

Improved Technique of Non-viral Gene Delivery into Cancer Cells

D. Vainauska, S. Kozireva, A. Karpovs, M. Chistyakovs and M. Baryshev

Abstract—Liposomal magnetofection is a simple, highly efficient technology for cell transfection, demonstrating better outcome than a number of other common gene delivery methods. However, aggregate complexes distribution over the cell surface is non-uniform due to the gradient of the permanent magnetic field. The aim of this study was to estimate the efficiency of liposomal magnetofection for prostate carcinoma PC3 cell line using newly designed device, “DynaFECTOR”, ensuring magnetofection in a dynamic gradient magnetic field. Liposomal magnetofection in a dynamic gradient magnetic field demonstrated the highest transfection efficiency for PC3 cells – it increased for 21% in comparison with liposomal magnetofection and for 42% in comparison with lipofection alone. The optimal incubation time under dynamic magnetic field for PC3 cell line was 5 minutes and the optimal rotation frequency of magnets – 5 rpm. The new approach also revealed lower cytotoxic effect to cells than liposomal magnetofection.

Keywords—Dynamic gradient magnetic field, gene delivery, liposomal magnetofection, prostate cancer cell line

I. INTRODUCTION

EFFICIENT DNA transfection is a critical factor for the development of new clinical therapy. Since the first reports on introduction of foreign genetic material into cultured cells by cationic polymers a substantial progress in nonviral gene delivery has been achieved.

Currently, among non-viral delivery systems cationic lipids, which allow maximize DNA complexation and membrane fusion, became the widely used delivery agents. The association of the lipid-based transfection reagent with nucleic acids results in tight compaction and protection of nucleic acids and these cationic complexes are mainly internalized by endocytosis. The main advantages of lipofection are its high efficiency, ability to transfect all types of nucleic acids in a wide range of cell types, ease of use, reproducibility and low toxicity. Nevertheless insufficient contact of this delivery system with target cells is a one of the main reason for their often observed limited efficiency.

A novel method exploring permanent magnetic field acting on nucleic acid vectors associated with magnetic particles in order to mediate the rapid contact of vectors with target cells was described in 2000 by Luo & Saltzman [1]. Using this method, termed magnetofection, magnetic particles containing nucleic acids are sedimented onto surface of the cells within

minutes overcoming by this way diffusion barrier. This process leads to considerable improvement in transfection efficiency compared to transfection carried out by biochemical methods (e.g. lipofection) [2]-[5].

Further enhancement of transfection efficiency (non-viral gene delivery) has been achieved by application of pulsed magnetic field in which alternating horizontal, perpendicular and oscillating movements of the magnetic particles are induced [6],[7].

Recently a liposomal magnetofection method, which combine the biochemical and physical nucleic acids delivery system, has been reported [8]-[11]. In this technique self-assembled complexes of enhancers like cationic lipids with nucleic acids and magnetic nanoparticles are formed and then concentrated on the surface of cells by applying a permanent magnetic field.

The magnetofection and liposomal magnetofection has been successfully used in *in vitro* applications for various types of nucleic acids and across a broad range of cell lines [8]-[15]. Nevertheless, there is a continued need for further improvements in terms of effectiveness. Here we report a simple approach that enhances gene delivery using dynamic gradient magnetic field.

II. MATERIALS AND METHODS

A. Cell Line

Human prostate cancer cell line PC3 (ScienCell, CA, USA) was maintained in RPMI-1640 culture medium supplemented with 10% fetal bovine serum (Invitrogen, CA, USA), penicillin (100 U/ml) and streptomycin (100 µg/ml) (Invitrogen, CA, USA). The cell line was cultured at 37 °C in a humidified incubator supplied with 5% CO₂.

B. Plasmid Preparation

The competent *Escherichia coli* (*E. coli*) XL1Blue (Promega, Wisconsin, USA) was transformed by pcDNA3.1LacZ (Invitrogen, CA, USA) and pECFP-ERp29 vectors (Clontech, Palo Alto, USA) according to standard protocol. Bacteria cells were cultured at optimal conditions (37°C, 200 rpm) overnight and plasmid vectors were purified using Midi kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. Plasmid DNA concentration was estimated by NanoDrop spectrophotometer (ThermoScientific, NC, USA).

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C. Transfection And Exposure To Magnetic Field

PC3 cells ($1.6\text{--}2.0 \times 10^5$) were seeded in 24-well plates, one day before transfection to obtain 80%–90% confluence. All incubations were done at 37°C and 5% CO₂. Before transfection, the growth medium was removed and 350 µl of Opti-MEM reduced serum medium (Invitrogen, CA, USA) was added. The expression vectors pcDNA3.1LacZ and pECFP-ERp29 encoding beta-galactosidase and ECFP-ERp29 fusion protein, respectively, were used for evaluating transfection efficiency of the PC3 cell line. Samples were prepared in triplicates. Lipofectamine2000 (Invitrogen, CA, USA) was used to transfect the cell line based on the manufacturer's recommendation. Briefly, 2 µl of Lipofectamine2000, 1 µg of pcDNA3.1LacZ or pECFP-ERp29 and an equal volume (1 µl) of CombiMag magnetic nanoparticles (Chemicell, Berlin, Germany) were diluted separately in 50 µl of Opti-MEM reduced serum medium and mixed gently. After 5 min incubation at room temperature, the CombiMag, the Lipofectamine2000 and pcDNA3.1LacZ or pECFP-ERp29 were combined, the mixture was gently pipetted up and down and incubated for an additional 25 min at room temperature to allow the plasmid-CombiMag-Lipofectamine2000 complexes to form. Subsequently 150 µl of the complexes was added to each well and the cell culture plates were placed on a neodymium–iron–boron (NdFeB) permanent magnet or on a dynamic magnetic field device “DynaFECTOR” for 5, 10, and 20 min, with specified magnets' rotation frequency 5, 25, 50, and 100 rpm.

D. Determination Of Gene Expression

After 24 hr incubation, LacZ gene expression was detected using β-Gal staining kit (Invitrogen, CA, USA) according to manufacturer's protocol. Transfection efficiency was determined by counting stained and unstained cells under a microscope and calculating the percentage of stained cells in the total population. Level of ECFP-ERp29 gene expression was determined by immunoblot with anti-ERp29 rabbit polyclonal antibody. Untransfected cells were included to account for a background.

E. Assessment Of Cell Viability

Cell viability was determined using Ethidium bromide/Acridine orange (EB/AO) staining as described by Ribble *et al.* [16]. After transfection the cell supernatants (medium and floating cells) were transferred to 1.5 ml tubes. PC3 cells were detached with PBS-EDTA containing 1 mM EDTA. The supernatant and detached cells from the same sample were pooled together in 1.5 ml tube, centrifuged at 1000 rpm for 5 min, and washed with 1 ml of cold PBS once. Cell pellets were re-suspended in 25 µl cold PBS and 2 µl EB/AO dye mix was added. Stained cell suspension was placed on a clean microscope slide and covered with a cover slip. Cells were viewed and counted using Nikon microscope at 400x magnification with excitation filter. Pictures were taken with a Nikon digital camera. Tests were done in triplicate counting a minimum of 100 total cells.

III. RESULTS

A. Optimal Conditions For Magnetofection In A Dynamic Gradient Magnetic Field

To determine the optimal exposure time to dynamic gradient magnetic field, the plasmid-Lipofectamine2000-CombiMag complexes were incubated on the “DynaFECTOR” for 5, 10 and 20 min with a magnets' rotation frequency 5 rpm. Exposure to dynamic gradient magnetic field resulted in transfection rates $65\% \pm 3$, $56\% \pm 5$ and $43\% \pm 7$, exposed for 5, 10, and 20 min, respectively (Fig. 1). The 5 min exposure time that demonstrates the highest transfection efficiency was further used to study the magnetically driven DNA delivery using various magnet rotation frequencies. Liposomal magnetofection in a permanent magnetic field for 5, 10, and 20 min was performed in parallel to evaluate the optimal conditions for this approach. Exposure of PC3 cells to permanent magnetic field for 5, 10 and 20 min gave transfection rates, corresponding to $55 \pm 2\%$, $47 \pm 4\%$ and $39 \pm 5\%$, respectively (data not shown). The highest transfection efficiency was obtained using 5 min exposure to permanent magnetic field.

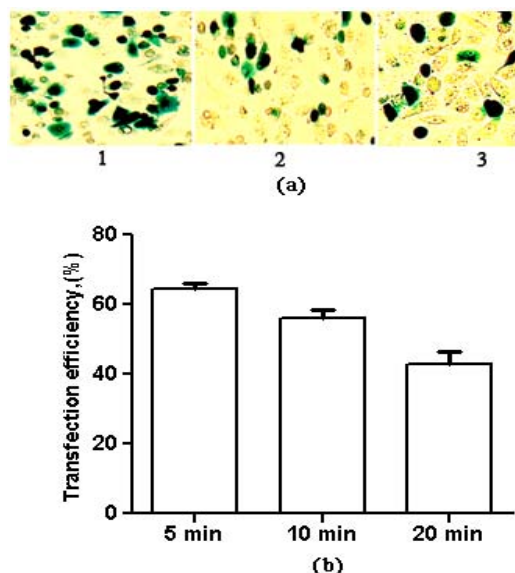


Fig. 1 Dependence of liposomal magnetofection on duration of exposure to dynamic gradient magnetic field. (a) Representative data showing β-galactosidase expression in PC3 cells at 24 h following liposomal magnetofection using 5(1), 10(2), and 20(3) min exposure to dynamic magnetic field; (b) Bar chart showing the proportion of transfected cells at 24 h following 5, 10 and 20 min exposure to dynamic magnetic field. Data represent the mean values of at least three independent experiments

In order to clarify an optimal magnet rotation frequency for dynamic gradient magnetic field, the plasmid-Lipofectamine2000-CombiMag complexes were incubated on the “DynaFECTOR” for 5 min with magnets' rotation frequency – 5, 25, 50, and 100 rpm. The variation of magnets' rotation frequencies to transfection efficiency values shows

rather parabolic tendency: $61\% \pm 5$ (5 rpm), $45\% \pm 3$ (25 rpm), $39\% \pm 3$ (50 rpm), and $52\% \pm 3$ (100 rpm) (Fig. 2).

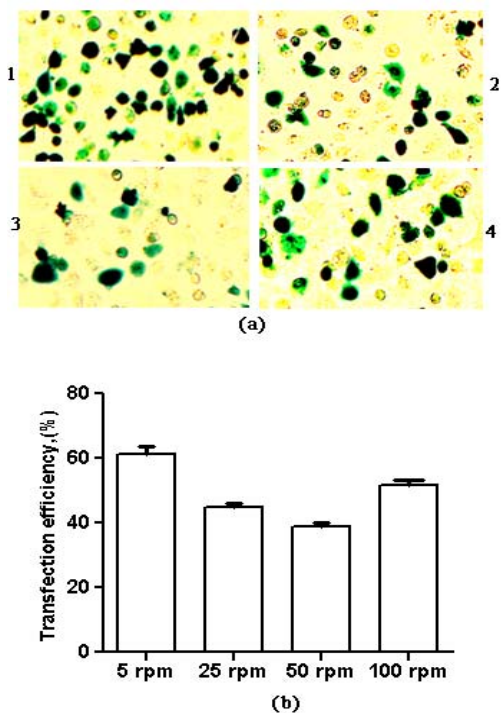


Fig. 2 Effect of magnets' rotation frequency on expression of β -galactosidase. (a) Representative data showing β -galactosidase expression in PC3 cells at 24 h following liposomal magnetofection under dynamic magnetic field with magnets' rotation frequency 5(1), 25(2), 50(3) and 100(4) rpm; (b) Bar chart showing the proportion of transfected cells at 24 h following liposomal magnetofection under dynamic magnetic field with magnets' rotation frequency 5, 25, 50 and 100 rpm. Data represent the mean values of at least three independent experiments

B. Dynamic Gradient Magnetic Field Enhances Transfection Efficiency

The obtained results from PC3 cells exposed to a dynamic gradient and permanent magnetic field, and cells not exposed to magnetic field, e.g. transfected by lipofection are presented in Fig. 3.

The highest transfection efficiency of $78 \pm 3\%$ was achieved using dynamic gradient magnetic with optimal parameters. It enhanced over those of the liposomal magnetofection for 21% and lipofection for 42%. Similar results, demonstrating 2-fold increased transfection efficiency in the presence of dynamic gradient magnetic field compared to liposomal magnetofection and 3.3-fold increased transfection efficiency in comparison with lipofection, were obtained using pECFP-ERp29 plasmid vector (Fig. 4).

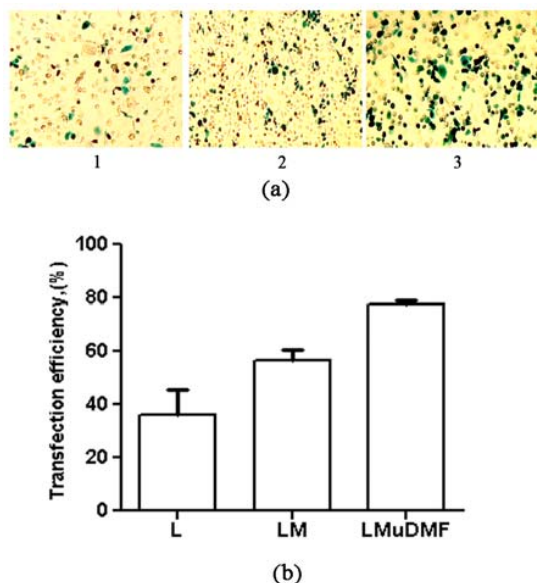


Fig. 3. Effect of dynamic gradient magnetic field on transfection efficiency of pcDNA3.1LacZ plasmid. (a) Representative data showing β -galactosidase expression in PC3 cells at 24 h following lipofection (1) liposomal magnetofection (2) and liposomal magnetofection under dynamic magnetic field (3); (b) Bar chart showing the proportion of transfected cells at 24 h following lipofection (L), liposomal magnetofection (LM) and liposomal magnetofection under dynamic magnetic field (LMuDMF). Data represent the mean values of at least three independent experiments

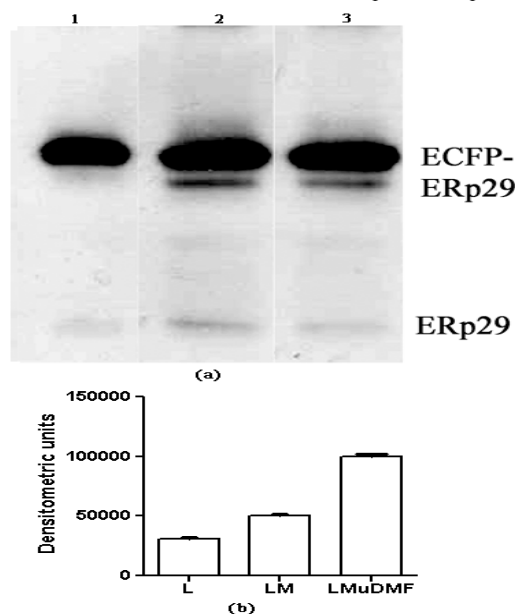


Fig. 4. Effect of dynamic gradient magnetic field on ECFP-ERp29 expression. (a) Representative image showing ECFP-ERp29 expression in PC3 cells at 24 h following lipofection (lane 1) liposomal magnetofection (lane 2) and liposomal magnetofection under dynamic magnetic field (lane 3); (b) Bar chart showing the proportion of transfected cells at 24 h following lipofection (L), liposomal magnetofection (LM) and liposomal magnetofection under dynamic magnetic field (LMuDMF). Data represent the mean values of at least three independent experiments.

C. Applying Dynamic Gradient Magnetic Field Leads To Less Cytotoxic Effect

As tested by AO/EB cell staining, the liposomal magnetofection in the presence of dynamic gradient magnetic field reproducibly gave for 16.5% lower cytotoxicity in comparison with liposomal magnetofection (Fig 5).

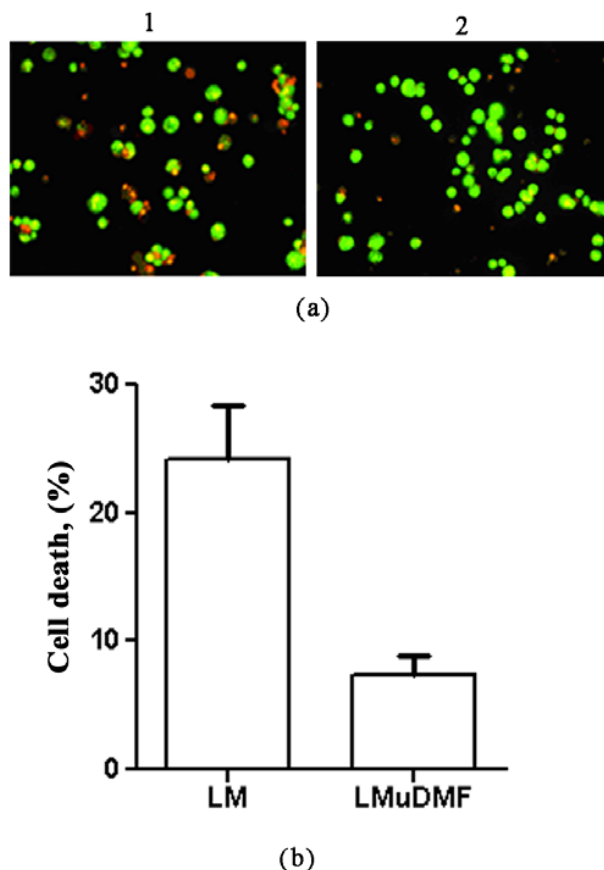


Fig. 5 Effect of dynamic gradient magnetic field on cell viability.

(a) Representative data showing cell viability at 24 h following liposomal magnetofection under permanent (1) and dynamic gradient (2) magnetic field. (b) Bar chart showing the proportion of dead cells at 24 h following liposomal magnetofection (LM) and liposomal magnetofection under dynamic magnetic field (LMuDMF). Data represent the mean values of at least three independent experiments

Thus, for our experiments, the following improvements in comparison to liposomal magnetofection were obtained by application of dynamic gradient magnetic field: a significant increase in transfection efficiency and significant decrease in cytotoxicity.

IV. DISCUSSION

In this study we describe an improved plasmid DNA delivery method based on the application of dynamic gradient magnetic field which enhances the non-viral gene delivery to prostate cancer cells. The presence of permanent magnetic field for 5 min was sufficiently to significantly increase transfection efficiency over lipofection.

Our findings are consistent with results of previous studies which demonstrated that permanent magnetic field enhanced the transfection efficiency [6],[12]. However, despite promising results showing high gene transfer rates, the permanent magnetic field has one disadvantage associated with non-uniform complexes distribution over the cell surface.

We hypothesize that dynamic gradient magnetic field provides more uniform distribution of complexes as well as more efficient cationic lipid : nucleic acid with magnetic nanoparticles complexes cellular uptake. This magnetic field forced cationic lipid : nucleic acid with magnetic nanoparticles complexes oscillate not only perpendicular, concentrating the complexes on the cells' surface, but also parallel to the cells' surface that leads to a slow rolling of the complexes over the cells' surface, facilitating by this mean cellular uptake.

Our results indicate that the efficiency of liposomal magnetofection in the presence of dynamic magnetic field depends on the exposure time and magnets' rotation frequency. The highest transfection efficiency exceeding that of routine magnetofection has been achieved in the presence of dynamic magnetic field for 5 min with magnets' rotation frequency 5 rpm.

The results strongly support the use of dynamic gradient magnetic field as a perspective tool for non-viral gene delivery. To confirm a universality of this approach, further studies using cell lines of various origins are required.

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