Bioelectrochemistry 74 (2008) 124–129

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

Optimization of bulk cell electrofusion in vitro for production of human–mouse heterohybridoma cells

Katja Trontelj [a], Matej Reberšek [a], Maša Kandušer [a], Vladka Čurin Šerbec [b,c], Marjana Šprohar [b], Damijan Miklavčič [a,*]

[a] University of Ljubljana, Faculty of Electrical Engineering, Tržaska 25, SI-1000 Ljubljana, Slovenia
[b] Blood Transfusion Centre of Slovenia, Šlajmerjeva 6, SI-1000 Ljubljana, Slovenia
[c] University of Ljubljana, Faculty of Chemistry and Chemical Technology, Aškerčeva 5 SI-1000, Ljubljana, Slovenia

ARTICLE INFO

Article history:
Received 11 December 2007
Received in revised form 14 May 2008
Accepted 2 June 2008
Available online 11 June 2008

Keywords:
Electrofusion
Electropermeabilization
Electric field direction
Heterohybridoma

ABSTRACT

Cell electrofusion is a phenomenon that occurs, when cells are in close contact and exposed to short high-voltage electric pulses. The consequence of exposure to pulses is transient and nonselective permeabilization of cell membranes. Cell electrofusion and permeabilization depend on the values of electric field parameters including amplitude, duration and number of electric pulses and direction of the electric field. In our study, we first investigated the influence of the direction of the electric field on cell fusion in two cell lines. In both cell lines, applications of pulses in two directions perpendicular to each other were the most successful. Cell electrofusion was finally used for production of human–mouse heterohybridoma cells with modified Koehler and Milstein hybridoma technology, which was not done previously. The results, obtained by cell electrofusion, are comparable to usually used polyethylene glycol mediated fusion on the same type of cells.

1. Introduction

The ability to fuse two different types of cells allows for creation of a third type of cells that are polynuclear and display hybrid characteristics of the two original types of cells. Cell fusion has been used for transfer of foreign receptors into the membrane of the living cell [1,2] and was also demonstrated as an important process in tissue regeneration in cell transplantation [3–5]. The later offers possibilities for targeted cell therapy for organ regeneration. In addition, hybrid cells can be useful especially in biotechnology for production of monoclonal antibodies [6,7] and in biomedicine for the production of hybrid cell vaccines for immunotherapy of cancer [8].

Hybridoma technology is the most often used procedure for producing monoclonal antibodies [12]. The critical step within this procedure is fusion of myeloma cells with B-lymphocytes to form hybridoma cells, which grow in culture and produce these important biological molecules. Myeloma cells are “fusion partner” cells that grow in culture and lymphocytes are the cells that produce antibodies. After fusion, cells are plated in HAT selection media to obtain only cells that are constituted from both types of cells. In some cases, where mouse or hen cells are used, fusion with polyethylene glycol [9] and electrofusion give good results [10,11], however the use of human lymphocytes is favored. The use of human lymphocytes would give us human monoclonal antibodies, which are more valuable than mouse monoclonal antibodies for use in human therapy.

Also promising are hybrid cells made of dendritic cells and autologus tumor cells. These hybrid cells could be used as a vaccine in cancer immunotherapy. Dendritic cells are most powerful antigen presenting cells that activate naive T lymphocytes to generate cytotoxic effectors (cytotoxic T lymphocytes). Hybrid autologus tumor–dendritic cells would thus express specific tumor antigens and be able to activate T cell mediated responses [8]. Due to low efficiency of fusion by means of polyethylene glycol, it is however not possible to produce hybrid cells in sufficient quantities for the therapy with this method. Hybrid cells must therefore be further grown in the culture, thus it is difficult to obtain sufficient number of cells for therapy in adequately short time.

Fusion of human cells is however most often unsuccessful. The compromise for hybridoma technology is fusing human cells or human B-lymphocytes with mouse or hen myeloma cells, respectively. The efficiency of such fusion with polyethylene glycol is however not good enough for efficient production of monoclonal antibodies. The alternative procedure for obtaining human monoclonal antibodies or hybrids of dendritic and autologus tumor cells, that can be more efficient than polyethylene glycol, is cell electrofusion. For cancer immunotherapy, investigators suggested that electrofusion is an effective method [13]; justified to be used in clinical trials besides previously used fusion by means of polyethylene glycol [14]. For production of human monoclonal antibodies from hybrids made of

* Corresponding author. Tel.: +386 1 4768456; fax: +386 1 4264658.
E-mail address: damijan.miklavcic@fe.uni-lj.si (D. Miklavčič).

1567-5394/$ – see front matter
doi:10.1016/j.bioelechem.2008.06.003

human lymphocytes and mouse or even human fusion partner cell lines, no such comparative study of both fusion techniques (polyethylene glycol and electrofusion) has been done before.

Cell electrofusion is a simple and safe method that does not introduce any substances in the cell suspension so it can be safely used in all clinical applications, which is clearly an advantage over chemical or viral methods. Furthermore, electrofusion effectiveness can be further improved by optimizing electrical parameters that affect its efficiency. Cells fuse only when they are brought into their fusogenic state. This fusogenic state seems to correlate well with the permeabilized state of the membrane [15]. To achieve cell fusion we must have cells with permeabilized membranes in close contact. Therefore, in order to achieve the highest fusion yield, we must choose values of electrical parameters, which cause membrane permeabilization and good survival of fusion partner cells.

The most important and known electrical parameters governing membrane electropermeabilization are pulse amplitude, which enlarges permeabilized area and pulse duration and number of pulses, which enlarge the density of membrane defects [16]. Another way of enlarging the permeabilized area without reducing the survival of the cells that has not been studied until lately is changing electric pulse direction [17]; i.e. delivering electrical pulses in different directions to the cells. From the theory of electroporation [18,19] and already performed experiments [20,21] it follows that applying pulses to cells in different directions causes permeabilization of different areas of the cell membrane. Application of pulses in different directions thus increases the total permeabilized area of the membrane.

It was demonstrated that a prerequisite for cell fusion is that membranes of both cell fusion partners in contact are in their fusogenic state [22]. Contact between cells after exposing them to electric pulses in electrofusion is most often established by centrifugation of cells in suspension. Since in centrifugation contacts between cells create randomly between already electroporated cells, increased permeabilized area should increase the probability of creating adequate contact between membranes in fusogenic state of two neighboring cells.

In our study, we therefore first investigated the influence of the electric field direction on electropermeabilization and subsequent electrofusion in two cell lines (B16F1 and CHOK1, respectively). In the second part of our study, electrofusion was used for the first time for production of human–mouse heterohybridoma cells with modified Koehler and Milstein hybridoma technology [12] and compared to the most often used polyethylene glycol mediated cell fusion.

2. Materials and methods

2.1. Cells

In the first part of our work, we used two adherent cell lines. Chinese hamster ovary cells (CHOK1) were grown in HAM medium with added 10% Fetal Calf Serum (both from Sigma, USA). Mouse melanoma cells (B16F1) were grown in Eagle's Minimum Essential Medium (EMEM) with added 10% Fetal Calf Serum (both from Sigma, USA). After trypsinization, cells were centrifuged for 5 min at 1000 rpm at 4 °C and resuspended in isoosmolar low conductance (pulsing medium) to obtain 5×10^6 cells/ml. This medium with pH 7.4 consists of 250 mM sucrose, 10 mM phosphate (K_2HPO_4/KH_2PO_4) and 1 mM $MgCl_2$ as was previously described elsewhere [23].

For production of heterohybridoma in the final part of our study, we used human spleen lymphoblasts and NS1—mouse myeloma cells—as fusion partners. Lymphoblasts were isolated and frozen in liquid nitrogen. One week before the experiment, they were thawed and kept in DMEM medium with added 13% Fetal Calf Serum (both from Sigma, USA). HAT-sensitive NS1 myeloma cells were also cultured in DMEM medium with added 13% Fetal Calf Serum. The myeloma cells were used for fusion when they were in exponential growth phase.

2.2. Electropermeabilization

A 100 µl droplet of cells suspended in the pulsing medium ($\approx5\times10^5$ cells) was taken and placed between four cylinder stainless steel electrodes [17] of diameter 2 mm, which were positioned in corners of a quadrant with a distance between the opposite electrodes $d=5$ mm. The entrapped droplet wetted all four electrodes and thus formed electric contact between all four of them (Fig. 1a).

Cells were exposed to three different combinations of 6 or 10 pulses with the amplitude of 400 or 500 V. In all experiments, pulses were 100 µs long and their repetition frequency was 77 Hz. Each combination of the pulses was further used in three different pulsing sequences (Fig. 1b) which resulted in different electric field directions of the pulses (same direction, opposite directions and two directions perpendicular to each other).

After exposure of cells to electric pulses, cell suspension was transferred by micropipette from the place between the electrodes to the 24-microtiter plate holes. Propidium iodide (Sigma, USA) was used to determine the degree of permeabilization of cells [24]. This nonpermeant fluorescent dye was added to the cell suspension before electroporation in quantity that gave 0.01 mM concentration of propidium iodide in the cell suspension.

Propidium iodide enters the cells when they are permeabilized as described earlier [24] and binds on cell's DNA. When bound, its fluorescence increases 1000 times. Propidium iodide is toxic and eventually enters in nonpermeabilized cells as well so all the measurements must be finished in less than 30 min after the addition of the dye.

Permeabilization was determined as the ratio between the number of fluorescent cells and the total number of cells in the field of view. We observed cells under the inverted fluorescent microscope Axiovert 200 (Zeiss, Germany). Phase contrast and fluorescence images of the same areas were taken between 5 and 9 min after the electroporation with digital IMAGO CCD camera VISICAM 1280 (Visitron, Germany) with the resolution 1280×1024 pixels and were analyzed with Meta Morph 5.0 (Visitron, Germany). Excitation was set at 510 nm

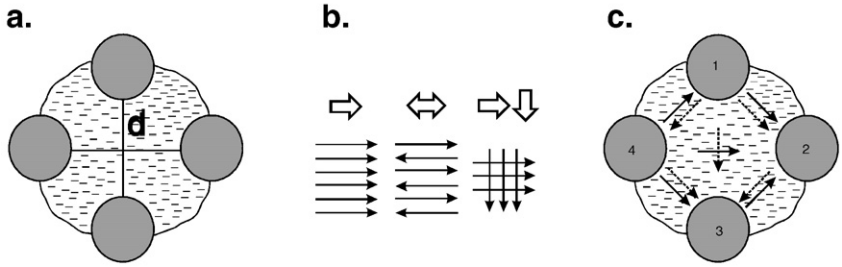

Fig. 1. a) Schematic of electrodes and the drop of cell suspension between them. b) Directions of pulsing sequences: pulses in same directions (⇨), pulses in opposite directions (⇔) and pulses in two directions perpendicular to each other (⇨ ⇩). c) At treatments with pulses in perpendicular directions, pulses were applied between opposite electrodes. Resulting electric field is depicted with dashed arrows (for pulses applied between electrodes 1 and 3) and solid arrows (for pulses applied between electrodes 2 and 4).