J Membrane Biol (2010) 236:87–95
DOI 10.1007/s00232-010-9280-3

Use of Collagen Gel as a Three-Dimensional In Vitro Model to Study Electropermeabilization and Gene Electrotransfer

Saša Haberl · Mojca Pavlin

Received: 21 December 2009 / Accepted: 22 June 2010 / Published online: 18 July 2010

Abstract Gene electrotransfer is a promising nonviral method that enables transfer of plasmid DNA into cells with electric pulses. Although many in vitro and in vivo studies have been performed, the question of the implied gene electrotransfer mechanisms is largely open. The main obstacle toward efficient gene electrotransfer in vivo is relatively poor mobility of DNA in tissues. Since cells are mechanically coupled to their extracellular environment and act differently compared to standard in vitro conditions, we developed a three-dimensional (3-D) in vitro model of CHO cells embedded in collagen gel as an ex vivo model of tissue to study electropermeabilization and different parameters of gene electrotransfer. For this purpose, we first used propidium iodide to detect electropermeabilization of CHO cells embedded in collagen gel. Then, we analyzed the influence of different concentrations of plasmid DNA and pulse duration on gene electrotransfer efficiency. Our results revealed that even if cells in collagen gel can be efficiently electropermeabilized, gene expression is significantly lower. Gene electrotransfer efficiency in our 3-D in vitro model had similar dependence on concentration of plasmid DNA and pulse duration comparable to in vivo studies, where longer (millisecond) pulses were shown to be more optimal compared to shorter (microsecond) pulses. The presented results demonstrate that our 3-D in vitro model resembles the in vivo situation more closely than conventional 2-D cell cultures and, thus, provides an environment closer to in vivo conditions to study mechanisms of gene electrotransfer.

Keywords 3-D in vitro model · Collagen gel · Gene electrotransfer · GFP · Electropermeabilization · Propidium iodide · CHO cell

S. Haberl · M. Pavlin (✉)
Faculty of Electrical Engineering, University of Ljubljana, Tržaška 25, 1000 Ljubljana, Slovenia
e-mail: mojca.pavlin@fe.uni-lj.si

Introduction

A variety of biochemical methods have been developed to transfer genes into cells, but many of them have either low efficiency or potential side effects (Curiel et al. 1991; Wagner et al. 1992; Cotten and Wagner 1993; Simoes et al. 1998; Marshall 1999; Kikuchi et al. 1999; Hacein-Bey-Abina et al. 2002). In the 1970s a physical method named "electropermeabilization," or "electroporation," was described to introduce molecules into cells, where a temporary increase in membrane permeability was achieved by electric pulses (Neumann and Rosenheck 1972). The first biomedical application of electropermeabilization was developed in the late 1980 s for introducing poorly permeant anticancer drugs into cutaneous and subcutaneous tumor nodules (Mir et al. 1991, 1998; Miklavcic et al. 1998; Sersa et al. 2000; Sersa 2006; Byrne and Thompson 2006). It was also shown that electroporation of cells in physical contact induces cell fusion, a process known as "electrofusion" (Vienken and Zimmermann 1985; Usaj et al. 2010). In the 1980s successful transfection of a gene into eukaryotic cells by applying electric pulses was achieved (Neumann et al. 1982; Potter 1988). Gene electrotransfer has since, due to its ease of application and efficiency, become a routine method for introducing foreign genes into bacterial (Drury 1996), yeast (Simon 1993), plant (Terzaghi and Cashmore 1997), and animal

(Andreason and Evans 1989; Rols and Teissie 1998) cells in vitro and into different tissues, including muscle (Aihara and Miyazaki 1998; Mir et al. 1999), tumors (Nishi et al. 1996; Rols et al. 1998), liver (Heller et al. 1996), and skin (Titomirov et al. 1991) in vivo. Nevertheless, mechanisms involved in gene electrotransfer in vitro or in vivo remain largely unknown.

The main obstacle for in vivo gene electrotransfer remains its efficiency since in tissues the diffusion of large molecules is impaired (Rols et al. 1998). Namely, extracellular matrix is thought to be one of the major barriers for successful gene electrotransfer in vivo (Zaharoff et al. 2002; Zaharoff and Yuan 2004; Cemazar et al. 2006).

Up to now many researchers have experimentally investigated various electroporation protocols to optimally deliver plasmid DNA into different tissues in vivo (Rols et al. 1998; Bettan et al. 2000; Cemazar et al. 2002; Andre et al. 2008; Tevz et al. 2008).

However, in vivo studies require large numbers of killed animals, and there are many factors (properties of tissue, such as differences in tissue organization, presence or absence of necrosis, overall tissue conductivity, the ability of cells to express transfected genes, cell density, and cell size) that influence gene electrotransfer efficiency (Somiari et al. 2000; Bettan et al. 2000). For this reason, the development of a reproducible three-dimensional (3-D) in vitro model of tissue is important since it would enable in vitro studies of gene electrotransfer, while classical in vitro experiments use plated monolayer cells which dramatically differ from cells in a 3-D environment.

Currently, different 3-D in vitro models of cell cultures are being employed in many areas of biomedical research, such as studying cell growth and mobility (Harkin and Hay 1996; Cukierman et al. 2001; Barralet et al. 2005; Hindie et al. 2006), tissue engineering (Chevallay and Herbage 2000) and cancer research (Kim et al. 2004; Lee et al. 2007). Also, studying cell behavior and differentiation in a simple 3-D in vitro model such as 3-D cell pellets has been reported (Ong et al. 2006; Bernstein et al. 2009). In addition 3-D spheroid models were used as models of tumors for analyzing transport of small molecules (Canatella et al. 2004; Wasungu et al. 2009) and for gene electrotransfer (Wasungu et al. 2009). However, up to now there has been no analysis of gene electrotransfer in a 3-D in vitro model made of collagen gel.

Therefore, the aim of our study was to develop a 3-D model which would enable studies of gene electrotransfer in an environment closer to in vivo conditions. We used CHO cells embedded in 3-D collagen gels to study electropermeabilization with uptake of propidium iodide (PI) and the effect of different plasmid concentrations and pulse durations on gene electrotransfer efficiency.

Materials and Methods

Preparation of Collagen Gel with Embedded Cells

Type I collagen from rat tail was obtained from Sigma-Aldrich (Deisenhofen, Germany) as a powder. Collagen solution was prepared on ice with diluted acetic acid (28.5 ml glacial acetic acid/l) to achieve a collagen concentration of 4.0 mg/ml and stored at 4°C.

After 24 h, collagen mixture was prepared by mixing 2.3 parts chilled collagen solution with 0.5 part Ham tissue culture medium for mammalian cells with 10% fetal bovine serum (FBS, Sigma-Aldrich) and 0.5 part 1× phosphate-buffered saline (PBS, pH 7.4). The pH of the mixture was adjusted to 7.2–7.6 with 0.1 M NaOH. To prevent gelation, the temperature of the mixture was maintained at 2–8°C.

Chinese hamster ovary cells (CHO-K1) were prepared as a cell suspension by 0.25% trypsin/EDTA solution (Sigma-Aldrich) and centrifuged for 5 min at 1,000 rpm ($180\times g$) at 4°C (Sigma-Aldrich). The cell pellet was resuspended with a liquid collagen mixture to a cell density of $\rho = 5.6 \times 10^5$ cells/ml. Collagen mixture (180 µl) with cells was pipetted into each space of a multiwell dish and stored for 1 h at 37°C in a humidified 5% CO_2 atmosphere in an incubator (Kambič, Semič, Slovenia). After raising the temperature to 37°C, collagen polymerized and formed a gel with embedded cells. Warm Ham culture medium for mammalian cells was gently added on top of the cells embedded in a gel as for normal culture. The plate was returned to the incubator and stored for 24 h at 37°C in a humidified 5% CO_2 atmosphere.

Plasmid DNA

Plasmid pEGFP-N1 (Clontech, Mountain View, CA) encoding green fluorescent protein (GFP) was amplified in DH5α strain of *Escherichia coli* and isolated with the HiSpeed Plasmid Maxi Kit (Qiagen, Hilden, Germany). The plasmid DNA concentration was spectrophotometrically determined at 260 nm and confirmed by gel electrophoresis.

Electropermeabilization

Standard electroporative medium (200 µl, pH 7.4; 10 mM NaH_2PO_4/Na_2HPO_4, 1 mM $MgCl_2$ and 250 mM sucrose) with 6 µl of 0.15 mM PI was added on top of CHO cells embedded in collagen gel. PI is a small molecule which enters a cell if the membrane of the cell is permeabilized (Ganeva et al. 1995). After 3-min incubation at room temperature (22°C), different pulsing protocols were used to monitor penetration of PI.

First, eight pulses lasting 5 ms at a frequency of 1 Hz were applied, with different electric field strengths

(0.2–1.2 kV/cm). Then, different pulse durations (8 × 200 μs, 8 × 1 ms, 8 × 5 ms, 8 × 10 ms) were used to deliver PI into the cells. In this experiment, an electric field strength of 0.8 kV/cm (applied voltage $U = 320$ V) and a repetition frequency of 1 Hz were used.

In all experiments, a pair of two-plate stainless-steel parallel electrodes with interelectrode distance $d = 4$ mm was used, which provided a homogenous electric field between the electrodes, $E = U/d$, where U is the applied voltage. Electrodes were dipped vertically into collagen gel, where cells were embedded and all cells in the 3-D collagen gel between the electrodes were exposed to the homogenous electric field.

For pulsing we used the GHT 1287 electroporator (Jouan, St. Herblain, France). An oscilloscope Wave Surfer^{TM} 422 (Le Croy, Chestnut Ridge, NY) monitored pulse shape. No electric pulses were applied to cells in the control sample.

PI transport into cells was monitored by observing the fluorescence of PI, which occurred when PI entered electropermeabilized cells. Fluorescence was detected by fluorescent microscopy (Axiovert 200; Zeiss, Gottingen, Germany) with excitation light at 530 nm generated with a monochromator system (PolyChrome IV; Visitron, Puchheim, Germany), and emission was detected at 617 nm. Images (see Fig. 2) were recorded using the MetaMorph imaging system (Visitron).

Gene Electrotransfer

Our study was divided into two sets of experiments. In the first part, the optimal concentration of added plasmid DNA was determined for our experimental conditions. In the second part, different pulse durations were tested for optimal concentration of plasmid DNA, as previously established. Electroporation was performed on 24-h-old cell culture with standard electroporative medium (pH 7.4; 10 mM NaH_2PO_4/Na_2HPO_4, 1 mM $MgCl_2$ and 250 mM sucrose).

On the day of the experiment, culture medium was removed and cells embedded in collagen gel were incubated with given concentrations of plasmid DNA that codes for GFP in electroporative media for 30 min at room temperature (22°C).

To determine the optimal concentration of plasmid DNA, cells embedded in collagen gel were incubated with different concentrations of plasmid DNA in electroporative medium (10, 60, 90, 125 μg/ml). The volume of added electroporative medium with DNA was 200 μl. A train of eight square pulses with duration of 5 ms and repetition frequency of 1 Hz was applied to deliver DNA into the cells. The amplitude of electric pulses applied was 320 V, which resulted in a homogeneous electric field between the electrodes of 0.8 kV/cm. No electric pulses were applied to cells in the control sample.

In the second part, the effect of different pulse durations on gene electrotransfer efficiency and cell viability was tested. Cells embedded in collagen gel were incubated with the optimal concentration of plasmid DNA, which was shown to be 90 μg/ml. Electric pulses of different durations were used to deliver DNA into the cells: 8 × 200 μs, 8 × 1 ms, 8 × 5 ms and 8 × 10 ms. Electric field strength was 0.8 kV/cm (applied voltage $U = 320$ V), with repetition frequency of 1 Hz for all pulsing protocols. For pulsing, the Jouan GHT 1287 electroporator was used. The distance between a pair of two-plate stainless-steel parallel electrodes was $d = 4$ mm. Electrodes were dipped vertically into collagen gel, where cells were embedded and all cells in the 3-D collagen gel between the electrodes were exposed to the homogenous electric field.

After exposing cells to electric pulses 70 μl of FCS (Sigma, St. Louis, MO) was added (35% of sample volume) to preserve cell viability. Cells were then incubated for 15 min at 37°C to allow cell membrane resealing and grown for 24 h in cell culture medium at 37°C in a humidified 5% CO_2 atmosphere in the incubator.

Gene electrotransfer efficiency was determined by fluorescent microscopy (Axiovert 200) with excitation light at 445 nm generated with a monochromator system (PolyChrome IV), and emission was detected at 488 nm. Images (see Fig. 1) were recorded using the MetaMorph imaging system. At least 10 fluorescence images were acquired in the area between the electrodes at ×10 objective magnification per parameter. Cells were counted manually and gene electrotransfer efficiency was determined by the ratio between the number of green fluorescent cells (successfully transfected) and the total number of cells. Two or three independent experiments were performed for each parameter, and the results are presented as mean values ± standard deviation.

Cell Viability

Cell viability was determined by measuring PI uptake 24 h after applying pulses with different durations. PI enters the cell if the membrane is damaged. Culture medium was removed and 200 μl of PBS with 6 μl of 0.15 mM PI was added to cells. After 5-min incubation, cell viability was determined by fluorescent microscopy. Images were recorded using the MetaMorph imaging system.

At least five fluorescence images were acquired in the area between the electrodes at ×10 objective magnification per parameter. Cells were counted manually and cell viability was determined by the ratio between the number of dead cells (cells with incorporated PI) and the total number of cells.

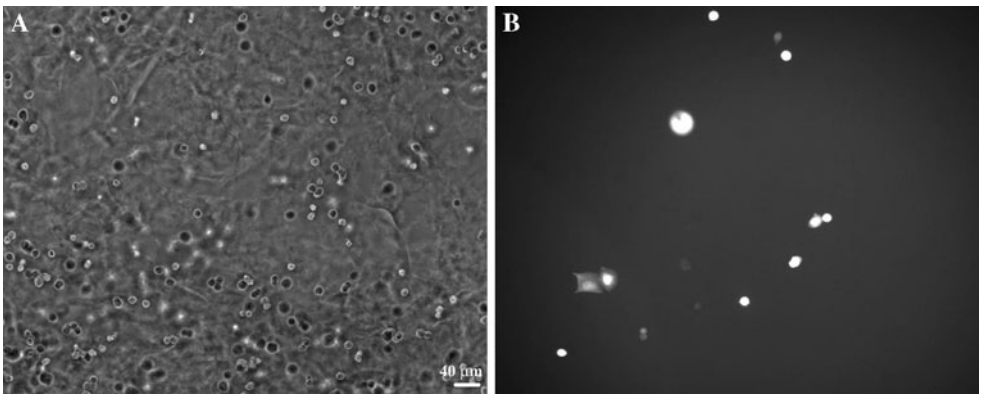

Fig. 1 Gene electrotransfer of CHO cells embedded in collagen gel 24 h after pulse application. Pulses of 8 × 10 ms ($E = 0.8$ kV/cm) were applied with repetition frequency of 1 Hz to deliver pEGFP (concentration of DNA in electroporative medium 90 μg/ml) into cells. **a** Phase-contrast image of treated cells. **b** Fluorescent image of cells expressing GFP protein (*white*). To visualize and quantify transfection, ×10 objective magnification was used

The total number of cells (for determining gene electrotransfer efficiency and cell viability) was difficult to obtain from phase-contrast imaging in 3-D. For this reason, we first determined at which pulsing parameters the entire cell population was permeabilized to PI. This we obtained for eight pulses lasting 5 ms, with an electric field strength of 1.2 kV/cm (applied voltage $U = 480$ V) and repetition frequency of 1 Hz. Therefore, after 5-min incubation with PI (described above), samples were exposed to electric pulses to permeabilize the whole cell population using the Jouan GHT 1287 electroporator. The distance between a pair of two-plate stainless-steel parallel electrodes was $d = 4$ mm. At least 10 fluorescence images were acquired in the area between the electrodes at ×10 objective magnification per parameter. Cells were counted manually.

Results

The main objective of our study was to develop a 3-D in vitro model of cells embedded in collagen gel which mimics the extracellular environment in tissues, to study the effect of different parameters on gene electrotransfer. Namely, a 3-D in vitro model would provide a more physiologically relevant approach for the analysis of gene electrotransfer than the conventional in vitro 2-D cell culture.

In the first part of the study, PI was used as a marker of efficient electropermeabilization and the electropermeabilization threshold was determined. In the second part of the study, gene electrotransfer of cells embedded in collagen gel was performed (Fig. 1). The effect of different concentrations of DNA on gene electrotransfer efficiency and the effects of pulse duration on gene electrotransfer efficiency and cell viability were analyzed.

Also, cell viability in our 3-D in vitro model was tested in control samples (samples where no electric pulses were applied), where >98% of cells survived (data not shown).

Electropermeabilization

Uptake of the impermeable dye PI was used to monitor electropermeabilization of CHO cells embedded in collagen gel and to determine the electropermeabilization threshold. Cells were subjected to eight pulses lasting 5 ms with different electric field strengths from 0.1 to 1.2 kV/cm. At 0.1 kV/cm, cells were not permeabilized (data not shown), while at 0.2 kV/cm, some cells were permeabilized (Fig. 2a). At 0.4 kV/cm approximately half of the cell population in collagen gel was permeabilized (Fig. 2b), and above 0.6 kV/cm the entire cell population was permeabilized to PI, meaning that for all cells the threshold for electropermeabilization was achieved (Fig. 2c–f).

We also tested different pulse durations (8 × 200 μs, 8 × 1 ms, 8 × 5 ms, 8 × 10 ms) to deliver PI into the cells embedded in collagen. The applied electric field strength was 0.8 kV/cm. At 8 × 200 μs, >80% of the cell population was permeabilized, while at 8 × 1, 8 × 5 and 8 × 10 ms, all cells in collagen gel were permeabilized (data not shown).

Gene Electrotransfer

To establish which plasmid DNA concentration is optimal for our protocol, we first determined the efficiency of gene electrotransfer for different plasmid concentrations. The efficiency of the transferred GFP gene was analyzed by fluorescent microscopy 24 h after electric pulse application. The electroporation protocol was the same for all concentrations of DNA: applied electric field strength 0.8 kV/cm, 8 × 5 ms pulses with 1-Hz repetition frequency.

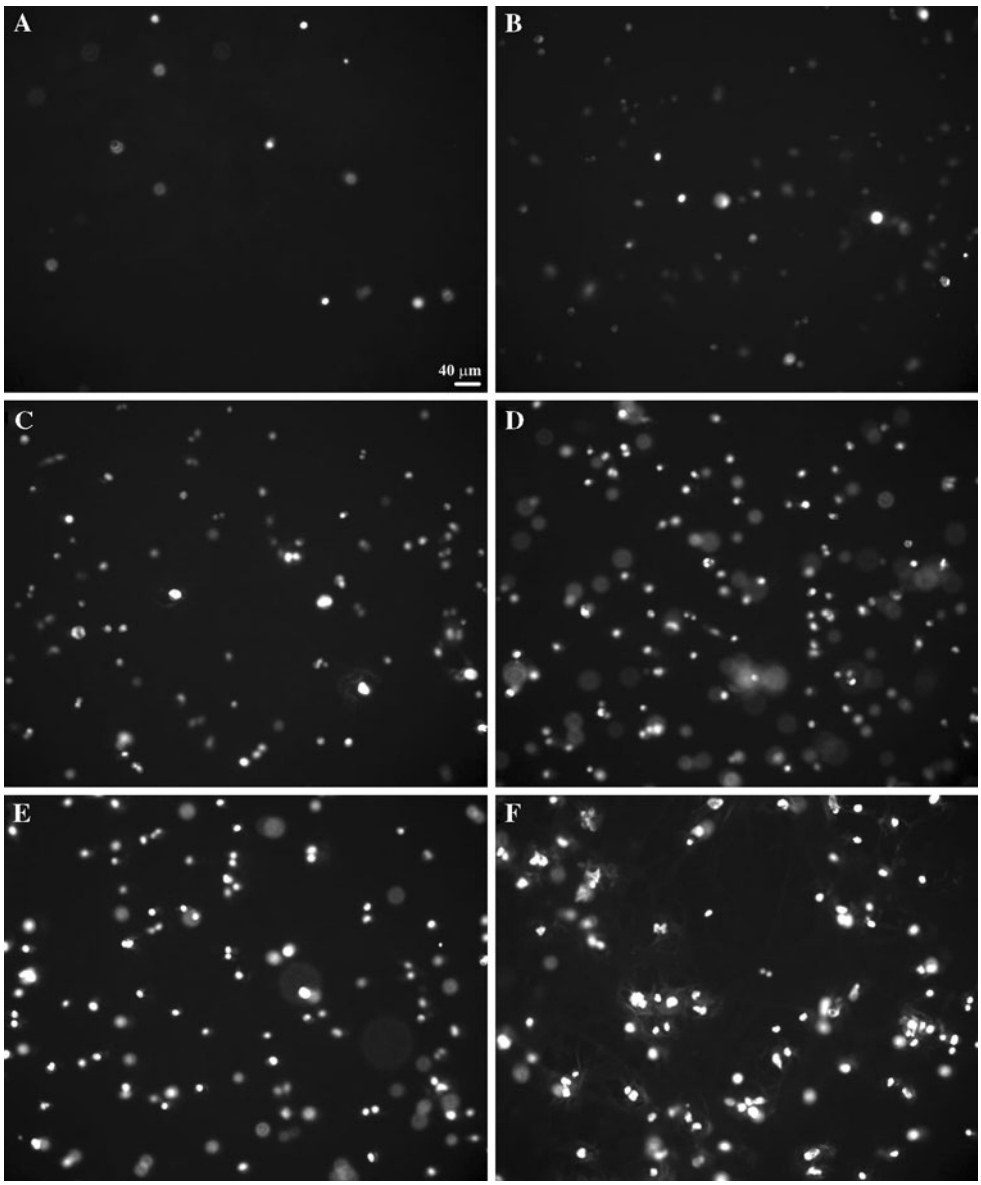

Fig. 2 Electropermeabilization of cells embedded in collagen gel. Pulses of 8 × 5 ms with repetition frequency of 1 Hz were applied to deliver PI into cells. Electric field strengths were as follows: **a** 0.2 kV/cm, **b** 0.4 kV/cm, **c** 0.6 kV/cm, **d** 0.8 kV/cm, **e** 1.0 kV/cm and **f** 1.2 kV/cm. To visualize permeabilization, ×10 objective magnification was used

As shown in Figure 3, the percentage of transfection, which represents the efficiency of gene electrotransfer, increased with increasing concentration of plasmid DNA up to 90 μg/ml, where 5.8% of cells were transfected. Increasing plasmid DNA concentration above 90 μg/ml did not result in increased gene electrotransfer efficiency. This observation correlated with a decrease in cell viability for DNA concentrations above 90 μg/ml (data not shown).

In the next part of our study we tested different pulse durations using the optimal plasmid DNA concentration, 90 μg/ml. The applied electric field of 0.8 kV/cm and repetition frequency of 1 Hz were the same for all pulse durations.

Figure 4 shows the percentage of transfection, which represents the gene electrotransfer efficiency for different pulse durations. We obtained a gradual increase in gene electrotransfer efficiency for increasing pulse durations. The highest efficiency was obtained when we applied a train of eight pulses with duration of 5 ms. Under this condition 2.5% of viable cells were transfected. With increasing pulse duration, a decrease in cell viability was observed (see Fig. 5).

Cell Viability

With increasing pulse duration, a decrease in cell viability was observed, as previously shown in vitro (Rols and Teissie 1998). Figure 5 shows the percentage of viable cells embedded in collagen gel for different pulse durations. The highest viability was observed when we applied shorter pulses (8 × 200 μs). Under this condition, 82% of cells survived. At longer pulses, the viability of cells was

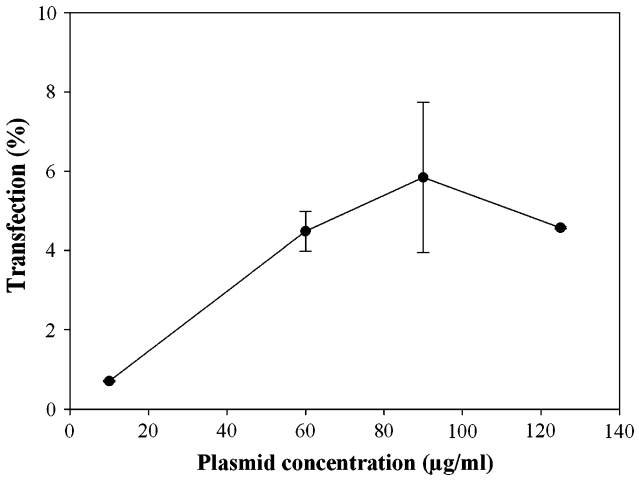

Fig. 3 Effect of different concentrations of plasmid DNA in electroporative medium on gene electrotransfer of cells embedded in collagen gel. Pulses of 8 × 5 ms ($E = 0.8$ kV/cm) with repetition frequency of 1 Hz were applied. The percentage of transfected cells is plotted as a function of different plasmid DNA concentrations. Each value represents the mean of two different experiments ± standard deviation

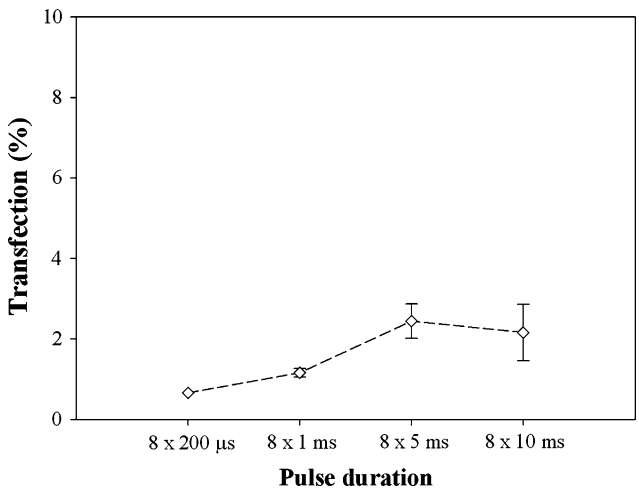

Fig. 4 Effect of different pulse durations on gene electrotransfer of cells embedded in collagen gel. Pulses of different durations with repetition frequency of 1 Hz and $E = 0.8$ kV/cm were applied. The percentage of transfected cells is plotted as a function of different pulse durations. Each value represents the mean of three different experiments ± standard deviation. Plasmid concentration in electroporative medium was 90 µg/ml

significantly lower. At 8 × 10 ms, only 41% of cells survived.

Discussion and Conclusion

Transfer of DNA into the cells by membrane electroporation is an established method for gene delivery both in vitro

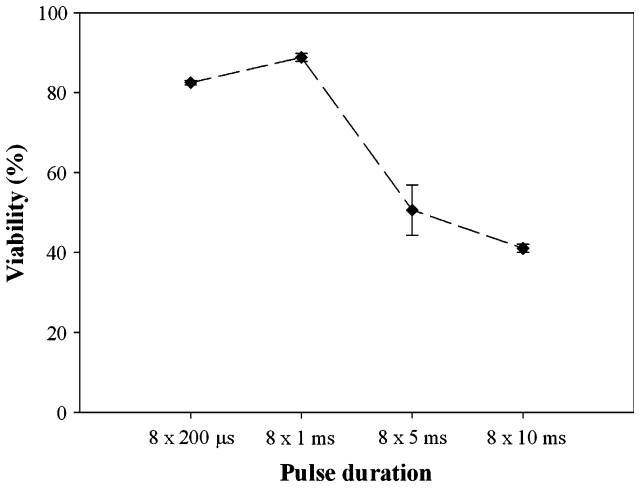

Fig. 5 Effect of different pulse durations on viability of cells embedded in collagen gel. Pulses of different durations with repetition frequency of 1 Hz and $E = 0.8$ kV/cm were applied. The percentage of viable cells is plotted as a function of different pulse durations. Each value represents the mean of two to three different experiments ± standard deviation. Plasmid concentration in electroporative medium was 90 µg/ml

and in vivo. The main problem in gene electrotransfer of mammalian cells in vivo is currently its relatively low efficiency (Rols et al. 1998; Bettan et al. 2000; Cemazar et al. 2002; Tevz et al. 2008). It has been shown (Zaharoff et al. 2002; Zaharoff and Yuan 2004; Cemazar et al. 2006) that extracellular matrix presents a major obstacle for diffusion of DNA through tissue, which hinders transport of DNA in the proximity of cells, consequently leading to relatively low transfection. In order to optimize protocols and to understand the mechanisms of gene electrotransfer in vivo, it is crucial to study gene electrotransfer in a realistic 3-D in vitro model.

Therefore, the aim of our study was to develop a 3-D in vitro model of cells embedded in the extracellular environment, which would more closely mimic in vivo conditions and enable successful electropermeabilization and gene electrotransfer of cells. For efficient gene electrotransfer it is crucial that cells are exposed to E above the electropermeabilization threshold (Wolf et al. 1994). Thus, in the first part of our study, PI was used to determine the threshold for CHO cells embedded in collagen type I gel. Electropermeabilization with our electric pulses (8 × 5 ms, 1 Hz) occurred for field strengths above 0.4 kV/cm. Increasing the applied field strength resulted in an increase in electropermeabilization efficiency (see Fig. 2), as obtained in vitro and in vivo (Wolf et al. 1994; Rols and Teissie 1998; Gehl and Mir 1999; Muller et al. 2001; Sukhorukov et al. 2005).

We further analyzed electropermeabilization in our 3-D in vitro model for different pulse durations. We found that uptake of PI was efficient for all pulse durations, where slightly higher uptake was achieved when longer pulses

were used (data not shown), which is in agreement with a previous study on plated cells (Rols and Teissie 1998).

In the second part of our study, we analyzed gene electrotransfer in our 3-D collagen model. First, we determined the optimal plasmid DNA concentration for our experimental conditions. Gene electrotransfer efficiency increased with increasing concentration of plasmid DNA up to a plasmid concentration of 90 μg/ml (total amount of DNA 34 μg per sample), where approximately 5.8% of cells were transfected, which is comparable to the results obtained from studies in vivo (Cemazar et al. 2002; Andre et al. 2008). Although in this case the standard deviation was quite high, further results showed that reproducibility of the experiments was successfully achieved. A further increase in the DNA concentration resulted in a decrease of gene electrotransfer efficiency. This observation is also in agreement with a study performed in vitro, where it was shown that a higher concentration of plasmid DNA reduces gene electrotransfer efficiency due to the fact that DNA acts as a toxic substance (Wolf et al. 1994).

The electric pulse protocol is one of the most important parameters that determine gene electrotransfer efficiency. As previously demonstrated, longer pulses substantially increase gene electrotransfer efficiency (Rols and Teissie 1998). Namely, electric pulses of longer duration are supposed to contribute to the electrophoretic mobility of DNA toward and into cells (Rols and Teissie 1998; Satkauskas et al. 2002; Cemazar et al. 2006; Kanduser et al. 2009; Pavlin et al. 2010). In our study we therefore used different pulse durations in order to determine the most efficient pulse duration for our 3-D in vitro model. In all experiments, $E = 0.8$ kV/cm was used, which is above the threshold for permeabilization of the cell membrane for all pulsing protocols. The result of our experiments showed that for optimal plasmid concentrations gene electrotransfer efficiency increased with increasing duration of pulses up to 8×5 ms. This is in agreement with studies in vivo on muscle (Bureau et al. 2000; Satkauskas et al. 2002, 2005), where longer pulses distinctly increased gene electrotransfer efficiency. However, at longer pulses cell viability in our 3-D in vitro model dropped (see Fig. 5), which is in agreement with other studies, where it was suggested that the pulsing protocol should be optimized to obtain sufficient gene electrotransfer efficiency and to avoid irreversible cell damage (Rols and Teissie 1998; Mir et al. 1999).

Since the main concern for in vivo gene electrotransfer efficiency is impaired DNA diffusion through the extracellular matrix, we examined whether GFP expression was homogenous in our 3-D model. As previously noticed by Wasungu et al. (2009) in spheroids, in our 3-D model more transfected cells were detected on the top of collagen gel (data not shown). Namely, in the 3-D model and in spheroids weak DNA diffusion was detected (in our model due to collagen gel mesh and in the spheroid model due to close cell contact). Since for efficient transfection DNA has to be in close proximity to cells, high transfection in the 3-D model was obtained only in part of the gel, where there was enough DNA. The lower layers of cells could not be transfected since DNA did not diffuse enough through the collagen gel, which acts as a physical barrier.

To conclude, 2-D cell cultures do not reproduce the morphology and biochemical features that cells possess in the original tissue. As an alternative, 3-D in vitro models of cells offer the possibility to study different parameters of gene electrotransfer under conditions that more closely resemble the in vivo situation. We successfully developed such a model using collagen gel with viable embedded CHO cells, which could be used to study mechanisms of gene electrotransfer as well as to design better protocols for in vivo gene electrotransfer.

We demonstrated that gene electrotransfer efficiency in our 3-D in vitro model depends on the concentration of DNA and that the pulse duration dependence is comparable to in vivo studies (Bureau et al. 2000; Satkauskas et al. 2002, 2005; Cemazar et al. 2002; Andre et al. 2008).

Our results revealed that although small molecules, such as PI, are efficiently transferred into cells in a 3-D in vitro model, gene expression was substantially lower due to poor diffusion of plasmid DNA in collagen gel. Even though some conditions need to be optimized for our 3-D model (better cell viability, better incorporation of cells in collagen mesh), it represents an intermediate step between 2-D in vitro and animal in vivo experiments. Thus, it can be used to optimize gene delivery in vivo and reduce the number of animals used for in vivo experiments.

References

Aihara H, Miyazaki J (1998) Gene transfer into muscle by electroporation in vivo. Nat Biotechnol 16:867–870

Andre F, Gehl J, Sersa G et al (2008) Efficiency of high and low voltage pulse combinations for gene electrotransfer in muscle, liver, tumor and skin. Hum Gene Ther 19:1261–1271

Andreason GL, Evans GA (1989) Optimization of electroporation for transfection of mammalian cell lines. Anal Biochem 180: 269–275

Barralet JE, Wang L, Lawson M et al (2005) Comparison of bone marrow cell growth on 2D and 3D alginate hydrogels. J Mater Sci Mater Med 16:515–519

Bernstein P, Dong M, Corbeil D et al (2009) Pellet culture elicits superior chondrogenic redifferentiation than alginate-based systems. Biotechnol Prog 25:1146–1152

Bettan M, Ivanov MA, Mir LM et al (2000) Efficient DNA electrotransfer into tumors. Bioelectrochemistry 52:83–90

Bureau MF, Gehl J, Deleuze V et al (2000) Importance of association between permeabilization and electrophoretic forces for intramuscular DNA electrotransfer. Biochim Biophys Acta 1474: 353–359

Byrne CM, Thompson JF (2006) Role of electrochemotherapy in the treatment of metastatic melanoma and other metastatic and primary skin tumors. Expert Rev Anticancer Ther 6:671–678

Canatella PJ, Black MM, Bonnichsen DM et al (2004) Tissue electroporation: quantification and analysis of heterogeneous transport in multicellular environments. Biophys J 86:3260–3268

Cemazar M, Sersa G, Wilson J et al (2002) Effective gene transfer to solid tumors using different nonviral gene delivery techniques: electroporation, liposomes, and integrin-targeted vector. Cancer Gene Ther 9:399–406

Cemazar M, Golzio M, Sersa G et al (2006) Electrically-assisted nucleic acids delivery to tissues in vivo: where do we stand? Curr Pharm Des 12:3817–3825

Chevallay B, Herbage D (2000) Collagen-based biomaterials as 3D scaffold for cell cultures: applications for tissue engineering and gene therapy. Med Biol Eng Comput 38:211–218

Cotten M, Wagner E (1993) Non-viral approaches to gene therapy. Curr Opin Biotechnol 4:705–710

Cukierman E, Pankov R, Stevens DR et al (2001) Taking cell–matrix adhesions to the third dimension. Science 294:1708–1712

Curiel DT, Agarwal S, Wagner E et al (1991) Adenovirus enhancement of transferring-polylysine-mediated gene delivery. Proc Natl Acad Sci USA 88:8850–8854

Drury L (1996) Transformation of bacteria by electroporation. Methods Mol Biol 58:249–256

Ganeva V, Galutzov B, Teissie J (1995) Electric field mediated loading of macromolecules in intact yeast cells is critically controlled at the wall level. Biochim Biophys Acta 1240: 229–236

Gehl J, Mir LM (1999) Determination of optimal parameters for in vivo gene transfer by electroporation, using a rapid in vivo test for cell permeabilization. Biochem Biophys Res Commun 261:377–380

Hacein-Bey-Abina S, Le Deist F, Carlier F et al (2002) Sustained correction of X-linked severe combined immunodeficiency by ex vivo gene therapy. N Engl J Med 346:1185–1193

Harkin DG, Hay ED (1996) Effects of electroporation on the tubulin cytoskeleton and directed migration of corneal fibroblasts cultured within collagen matrices. Cell Motil Cytoskeleton 35:345–357

Heller R, Jaroszeski M, Atkin A et al (1996) In vivo gene electroinjection and expression in rat liver. FEBS Lett 389: 225–228

Hindie M, Vayssade M, Dufresne M et al (2006) Interactions of B16F10 melanoma cells aggregated on a cellulose substrate. J Cell Biochem 99:96–104

Kanduser M, Miklavcic D, Pavlin M (2009) Mechanisms involved in gene electrotransfer using high- and low-voltage pulses—an in vitro study. Bioelectrochemistry 74:265–271

Kikuchi A, Aoki Y, Sugaya S et al (1999) Development of novel cationic liposomes for efficient gene transfer into peritoneal disseminated tumor. Hum Gene Ther 10:947–955

Kim JB, Stein R, O'Hare MJ (2004) Three-dimensional in vitro tissue culture models of breast cancer—a review. Breast Cancer Res Treat 85:281–291

Lee GY, Kenny PA, Lee EH et al (2007) Three-dimensional culture models of normal and malignant breast epithelial cells. Nat Methods 4:359–365

Marshall E (1999) Gene therapy death prompts review of adenovirus vector. Science 286:2244–2245

Miklavcic D, Beravs K, Semrov D et al (1998) The importance of electric field distribution for effective in vivo electroporation of tissues. Biophys J 74:2152–2158

Mir LM, Belehradek M, Domenge C et al (1991) Electrochemotherapy, a new antitumor treatment: first clinical trial. C R Acad Sci III 313:613–618

Mir LM, Glass LF, Sersa G et al (1998) Effective treatment of cutaneous and subcutaneous malignant tumors by electrochemotherapy. Br J Cancer 77:2336–2342

Mir LM, Bureau MF, Gehl J et al (1999) High-efficiency gene transfer into skeletal muscle mediated by electric pulses. Proc Natl Acad Sci USA 96:4262–4267

Muller KJ, Sukhorukov VL, Zimmermann U (2001) Reversible electropermeabilization of mammalian cells by high-intensity, ultra-short pulses of submicrosecond duration. J Membr Biol 184:161–170

Neumann E, Rosenheck K (1972) Permeability changes induced by electric impulses in vesicular membranes. J Membr Biol 10:279–290

Neumann E, Schaefer-Ridder M, Wang Y et al (1982) Gene transfer into mouse lyoma cells by electroporation in high electric fields. EMBO J 7:841–845

Nishi T, Yoshizato K, Yamashiro S et al (1996) High-efficiency in vivo gene transfer using intraarterial plasmid DNA injection following in vivo electroporation. Cancer Res 56:1050–1055

Ong SY, Dai H, Leong KW (2006) Inducing hepatic differentiation of human mesenchymal stem cells in pellet culture. Biomaterials 27:4087–4097

Pavlin M, Flisar K, Kandušer M (2010) The role of electrophoresis in gene electrotransfer. J Membr Biol. doi:10.1007/s00232-010-9276-z

Potter H (1988) Electroporation in biology: methods, applications, and instrumentation. Anal Biochem 174:361–373

Rols MP, Teissie J (1998) Electropermeabilization of mammalian cells to macromolecules: control by pulse duration. Biophys J 75:1415–1423

Rols MP, Delteil C, Golzio M et al (1998) In vivo electrically mediated protein and gene transfer in murine melanoma. Nat Biotechnol 16:168–171

Satkauskas S, Bureau MF, Puc M et al (2002) Mechanisms of in vivo DNA electrotransfer: respective contributions of cell electropermeabilization and DNA electrophoresis. Mol Ther 5:133–140

Satkauskas S, Andre F, Bureau MF et al (2005) Electrophoretic component of electric pulses determines the efficacy of in vivo DNA electrotransfer. Hum Gene Ther 16:1194–1201

Sersa G (2006) The state-of-the-art of electrochemotherapy before the ESOPE study: advantages and clinical uses. Eur J Cancer Suppl 4:52–59

Sersa G, Stabuc B, Cemazar M et al (2000) Electrochemotherapy with cisplatin: clinical experience in malignant melanoma patients. Clin Cancer Res 6:863–867

Simoes S, Slepushkin V, Gaspar R et al (1998) Gene delivery by negatively charged ternary complexes of DNA, cationic liposomes and transferrin or fusogenic peptides. Gene Ther 5:955–964

Simon JR (1993) Transformation of intact yeast cells by electroporation. Methods Enzymol 217:478–483

Somiari S, Glasspool-Malone J, Drabick JJ et al (2000) Theory and in vivo application of electroporative gene delivery. Mol Ther 2:178–187

Sukhorukov VL, Reuss R, Zimmermann D et al (2005) Surviving high-intensity field pulses: strategies for improving robustness and performance of electrotransfection and electrofusion. J Membr Biol 206:187–201

Terzaghi WB, Cashmore AR (1997) Plant cell transfection by electroporation. Methods Mol Biol 62:453–462

Tevz G, Pavlin D, Kamensek U et al (2008) Gene electrotransfer into murine skeletal muscle: a systematic analysis of parameters for long-term gene expression. Technol Cancer Res Treat 7:91–101

Titomirov A, Sukarev S, Kistanova E (1991) In vivo electroporation and stable transformation of skin cells of newborn mice by plasmid DNA. Biochim Biophys Acta 1088:131–134

Ušaj M, Trontelj K, Miklavčič D, Kandušer M (2010) Cell–cell electrofusion: optimization of electric field amplitude and hypotonic treatment for mouse melanoma (B16-F1) and Chinese hamster ovary (CHO) cells. J Membr Biol. doi:10.1007/s00232-010-9272-3

Vienken J, Zimmermann U (1985) An improved electrofusion technique for production of mouse hybridoma cells. FEBS Lett 182:278–280

Wagner E, Plank C, Zatloukal K et al (1992) Influenza virus hemagglutinin HA-2N-terminal fusogenic peptides augment gene transfer by transferring-polylysine-DNA complexes: toward a synthetic virus-like gene-transfer vehicle. Proc Natl Acad Sci USA 89:7934–7938

Wasungu L, Escoffre JM, Valette A et al (2009) A 3D in vitro spheroid model as a way to study the mechanisms of electroporation. Int J Pharm 379:278–284

Wolf H, Rols MP, Boldt E et al (1994) Control by pulse parameters of electric field-mediated gene transfer in mammalian cells. Biophys J 66:524–531

Zaharoff DA, Yuan F (2004) Effects of pulse strength and pulse duration on in vitro DNA electromobility. Bioelectrochemistry 62:37–45

Zaharoff DA, Barr RC, Li CY et al (2002) Electromobility of plasmid DNA in tumor tissues during electric field-mediated gene delivery. Gene Ther 9:1286–1290