L2 Visualization of local ablation zone distribution between two needle electrodes

Tjaša Potočnik, Alenka Maček Lebar
University of Ljubljana, Faculty of Electrical Engineering

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 visualize local ablation zone distribution between two needle electrodes with increasing pulse amplitude using trypan blue.

THEORETICAL BACKGROUND

Electroporation is the method in which by applying external electric field of sufficient amplitude and duration membrane of exposed cells becomes permeabilized for molecules that otherwise cannot pass cell membrane [1, 2]. After reversible electroporation cell membrane reseals. With increasing amplitude of electric field the level of cell membrane permeabilization and the number of cells that are permeabilized increases. When pulses with sufficient magnitude and duration are applied, cell death is achieved and the process is defined as irreversible electroporation (IRE) [3]. IRE is an emerging ablation technique inducing cell death in successfully treated cells or tissues. Usually there is a sharp border between treated and untreated tissue regions because only the cells that are exposed to high enough electric field are ablated. Effective prediction of electric field can be obtained by numerical modeling, which includes the shape and position of the electrodes and parameters of electric pulses (amplitude, duration, number, frequency) used as well as electrical properties of the tissue [4, 5]. Using treatment planning, IRE offers benefits over other cancer therapies because it can be performed near large blood vessels, nerves, and ducts without causing damage to these structures, sparing extracellular matrix.
Electroporation can be detected by measuring increased transport of molecules across the membrane. Cell uptake of dyes, either fluorescent molecules (lucifer yellow, yo-pro-1, propidium iodide) or colour stains (such as trypan blue), is most often used for *in vitro* electroporation detection [6, 7]. Trypan blue can be used as an indicator of plasma membrane integrity and cell viability. Trypan blue is normally impermeant to viable cells. When cell membrane integrity is compromised, the dye is able to enter the cell and stains cellular structures blue, especially nuclei, making the cell appear blue. Cells that take up this dye several hours after exposure to electrical pulses are usually considered dead or dying.

EXPERIMENT

We will visualize local ablation zone distribution between two needle electrodes using trypan blue. The effect of the pulse amplitude on the local ablation zone distribution between two needle electrodes will be determined for a train of eight 100 µs rectangular pulses delivered with the repetition frequency 1 Hz.
Protocol: You will use Chinese hamster ovary cells (CHO), plated 48 h before experiment in concentration 2.5 x 10^5 cells per tissue culture dish. Cells are attached to the culture dish surface. Immediately before electric pulses are applied replace the growth medium with electroporation buffer. As electroporation buffer you will use isotonic 10 mM K_2HPO_4/KH_2PO_4 containing 1 mM $MgCl_2$ and 250 mM sucrose with pH 7.4.

You will use needle electrodes 1 mm apart. For pulse delivery Gemini X2 electroporator (Hardvard apparatus BTX, USA) will be used. It can produce rectangular and exponential pulses. During the experiment current will be monitored with an oscilloscope and a current probe.

Remove the tissue culture dish from the incubator and replace the growth medium with 500 µl of electroporation buffer. Carefully place needle electrodes on edge of tissue culture dish and apply electric pulses. Electric pulse parameters used are: 8 rectangular pulses, 100 µs duration and pulse repetition frequency 1 Hz, while pulse amplitude increases gradually. Increase the pulse amplitude from 0 V to 100 V, 300 V, 500 V and 700 V. After electroporation leave cells for 10 minutes at room temperature. Remove electroporation buffer and add 500 µl of trypan blue to tissue culture dish. Leave the cells for 5 minutes at room temperature then replace the trypan blue with 500 µl of fresh electroporation buffer. For visualization of local ablation zone, EVOS XL Core Imaging System (Invitrogen™) will be used.

REFERENCES:

[1] Rols M.P. Electropermeabilization, a physical method for the delivery of therapeutic molecules into cells. *Biochim Biophys Acta*, 1758:423-428, 2006.

[2] Geboers B., Scheffer H.J., Graybill P.M., et al. High-voltage electrical pulses in oncology: Irreversible electroporation, electrochemotherapy, gene electrotransfer, electrofusion, and electroimmunotherapy. *Radiology,* 295:254–272, 2020.

[3] Davalos R.V., Mir L.M., Rubinsky B. Tissue ablation with irreversible electroporation. *Ann Biomed Eng,* 33:223-31, 2005.

[4] Čorović S., Pavlin M., Miklavčič D. Analytical and numerical quantification and comparison of the local electric field in the tissue for different electrode configurations. *Biomed Eng Online,* 6:37, 2007.

[5] Dermol J., Miklavčič D. Predicting electroporation of cells in an inhomogeneous electric field based on mathematical modeling and experimental CHO-cell permeabilization to propidium iodide determination. *Bioelectrochemistry*, 100:52-61, 2014.

[6] Batista Napotnik T., Miklavčič D. In vitro electroporation detection methods – An overview. *Bioelectrochemistry*, 120:166-182, 2018.

[7] Casciola M., Feaster T.K., Caiola M.J., Keck D., Blinova K. Human in vitro assay for irreversible electroporation cardiac ablation. *Front Physiol*, 13:1064168, 2023.

EXPECTED RESULTS

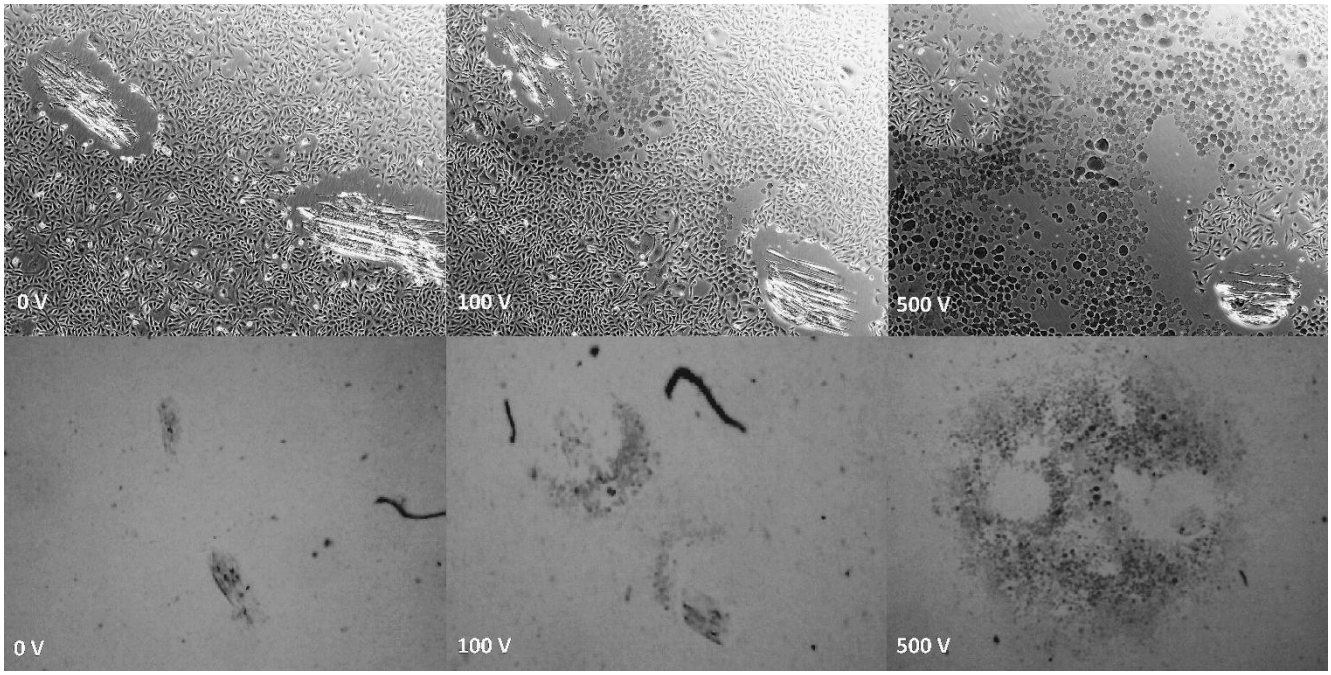
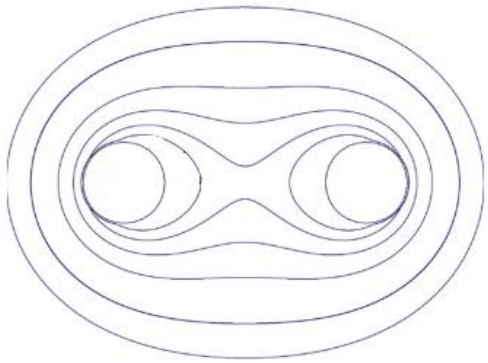


Figure 1: The sequence of the images of local ablation zone after cells were exposed to electric pulses with increasing pulse amplitude. The images were obtained by light microscopy under 10 × objective magnifications (top row) and under 5 × objective magnifications (bottom row).

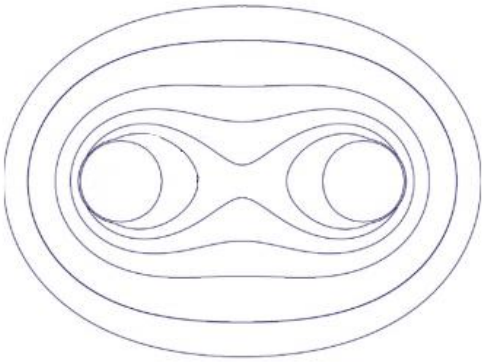
The area of blue cells, which is a consequence of efficient ablation, increases with increasing pulse amplitude, as presented in Figure 1.

NOTES & RESULTS

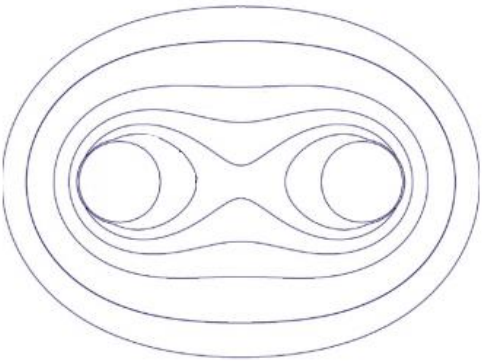
Sketch the blue-shaded area around the electrodes at different applied voltages.



100 V



500 V



700 V