Cell electrofusion L6

Katja Trontelj, Marko Ušaj
University of Ljubljana, Faculty of Electrical Engineering

Duration of the experiments: 90 min + 60 min
Max. number of participants: 4
Location: Cell culture laboratory 1 and Laboratory of Biocybernetics
Level: basic

PREREQUISITES

Participants should be familiar with the Safety rules and Rules for sterile work in cell culture laboratory (following the recommendations of the Good laboratory practice). No other specific knowledge is required for this laboratory practice.

THEORETICAL BACKGROUND

Electrofusion is achieved when cells in close physical contact are brought into their fusogenic state by means of high-voltage electric pulses (electropermeabilization). The efficiency of electrofusion depends on various parameters that affect two parts of the electrofusion process. First part of the electrofusion process is achievement of the close physical contact between cells, which can be obtained with different methods. The adherence method can be used efficiently due to spontaneously established cell contacts between cells. Contact between cells can also benefit from osmotic swelling of the cells when osmotic treatment is properly used. Second part of the electrofusion process is achieving the fusogenic state of the cell membranes. Fusogenic state correlates well and is governed by similar parameters of the electric pulses as optimal electropermeabilization of cell membranes.
Determination of fusion yield is usually assessed by labeling the cytoplasm of cells with different fluorescent cell tracker dyes. Cell electrofusion is a safe, non-viral and non-chemical method that can be used for preparing hybrid cells for human therapy. It is an effective method since it can be properly adjusted to any different types of cells.

The aim of this exercise is to demonstrate how different electric field amplitudes influence on electrofusion yield.

EXPERIMENT

We will detect cell electrofusion using two fluorescent cell tracker dyes; CMRA (orange) and CMFDA (green). From the images acquired during the experiment, we will determine the effect of the pulse amplitude on the efficiency of cell electrofusion. For achieving contacts between cells we will use modified adherence method.

Protocol: Perform the experiments on previously prepared mouse myeloma cells (NS1). Cells will be grown to 70-80% confluence in two separated culture flask and rinsed twice with bicarbonate-free Krebs-Hepes buffer. Dye loading will be performed as follows: cells from one flask will be loaded with 7 μM CellTracker Green CMFDA and cells from another flask will be loaded with 7 μM CellTracker Orange CMRA for 30 minutes at 37°C in bicarbonate-free Krebs-Hepes buffer. After loading, cells will be rinsed once with culture medium. Both cells (green and red) will be mixed together and suspension will be placed in 24 multiwell plates ($5x10^5$ cells/well). Cells will be incubated in 5% CO_2 at 37°C for 8 hours to allow them to slightly attach to the plate surface and establish cell contacts.
Remove culture media and wash cells with 1 ml of izoosomolar potassium phosphate buffer (10 mM KH_2PO_4, 10 mM K_2HPO_4, 1mM $MgCl_2$, 250 mM sucrose). Add hypoosmolar

potassium phosphate buffer (10 mM KH_2PO_4, 10 mM K_2HPO_4, 1mM $MgCl_2$, 175 mM sucrose) in order to induce cell swelling. After 2-3 min, when cells are close to their maximum volumes, apply a train of 8 rectangular pulses (100 μs, 1 Hz) with electric pulse generator Juan PS 10 (Jouan, France) to each sample and monitor the pulses with the oscilloscope LeCroy 9310C (LeCroy, ZDA). Use three different amplitudes of the pulses for sub-optimal, optimal and supra-optimal permeabilization of the cells: 200 V, 500 V and 800 V. Treat the cells in control the same as other samples except for the electric pulses.
Leave the cells undisturbed for 10 min and then determine the fusion yield by means of fluorescent and phase contrast microscopy (Zeiss 200, Axiovert, Germany). Capture five triplets of images per parameter with cooled CCD camera VisiCam 1280 (Visitron, Germany): phase contrast, CMRA fluorescence (excitation at 548 nm) and CMFDA fluorescence (excitation at 492 nm) using MetaMorph 7.0 (Molecular Devices, USA), objective magnification 20x. Create three channel images from each image triplet (phase contrast, orange and green fluorescence) with Image-J (NIH Image, USA). Determine the percentage of dually fluorescent cells for all amplitudes by dividing the number of dually fluorescent cells with the number of all cells.

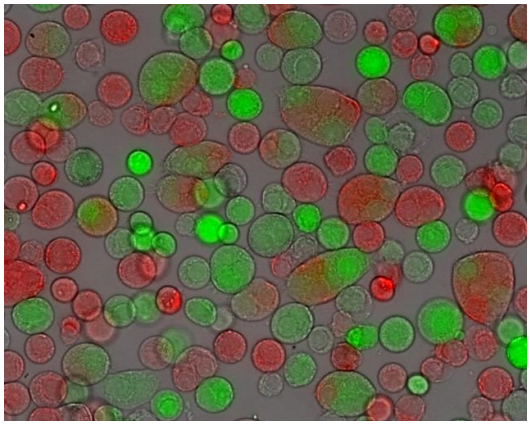

Figure1: Three channel microscopy image of B16F1 cells after electrofusion: phase contrast, CMRA fluorescence (excitation at 548 nm) and CMFDA fluorescence (excitation at 492 nm), objective magnification 20x

FURTHER READING:

Zimmermann, U. (1982) Electric field-mediated fusion and related electrical phenomena. Biochimica et Biophysica Acta 696, 227-277

Rols, M.P., Teissie, J. (1990) Modulation of electrically induced permeabilization and fusion of Chinese hamster ovary cells by osmotic pressure. Biochemistry 29, 2960-2966

Gabrijel, M., Repnik, U., Kreft, M., Grilc, S., Jeras, M., Zorec, R. (2004) Quantification of cell hybridoma yields with confocal microscopy and flow cytometry. Biochemical and biophysical research communications 314, 717-723

Trontelj, K., Reberšek, M., Kandušer, M., Šerbec, V.Č., Šprohar, M., Miklavčič, D. (2008) Optimization of bulk cell electrofusion in vitro for production of human-mouse heterohybridoma cells. Bioelectrochemistry 74, 124-129

Usaj, M., Trontelj, K., Kanduser, M., Miklavcic, D. (2009) Cell size dynamics and viability of cells exposed to hypotonic treatment and electroporation for electrofusion optimization. Radiology and Oncology 43(2), in press.

NOTES & RESULTS

voltage [V] (E [V/cm])	control (0)	200 (400)	500 (1000)	800 (1600)
number of green cells				
number of orange cells				
number of dually fluorescent cells				
% of dually fluorescent cells				