Cell electrofusion L7

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Duration of the experiments: 60 min (laboratory work) and 60 min (computer work)
Max. number of participants: 4
Location: Cell Culture Laboratory 3 and Laboratory of Biocybernetics
Level: Basic

PREREQUISITES

Participants should be familiar with the Safety rules and Rules for sterile work in cell culture laboratory and handling with cells and devices. 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 (electroporation). The efficiency of electrofusion depends on various parameters that affect two phases of the electrofusion process. First phase of the electrofusion process is achieving a close physical contact between cells and the second is achieving a fusogenic state of the cell membranes. A close physical contact between cells can be obtained with different methods. The adherence method can be used efficiently due to spontaneously established cell contacts. The contact between cells is additionally improved by osmotic swelling of the cells when the osmotic treatment is properly used. Cells in the contact fuse only if their membranes are in the fusogenic state. The fusogenic state of the cell membranes correlates well and is governed by similar parameters of the electric pulses as an optimal permeabilization of cell membranes obtained by electroporation.

The determination of the fusion yield is usually assessed by labelling the cell cytoplasm with different fluorescent cell trackers. Cell electrofusion is a safe, non-viral and non-chemical method that is used for preparing hybrid cells for human therapy. It is an effective method since it can be adjusted to different cell types.

The aim of this laboratory practice is to demonstrate how electroporation induces cell fusion.

EXPERIMENT

We will detect the cell fusion using two vital fluorescent cell trackers; CMRA (red) and CMFDA (green). From the images acquired during the experiment, we will determine the effect of the electroporation on cell fusion. For achieving contacts between cells we will use modified adherence method.

Experimental protocol 1/2 (cell culture laboratory)

Perform the experiments on previously prepared mouse melanoma cells (B16-F1). The cells will be grown to 70-80 % confluence in two separated culture flasks and rinsed twice with bicarbonate-free Krebs-Hepes buffer. A dye loading will be performed as follows: cells from one flask will be loaded with 7 µM cell tracker CMRA and the cells from another flask will

be loaded with 7 μM cell tracker CMFDA for 30 minutes at 37 °C in bicarbonate-free Krebs-Hepes buffer. After loading, cells will be rinsed once and incubated for 1 hour at 37 °C in complete culture medium. Both cells (red and green) will be mixed together to obtain concentration 2×10^6 cells/ml. 40 μl drops of this suspension will be placed in 24 multiwell plates (in the middle of the wells). The cells will be incubated in 5 % CO_2 at 37 °C for 20 minutes to allow them to slightly attach to the plate surface and to establish cell contacts.

Remove culture medium and wash the cells with 500 μl of isotonic potassium phosphate buffer (10 mM KH_2PO_4, 10 mM K_2HPO_4, 1 mM $MgCl_2$, 250 mM sucrose). Add 500 μl of hypotonic potassium phosphate buffer (10 mM KH_2PO_4, 10 mM K_2HPO_4, 1mM $MgCl_2$, 75 mM sucrose) in order to induce cell swelling. Avoid pipetting directly to the cells. After 2 min, when the cells are close to their maximum sizes, apply a train of 8 rectangular pulses (100 μs, 1 Hz) with electric pulse generator Jouan. Use the amplitude of 500 V with custom made parallel wired electrodes (5 mm gap) for efficient cell electroporation. Treat the cells in control in the same way except for the electric pulses. Leave the cells undisturbed for 10 minutes and then determine the fusion yield by fluorescent and phase contrast microscopy (Zeiss AxioVert 200, Germany). Capture four triplets of images (Fig. 1) per parameter with cooled CCD camera VisiCam 1280 (Visitron, Germany): phase contrast, CMRA fluorescence (excitation at 548 nm, emission filter D605/55m) and CMFDA fluorescence (excitation at 492 nm, emission filter HQ535/30m) using MetaMorph 7.0 (Molecular Devices, USA) under 20× objective magnification.

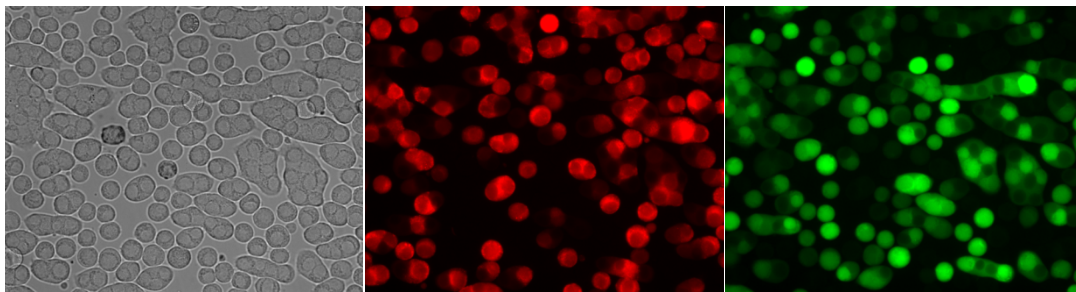

Fig. 1 The electrofused B16-F1 cells 10 minutes after their electroporation by the train of pulses (500 V, 8×100 μs, 1 Hz). *Left:* the phase contrast image of the cells. *Middle:* the red fluorescence image of the cells. *Right:* the green fluorescence image of the cells.

Experimental protocol 2/2 (laboratory of biocybernetics)

Create three channel images (Fig. 2) from each image triplet (phase contrast, red and green fluorescence) with ImageJ software plug-in *RGB Gray Merge* (NIH Image, USA). If it is necessary adjust image contrast with the ImageJ dedicated function (*Image/Adjust/Brightness&Contrast*). Separately count double and single color cells by using ImageJ plugin *CellCounter*. Determine the yield of the electrofusion (F) in each three channel image as a ratio between the number of double color cells (N_D) and the number of all cells (i.e. single color - N_S and double color cells together):

$$F = \frac{N_D}{(N_D + N_S)} \times 100$$

Calculate the final electrofusion yield by averaging those yields obtained from separate images.

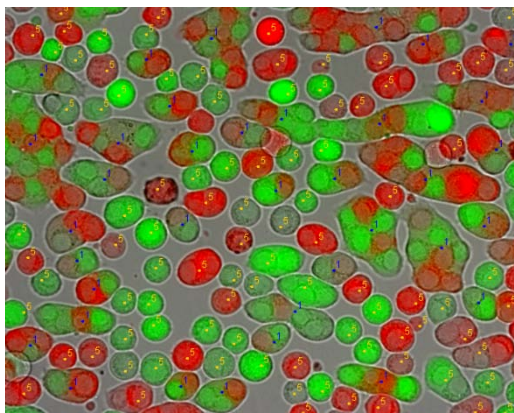

Fig. 2 The example of the counted three channel microscopy image of fused B16-F1 cells: phase contrast, red fluorescence (CMRA) and green fluorescence (CMFDA). The three channel image was created and counted using ImageJ software. The fused double color cells are marked with “1” and single color cells are marked with “5”. Objective magnification: 20×.

FURTHER READING:

Rols MP, Teissie J, Modulation of electrically induced permeabilization and fusion of Chinese hamster ovary cells by osmotic pressure. *Biochemistry*, 29: 2960-2966, 1990

Gabrijel M, Repnik U, Kreft M, Grilc S, Jeras M, Zorec R, Quantification of cell hybridoma yields with confocal microscopy and flow cytometry, *Biochem. Biophys. Res. Commun,* 314: 717-723, 2004.

Ušaj M, Trontelj K, Miklavčič D, Kandušer M, Cell–cell electrofusion: Optimization of electric field amplitude and hypotonic treatment for mouse melanoma (B16-F1) and Chinese hamster ovary (CHO) cells, *J. Membrane Biol.* 236: 107-116, 2010.

Video Article:

Trontelj K, Ušaj M, Miklavčič D, Cell electrofusion visualized with fluorescence microscopy, *J. Visual Exp*. 41: 1991, 2010.

NOTES & RESULTS

voltage [V]	0	500
number of red or green cells		
number of double color cells		
electrofusion yield [%]		

NOTES & RESULTS