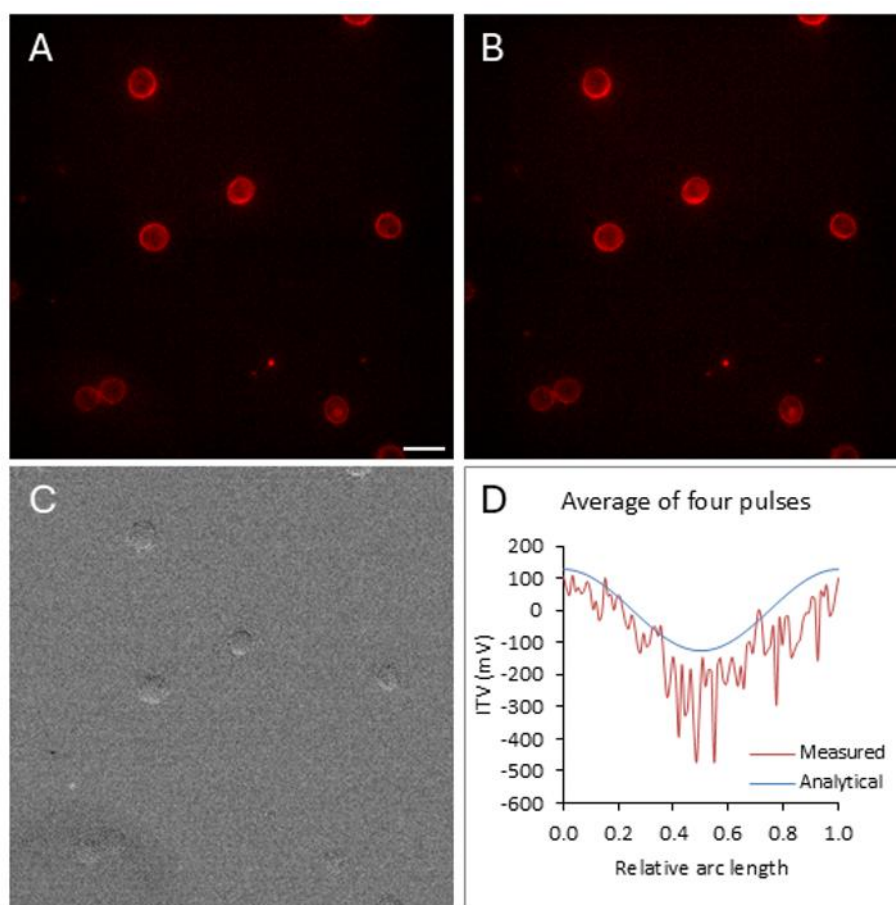


Figure 1: Expected experimental results showing the induced transmembrane voltage (ITV) on an irregularly shaped CHO-K1 cell. (A) A control fluorescence image of a cell stained with ElectroFluor630. Bar represents 20 μm . (B) Fluorescence image acquired during the exposure to a 50 V (~ 100 V/cm), 50 ms rectangular pulse. (C) Changes in fluorescence of a cell obtained by subtracting the control image A from the image with pulse B. The brightness of the image was automatically enhanced. (D) Results of measurements of the induced transmembrane voltage (average of four measurements), compared to the analytical calculation for the ITV on a spherical cell (Schwann equation).

NOTES & RESULTS
