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

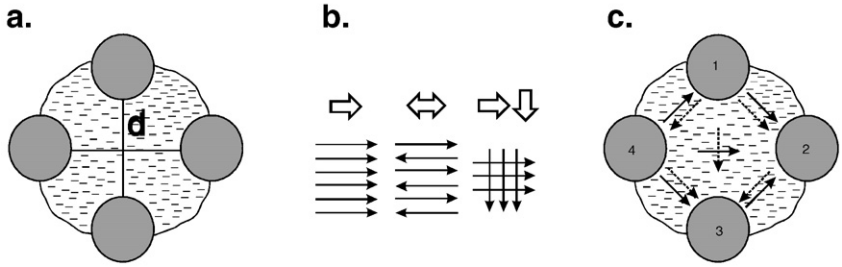

Fig. 1. a) Schematic of electrodes and the drop of cell suspension between them. b) Directions of pulsing sequences: pulses in same directions (⇨), pulses in opposite directions (⇔) and pulses in two directions perpendicular to each other (⇨ ⇩). c) At treatments with pulses in perpendicular directions, 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).

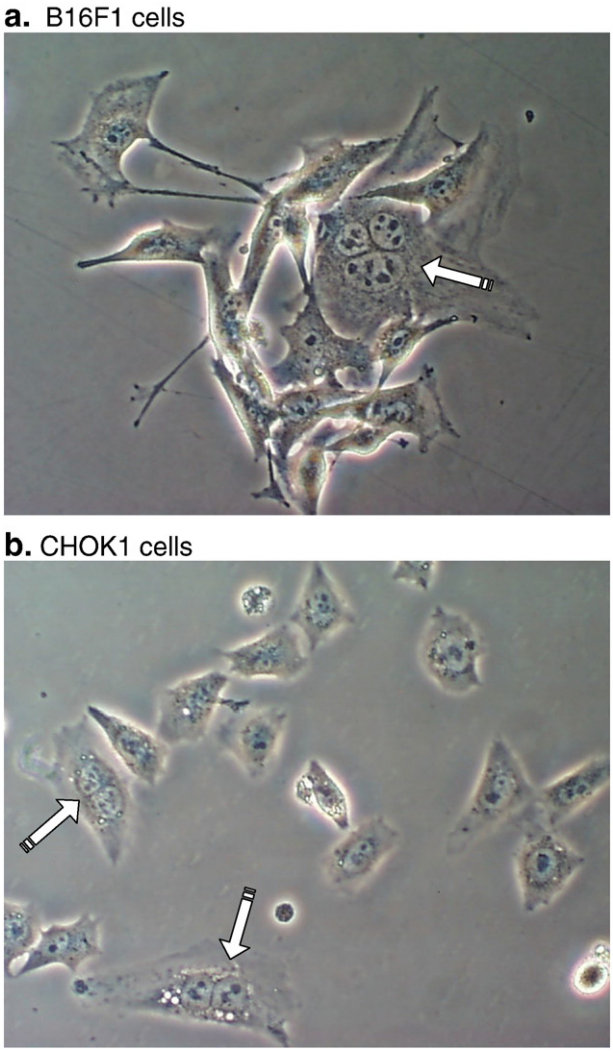

Fig. 2. Phase contrast pictures with 20× magnification a) B16F1 cells, cell marked with an arrow has three nuclei. b) CHOK1 cells: cells, marked with arrows have two nuclei.

wavelength and emission detected between 565 nm and 595 nm (Rhodamin filter BP 580/30).

2.3. Electrofusion

For electrofusion, we used the protocol where contact between cells was established by means of centrifugation as previously described [25,26]. After exposing cells to electric field (see Section 2.2), cell suspension was transferred by micropipette to a centrifuge tube and in a centrifuge in less than 20 s after the electroporation. The electroporated cells were centrifuged 5 min at 525 rpm at 4 °C. Next 10 min cells in centrifuge tubes were incubated at 37 °C and 5% CO_2. After this procedure, cells were placed in Petri dishes and incubated at 37 °C and 5% CO_2 for 24 h.

We observed adhered cells with inverted optical microscope CK40 (Olympus, Japan). Images were taken from live cells in the medium or from cells fixed first with absolute methanol and dyed with Giemsa dye. For each experiment, we counted at least 400 cells. All experiments were repeated three times.

Fusion yield FY was calculated as a number of nuclei in multinucleated cells divided by number of all nuclei.

$$FY = \frac{\text{number of nuclei in multinucleated cells}}{\text{number of all nuclei}} \quad (1)$$

FY values can be from zero to one. Zero means there are no polynucleated cells, at one all cells would be polynucleated. In the next step, we considered the survival of the cells (S) in each treatment. We defined number of nuclei in control treatment (no electric pulses) as 100% (all cells survive). We determined the survival S in different treatments according to this number. Actual fusion yield FY* was then calculated considered cell survival, thus correcting FY.

$$FY^* = FY \cdot S[\%] \quad (2)$$

FY* values can be from zero to one, because FY is between zero and one and survival S is between zero and hundred percent, but FY* is always smaller than FY since S is smaller than 100%. Furthermore, in practical use of fused cells, we are usually not interested in their nuclei number but in the yield of functional fused cells. Namely some fused cells can contain large (>2) number of nuclei, which reduces the number of cells obtained. On the picture 2a, B16F1 cell with three nuclei can be seen and on the picture 2b, two CHOK1 cells with two nuclei can be seen.

We thus considered the average number of nuclei in the polynucleated cells for different treatments, so called index of

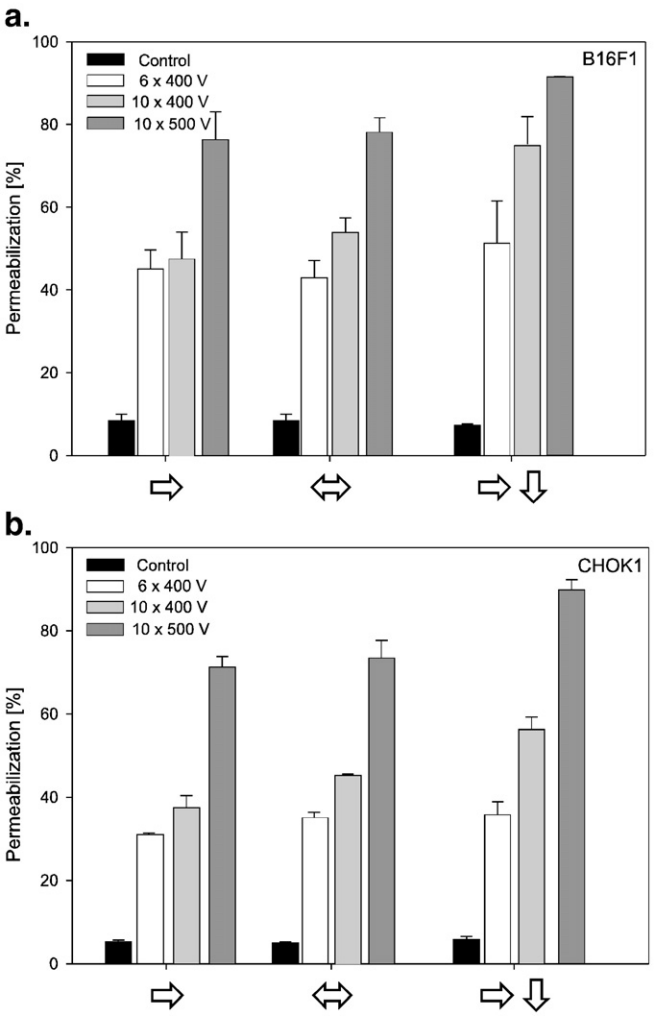

Fig. 3. Permeabilization of a) B16F1 and b) CHOK1 cells. Cells were pulsed in suspension. The length of square-wave pulses was 100 μs and repetition frequency of pulses was 77 Hz. Numbers of pulses applied were 6 and 10. The pulse amplitudes were 400 V and 500 V. Three different combinations of pulse directions were used: pulses applied in same directions (⇨), pulses applied in opposite directions (⇔) and pulses applied in two directions perpendicular to each other (⇨ ⇩). Values are given as a mean±SD.

polynucleation I_P. To obtain the yield of fused cells we calculated FY** as

$$FY^{**} = FY^{*} \cdot 2/I_P \quad (3)$$

I_p in ideal situation of the fusion would be two, meaning that each cell fused with one another cell resulting in all polynucleated cells having two nuclei. If the number of nuclei is higher than two, FY** is smaller than FY*. FY** is thus the most conservative estimate of fusion yield taking into account also survival and index of polynucleation (Fig. 2).

2.4. Hybridoma technology

For production of heterohybridoma, we used human spleen lymphoblasts and mouse myeloma NS1 cells. We mixed both cells in the ratio of 1:1 in the pulsing medium at the same concentration as for CHOK1 or B16F1 cells (5×10^6 cells/ml) and then fused them by using the same protocol as for CHOK1 or B16F1 cells.

After exposing cells to the electric field, as we described in Section 2.2, the cell suspension was transferred by micropipette to a centrifuge tube and into a centrifuge in less than 20 s after the electroporation. The electroporated cells were centrifuged for 5 min at 525 rpm at 4 °C. Next 10 min cells in centrifuge tubes were incubated at 37 °C and 5% CO_2.

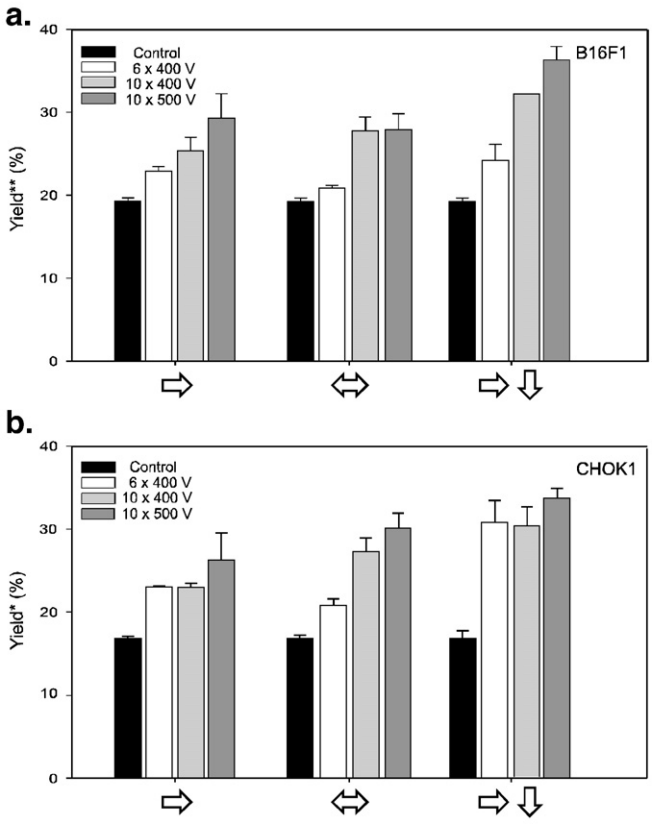

Fig. 4. Fusion yield of a) B16F1 and b) CHOK1 cells. Note: FY** is given for B16F1 and FY* is given for CHOK1. Cells were pulsed in suspension. The length of square-wave pulses was 100 μs and frequency of pulses was 77 Hz in all experiments. Numbers of pulses applied were 6 and 10. The pulse amplitudes were 400 V and 500 V. Three different combinations of pulse directions were used: pulses applied in same directions (⇨), pulses applied in opposite directions (⇔) and pulses applied in two directions perpendicular to each other (⇨ ⇩). Fusion yield presented is corrected for survival and polynucleation of cells in each treatment for B16F1 cells and for survival only for CHOK1 cells. Values are given as a mean±SD.

Table 1
Survival of B16F1 and CHOK1 cells in the experiments of cell fusion

Survival of cells [%]		6 pulses, 100 μs, 400 V	10 pulses, 100 μs, 400 V	10 pulses, 100 μs, 500 V
B16F1	⇨	101.8±0.02	103.6±0.05	104.1±0.1
	⇔	95.0±0.1	103.5±0.2	96.7±0.1
	⇨ ⇩	91.9±0.1	92.1±0.1	86.9±0.1
CHOK1	⇨	90.2±0.1	98.8±0.3	87.9±0.1
	⇔	85.1±0.2	86.5±0.2	84.1±0.2
	⇨ ⇩	82.6±0.1	80.3±0.1	77.2±0.2

Values are given as a mean±SD.

After the electrofusion procedure, cells were resuspended in DMEM medium with 13% FCS and transferred in units of 5×10^4 cells to the 96-well culture plates.

After 24 h, HAT selection medium was added and replaced with HT medium after two weeks. About 3 weeks later heterohybridomas were counted under inverted microscope and transferred into conventional 24-well culture plates for growing.

Results (permeabilization and fusion yield) are given in a form of multiple bar graphs (SigmaPlot 9.0, Systat, USA) where every point represents the mean of three independent experiments and the error bars indicate the standard deviation (Figs. 3 and 4). Statistical test, One way analysis of variance (One way ANOVA), was performed on all results (SigmaStat 3.1, Systat, USA). Bonferroni *t*-test was performed on results if there was indication of a statistically significant difference between different electric field protocols used.

3. Results

3.1. Electropermeabilization

The permeabilization of cell membrane and the percentage of permeabilized cells in suspension are increased with an increase in the pulse amplitude and with the number of pulses as expected and described before, and can be seen in Fig. 3a and b (pulses delivered in the same directions). The effect of electric field direction is however more interesting because its role in cell electrofusion has not been extensively studied yet.

Pulsing cells in two opposite directions caused almost no difference in permeabilization in comparison to pulsing cells in one direction while permeabilization is increased for the same pulse amplitude and number of pulses by using pulses in two perpendicular directions ($p<0.001$). The effects of the observed parameters were similar for both cell lines under investigation, the B16F1 and CHOK1 cells (Fig. 3a and b).

3.2. Electrofusion

The fusion yield FY was generally increased with an increase in the pulse amplitude and in the number of pulses (Fig. 4) although number of pulses and pulse amplitudes were chosen close together so that the

Table 2
Polynucleation index for fusion of B16F1 and CHOK1 cells

Polynucleation index [I_P]		6 pulses, 100 μs, 400 V	10 pulses, 100 μs, 400 V	10 pulses, 100 μs, 500 V
B16F1	⇨	2.3±0.2	2.2±0.2	2.2±0.1
	⇔	2.3±0.2	2.2±0.2	2.2±0.1
	⇨ ⇩	2.2±0.1	2.3±0.1	2.2±0.1
CHOK1	⇨	2.1±0.05	2.1±0.1	2.1±0.05
	⇔	2.1±0.1	2.0±0.1	2.1±0.02
	⇨ ⇩	2.4±0.2	2.2±0.2	2.3±0.2

Values are given as a mean±SD.

Table 3
Results of fusion of human lymphoblasts and myeloma cells

Pulse amplitude [V]	Number of pulses	Length of pulses [μs]	Wells seeded	Wells where clones appeared	Viable hybridoma
400	6	100	27	/	/
400	10	100	27	/	/
500	10	100	70	21 [30%]	5
500	10	200	18	6 [33%]	1
500	10	500	27	2 [7%]	/

differences were small. In control samples (without electrical pulses delivered), we observed polynucleated cells that are always present in the cell culture. For CHO cells there was approximately 17% and for B16F1 cells 19% of inherently polynucleated cells.

The fusion yields shown are multiplied with the cell survival (Table 1) according to Eq. (2) in order to take into account also the cell survival. Survival of CHOK1 cells was consistently lower than the survival of B16F1 cells for about 10%.

For each pulse combination, we also evaluated index of polynucleation—i.e. the average number of nuclei in one polynucleated cell (Table 2). The level of polynucleation was between 2.0 and 2.4; it was the same in both cell lines and did not depend on different protocols used. When we took into account the level of polynucleation (Eq. (3)), most of the significant differences between treatments for CHOK1 cells were no longer present. The differences in fusion yield between treatments with different number of pulses remained noticeable also when we took into account the level of polynucleation.

3.3. Hybridoma technology

In our present work, we also used electrofusion to produce human–mouse heterohybridoma cells from human lymphocytes and mouse myeloma cell line NS1 with modified Koehler and Milstein hybridoma technology. We were able to obtain viable human–mouse heterohybridoma in amounts that are comparable to those obtained by fusing the same cells by means of polyethylene glycol (data not published).

We used the treatment procedures that worked best on B16F1 and CHOK1 cells (pulses in two directions perpendicular to each other) and also treatments with prolonged pulses (Table 3). Our results show that treatments with pulse amplitudes of 400 V are suboptimal for making lymphocytes fusogene and treatments with ten pulses 500 μs long were probably too strong for myeloma cells to survive.

4. Discussion

In our present study, we examined the effect of different electric pulse parameters on electropermeabilization and electrofusion in two cell lines. We were especially interested in the effect that the direction of the electric field might have on cell fusion. Even though we intentionally worked at suboptimal conditions in order to be able to detect expected differences with changing the direction of electric field, fusion yield obtained in our study was comparable to the fusion yield obtained by means of electrofusion by other researchers [27–29]. At optimal values of studied parameters, the values of fusion yields, after the subtraction of the polynucleated cells in control samples, are approaching 20%.

The effect of the electric field direction on fusion yield is similar as for permeabilization of cells. We did not observe the difference between pulsing cells in one direction and pulsing cells in two opposite directions. Fusion yield is however increased when pulsing cells in two perpendicular directions for the same pulse amplitude and number of pulses.

We observed consistently lower survival of CHOK1 cells than of B16F1 cells for about 10%. Since B16F1 cells are somewhat larger than CHOK1 cells, the difference in the survival is not due to the cell size effect on the sensitivity of the cells to the electric field. It was shown that B16F1 cells are less sensitive to electric field due to their biological properties [30]. The other reason is probably also the larger sensitivity of CHOK1 cells to mechanical and other manipulation.

The level of polynucleation was between 2.0 and 2.4 and did not depend on different protocols used. The level of polynucleation is however important, because obtaining cells with huge number of nuclei reduces the number of polynucleated cells and additionally large cells are less likely to survive and divide. The ideal level of polynucleation values therefore should not be much higher than 2.

The observed effects due to different parameters used, were similar for both investigated cell lines although the differences were more pronounced for the B16F1 cell line (Fig. 4). For CHOK1 cells we have shown only the FY*, because at the FY** level significant differences between different treatments were not apparent, while for the B16F1 cells the differences remained visible also when we further took into account the level of polynucleation.

The degree of permeabilization and fusion yield was higher at treatments with higher pulse amplitude and larger number of pulses. This effect of pulse amplitude and number is in agreement with Teissie and Ramos, 98 [15]. In their study, they observed a strong correlation between permeabilization and fusion.

In our study, we focused on observing differences between the treatments delivering pulses in different directions. We obtained the highest fusion yield when delivering pulses in two directions perpendicular to each other, while the effect of using pulses in two opposite directions was not significantly different when compared to delivering pulses in the same direction. That was expected and it is in agreement with the theory of electroporation [17,18] and experiments done by others [20,31].

The differences between different electric protocols used, however, are not large. The reason for that in our opinion is in the design of the electrodes. Only part of the cells in suspension drop was actually exposed to the pulses in claimed directions. This effect is most obvious when pulses are applied in two directions perpendicular to each other. Large outer part of the cell suspension is actually exposed only to the field in the same or in two opposite directions (Fig. 1c). The design of the electrodes has since been improved in order to resolve this problem [32].

In our study, we also noticed that survival for treatments, where the electric field direction was changed, was lower than in the treatments where pulses were applied in only one direction. The reason for that is probably in the fact that in the case of changing electric field more cells is in close proximity to an energized electrode and therefore a high electric field. In our design of new electrodes, we have predicted the possibility of covering the surface of the electrodes with the filler material, which would conduct the electric current in the same range as our medium and on the other hand, it would function as a mechanical barrier between the cells and the electrode surface. This barrier would exclude cells from the proximity of the electrode surface and thus reduce the number of cells being killed while applying electric field. As it was suggested already by Schmeer [33].

The advantage of our method of detection of fused cells by counting nuclei after 20 to 24 h is that we count only the cells that actually survived. This is important, because only cells that actually survive and divide are useful in obtaining hybrid cells for monoclonal antibodies or hybrid autologus tumor–dendritic cells production. On the other hand, this method has a drawback that nuclei in fused cells can also fuse [34]. Therefore, in estimation of fusion yield we depended also on cell and nuclei sizes, which are not linearly dependent on nuclei number in the cell and therefore cannot be relied on completely.

In the second part of our study, we used electrofusion technique in hybridoma technology for fusion of smaller human lymphoblasts with

larger mouse myeloma cells NS1 as fusion partners. The yield of heterohybridomas is never as good as the yield of mouse–mouse or human–human hybridomas due to the chromosome loss [12]. We succeeded in producing heterohybridomas in the amounts that are comparable to those previously obtained by the means of polyethylene glycol.

Furthermore, we used electrical conditions that were the most successful in the first part of our study. This can be useful for comparing the results but was not necessarily optimal for fusion of human lymphoblasts with NS1 cells. Lymphoblasts are namely approximately twice smaller in diameter than NS1 cells and are therefore less prone to electropermeabilization under the same conditions than larger NS1 cells. For permeabilization of lymphoblasts, we would need higher pulse amplitude or number of pulses than for permeabilization of myeloma NS1 cells [35]. On the other hand, it was shown in the case of primary and transformed human amnion cells that primary cells are more sensitive to electric field than transformed cells [36]. Since lymphoblasts are primary cells, they could be more sensitive to electric field even though they are of smaller size.

In our experiments, we observed that after exposure to electric pulses myeloma cells were in bad shape. That makes us believe that they were seriously damaged by exposure to electric pulses. At the same time, treatments with longer pulses, higher pulse amplitude or more pulses were needed for obtaining hybridoma (Table 3). This supports our conclusion that lymphoblasts were fusogenic only after treatments with longer pulses, larger pulse amplitude or more pulses. We expect that pulses with even larger amplitude could be more successful due to enlarged area of permeabilized membrane [37] but current electrode design and pulse generator limitations did not allow us to use them. If we could use larger amplitudes, we would most probably encounter a problem of viability of the NS1 cells, which were permeabilized at the same time with the lymphoblasts. Therefore, we suggest raise of myeloma cells fraction from half as used in our study to 90% or separate permeabilization of different types of cells and consequent fusion in order to achieve optimal fusogenicity and survival of both fusion partner cells.

Acknowledgements

This research was in part supported by the Agency for Research (ARRS) of the Republic of Slovenia, the Proteus program of scientific, technological and cultural cooperation between CNRS (project no. 5386), France and Ministry for research and higher education (Proteus program), Slovenia and the PICS program between CNRS (project no. 3212), France and Ministry for research and higher education (PICS program), Slovenia. We thank Dr. Justin Teissie for valuable discussion of procedures and results.

References

[1] R. Heller, R.J. Grasso, Transfer of human membrane surface components by incorporating human cells into intact animal tissue by cell-tissue electrofusion in vivo, Biochim. Biophys. Acta 1024 (1990) 185–188.
[2] J. Teissie, Transfer of foreign receptors to living cell surfaces: the bioelectrochemical approach, Bioelectrochemistry and Bioenergetics 46 (1998) 115–120.
[3] X. Wang, H. Willenbring, Y. Akkari, Y. Torimaru, M. Foster, M.A. Dhalimy, E. Lagasse, M. Finegold, S. Olson, M. Grompe, Cell fusion is the principal source of bone-marrow-derived hepatocytes, Nature 422 (2003) 897–901.
[4] G. Vassilopoulos, P.R. Wang, D.W. Russell, Transplanted bone marrow regenerates liver by cell fusion, Nature 422 (2003) 901–904.
[5] M. Alvarez-Dolado, R. Pardal, J.M. Garcia-Verdugo, J.R. Fike, H.O. Lee, K. Pfeffer, C. Lois, S.J. Morrison, A. Alvarez-Buylla, usion of bone-marrow-derived cells with Purkinje neurons, cardiomyocytes and hepatocytes, Nature 425 (2003) 968–973.
[6] P. Kufer, R. Lutterbuese, P.A. Baeuerle, A revival of bispecific antibodies, Trends Biotechnol. 22 (2004) 238–244.
[7] Martin J. Glennie, Peter W.M. Johnson, Clinical trials of antibody therapy, Immunology oday 21 (2000) 403–410.
[8] T.H. Scott-Taylor, R. Pettengell, I. Clarke, G. Stuhler, M.C. La Barthe, P. Walden, A.G. Dalgleish, Human tumor and dendritic cell hybrids generated by electrofusion: potential for cancer vaccines, Biochim. Biophys. Acta 1500 (2000) 265–279.
[9] E. Harlow, D. Lane, Antibodies — Laboratory Manual, Cold Spring Harbor Laboratory, 1988.
[10] L.H. Li, M.L. Hensen, Y.L. Zhao, S.W. Hui, Electrofusion between heterogeneous-sized mammalian cells in a pellet: potential applications in drug delivery and hybridoma formation, Biophys. J. 71 (1996) 479–486.
[11] S.W. Hui, The application of electroporation to transfect hematopoietic cells and to deliver drugs and vaccines transcutaneously for cancer treatment, Technol. Cancer Res. Treat. 1 (2002) 373–384.
[12] M.A. Ritter, H.M. Ladyman, Monoclonal Antibodies, Production, Engineering and Clinical Application, Cambridge University Press, 1995.
[13] P.A. Elder, P.T. Gaynor, J.G. Lewis, P.S. Bodger, L.M. Bason, Generation of monoclonal progesterone antibodies by electrofusion techniques, Bioelectrochem. Bioenerg. 43 (1997) 35–40.
[14] M. Lindner, V. Schirrmacher, Tumour cell–dendritic cell fusion for cancer immunotherapy: comparison of therapeutic efficiency of polyethylen-glycol versus electro-fusion protocols, Eur. J. Clinic. Inv. 32 (2002) 207–217.
[15] J. Teissie, C. Ramos, Correlation between electric field pulse induced long-lived permeabilization and fusogenicity in cell membranes, Biophys. J. 74 (1998) 1889–1898.
[16] C. Ramos, J. Teissie, Tension–voltage relationship in membrane fusion and its implication in exocytosis, FEBS Lett. 465 (2000) 141–144.
[17] M. Reberšek, C. Faurie, M. Kandušer, S. Čorović, J. Teissie, M.P. Rols, D. Miklavčič, Electroporator with automatic change of electric field direction improves gene electrotransfer *in-vitro*, Biomed. Eng. (OnLine) 6 (2007).
[18] B. Valič, M. Golzio, M. Pavlin, A. Schatz, C. Faurie, B. Gabriel, J. Teissie, M.P. Rols, D. Miklavčič, Effect of electric field induced transmembrane potential on spheroidal cells: theory and experiment, Eur. Biophys. J. 32 (2003) 519–528.
[19] T. Kotnik, L.M. Mir, K. Flisar, M. Puc, D. Miklavčič, Cell membrane electropermeabilization by symmetrical bipolar rectangular pulses Part I, Increased efficiency of permeabilization, Bioelectrochemistry 54 (2001) 83–90.
[20] D. Miklavčič, Electrodes and corresponding electric field distribution for effective in vivo electroporation, in: T. Kotnik, D. Miklavčič (Eds.), Medicon 2001, 2001, pp. 5–9.
[21] C. Faurie, E. Phez, M. Golzio, C. Vossen, J.C. Lesbordes, C. Delteil, J. Teissie, M.P. Rols, Effect of electric field vectoriality on electrically mediated gene delivery in mammalian cells, Biochim. Biophys. Acta 1665 (2004) 92–100.
[22] G.B. Melikyan, L.V. Chernomordik, Electrofusion of lipid bilayers, in: E. Neumann, A.E. Sowers, C.A. Jordan (Eds.), Electroporation and Electrofusion in Cell Biology, Plenum Press, New York, 1989, pp. 181–192.
[23] C. Blangero, J. Teissie, Ionic modulation of electrically induced fusion of mammalian-cells, J. Membr. Biol. 86 (1985) 247–253.
[24] M.P. Rols, J. Teissie, Flow cytometry quantification of electroporation, in: M. Jaroszeski, R. Heller (Eds.), Methods in Molecular Biology: Flow Cytometry Protocols, Humana Press, 1998, pp. 141–148.
[25] J. Teissie, M.P. Rols, Fusion of mammalian cells in culture is obtained by creating the contact between cells after their electropermeabilization, Biochem. Biophys. Res. Commun. 140 (1986) 258–266.
[26] A.E. Sowers, A long-lived fusogenic state is induced in erythrocyte ghosts by electric pulses, J. Cell Biol. 102 (1986) 1358–1362.
[27] C. Ramos, D. Bonenfant, J. Teissie, Cell hybridization by electrofusion on filters, Anal. Biochem. 302 (2002) 213–219.
[28] M.P. Rols, J. Teissie, Ionic-strength modulation of electrically induced permeabilization and associated fusion of mammalian cells, Eur. J. Biochem. 179 (1989) 109–115.
[29] M.P. Rols, J. Teissie, Modulation of electrically induced permeabilization and fusion of Chinese hamster ovary cells by osmotic pressure, Biochemistry 29 (1990) 4561–4567.
[30] M. Kanduser, M. Sentjurc, D. Miklavcic, Cell membrane fluidity related to electroporation and resealing, Eur. Biophys. J. 35 (2006) 196–204.
[31] B. Gabriel, J. Teissie, Mammalian cell electropermeabilization as revealed by millisecond imaging of fluorescence changes of ethidium bromide in interaction with the membrane, Bioelectrochem. Bioenerg. 47 (1998) 113–118.
[32] K. Trontelj, M. Reberšek, D. Miklavčič, The tip electrode chamber for small volume electroporation, electrofusion and gene transfection, PCT/SI2007/000036.
[33] M. Schmeer, Elektrische Kenngrossen für die elektroporative Wirkstoff-Einschleusung und den DNA-Transfer in Zentrifugal-Aggregate von CHO-Zellen als Gewebemodell, PhD Thesis. Bielefeld, Bielefeld University, 2004, p 103.
[34] D.J. Prockop, C.A. Gregory, J.L. Spees, One strategy for cell and gene therapy: harnessing the power of adult stem cells to repair tissues, PNAS 100 (2003) 11917–11923.
[35] S. Sixou, J. Teissie, Specific electropermeabilization of leucocytes in a blood sample and application to large volumes of cells, Biochim. Biophys. Acta 1028 (1990) 154–160.
[36] S. Kwee, B. Gesser, J.E. Celis, Electroporation of human cultured cells grown in monolayers Part 3. Transformed cells and primary cells, Bioelectrochem. Bioenerg. 28 (1992) 269–278.
[37] C. Ramos, J. Teissie, Electrofusion: a biophysical modification of cell membrane and a mechanism in exocytosis, Biochimie 82 (2000) 511–518.