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A Novel Approach for Nucleic Acid Delivery Into Cancer Cells

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***Key words**: liposomal magnetofection; dynamic gradient magnetic field; gene delivery; cancer cells.*

***Summary.** Background. Liposomal magnetofection is based on the use of superparamagnetic particles and cationic lipids and shows better transfection efficiency than other common nonviral gene delivery methods; however, the distribution of aggregate complexes over the cell surface may be ununiform. The use of a dynamic gradient magnetic field could overcome this limitation. A newly developed device for magnetofection under a dynamic magnetic field was used to compare the transfection efficiency of prostate carcinoma cell line PC3 with that obtained by lipofection and magnetofection.*

Material and Methods. Reporter plasmid pcDNA3.1LacZ DNA was used in combination with Lipofectamine2000 reagent and superparamagnetic nanoparticles CombiMag. The effects of incubation time under a dynamic magnetic field and a rotation frequency of magnets on transfection efficiency for PC3 cell line were determined. Alternatively, lipofection and liposomal magnetofection were carried out. Transfection efficiency of delivery methods was estimated by β-galactosidase staining; cell viability, by acridine orange/ethidium bromide staining.

Results. Liposomal magnetofection under a dynamic gradient magnetic field demonstrated the highest transfection efficiency: it was greater by almost 21% and 42% in comparison with liposomal magnetofection and lipofection, respectively. The optimal incubation time under dynamic magnetic field and the optimal magnet rotation frequency were 5 minutes and 5 rpm, respectively. Liposomal magnetofection under a dynamic gradient magnetic field was less cytotoxic (7%) than that under a permanent magnetic field (17%) and lipofection (11%).

Conclusions. Our new approach, based on the use of a dynamic gradient magnetic field, enhanced the transfection efficiency and had a less cytotoxic effect on prostate cancer cells in comparison with the standard magnetofection and lipofection.

Introduction

Efficient DNA and siRNA transfection is a critical factor for the development of new clinical therapy. Since the first reports on the introduction of foreign genetic material into cultured cells by cationic polymers, a substantial progress in nonviral gene delivery has been achieved (1, 2). Currently, cationic lipids that allow maximizing DNA complexation and membrane fusion have become widely used delivery agents among nonviral delivery systems (3). The association of the lipid-based transfection reagent with nucleic acids results in a tight compaction and protection of nucleic acids, and these cationic complexes are mainly internalized by endocytosis (3, 4). 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 (5, 6). Nevertheless, an insufficient contact of this delivery system with target cells is one of the main reasons for their often observed limited efficiency (7). A novel method exploring a permanent magnetic field acting on nucleic acid vectors associated with magnetic particles in order to mediate a rapid contact of vectors with target cells was described in 2000 by Luo and Saltzman (8). Using this method, termed magnetofection, magnetic particles containing nucleic acids are sedimented onto the surface of cells within minutes overcoming a diffusion barrier by this way. This process leads to a considerable improvement in transfection efficiency compared with transfection carried out by biochemical methods (e.g., lipofection) (9–12). Further enhancement of magnetofection efficiency has been achieved by the application of a pulsed magnetic field in which alternating horizontal, perpendicular, and oscillating movements of the magnetic particles are induced (13, 14). Recently, the method of liposomal magnetofection, which combines biochemical and physical delivery systems of nucleic acids, has been reported (15–18). In this technique, the self-

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assembled complexes of enhancers such as cationic lipids with nucleic acids and magnetic nanoparticles are formed and then concentrated on the surface of cells by applying a permanent magnetic field. Magnetofection and liposomal magnetofection have been successfully used in in vitro applications for various types of nucleic acids and across a broad range of cell lines (15–22). Nevertheless, there is a continued need for further improvements in terms of transfection efficiency. Here we report a new approach, based on the application of a dynamic gradient magnetic field, which enhances gene delivery in prostate cancer cells.

Material and Methods

Materials

CombiMag magnetic nanoparticles were obtained from Chemicell GmbH. They are composed of magnetite (Fe_3O_4) and are 100 nm in hydrodynamic diameter on the average. Cylindrical rare-earth neodymium-iron-boron (NdFeB) N38 grade 1.26-T induction magnets were purchased from Semicom Ltd. Lipofectamine2000, a transfection reagent, was purchased from Invitrogen Corp.

Cell Line

Human prostate cancer cell line PC3 was grown in RPMI-1640 culture medium supplemented with 10% fetal bovine serum (Invitrogen Corp.), penicillin (100 U/mL), and streptomycin (100 μg/mL) (Invitrogen Corp.). The cell line was cultured at 37°C in a humidified incubator supplied with 5% CO_2.

Plasmid Preparation

The competent *Escherichia coli* XL1Blue strain (Promega Corp.) was transformed by pcDNA3.1LacZ (Invitrogen Corp.) according to the standard protocol. Bacterial cells were cultured under optimal conditions (37°C, 200 rpm) overnight, and the plasmid vector was purified using a Plasmid Midi kit (QIAGEN GmbH) according to the manufacturer's protocol. Plasmid DNA concentration was detected by a NanoDrop spectrophotometer (Thermo Fisher Scientific Inc.).

Transfection and Exposure to Magnetic Field

PC3 cells were seeded in 24-well plates at a density of 1.8×10^5 per well one day before transfection to obtain 80% confluence. All incubations were performed at 37ºC and 5% CO_2. Before transfection, the growth medium was removed, and 350 μL of Opti-MEM reduced serum medium (Invitrogen Corp.) was added. The expression vector pcDNA3.1LacZ encoding beta-galactosidase was used to evaluate the transfection efficiency of the PC3 cell line. The samples were prepared in triplicate. Lipofectamine2000 (Invitrogen Corp.) was used to transfect the cell line based on the manufacturer's recommendations.

For the transfection of PC3 cells, 2 μL of Lipofectamine2000, 1 μg of pcDNA3.1LacZ, and an equal volume (1 μL) of CombiMag magnetic nanoparticles (Chemicell GmbH) were diluted separately in 50 μL of Opti-MEM reduced serum medium and mixed gently. After the 5-minute incubation at room temperature, CombiMag, Lipofectamine2000, and pcDNA3.1LacZ were combined; the mixture was gently pipetted up and down and incubated for additional 25 minutes 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 either on an NdFeB permanent magnet or incubated on a dynamic magnetic field device "DynaFECTOR" for 5, 10, and 20 minutes with a specified magnet rotation frequency of 5, 25, 50, and 100 rpm. Alternatively, lipofection was carried out. For liposome formation, 2 μL of Lipofectamine2000 and 1 μg of pcDNA3.1LacZ were diluted separately in 75 μL of Opti-MEM reduced serum medium and mixed gently. After the 5-minute incubation at room temperature, Lipofectamine2000 and pcDNA3.1LacZ were combined; the mixture was gently pipetted up and down and incubated for additional 25 minutes at room temperature to allow the plasmid-Lipofectamine2000 complexes to form. After the incubation, 150 μL of the complexes was added to each well, and the plate was placed into a CO_2 incubator for 24 hours; no magnetic field was applied for lipofection.

Determination of LacZ Gene Expression

After the 24-hour incubation, *LacZ* gene expression was detected using a *b*-Gal staining kit (Invitrogen Corp.) according to the 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. The untransfected cells were included to account for a background.

Analysis of Cell Viability

Cell viability was determined using ethidium bromide/acridine orange (EB/AO) staining as described by Ribble et al. (23). For analysis, cells were cultivated on cover slips in 24-well plates. Twenty-four hours after the transfection, the cells were gently washed with 1 mL of cold PBS, and the EB/AO dye mix containing 10 μg/mL of each dye was added to the cells. The number of viable (green) and dead (yellow/red) cells was counted using a Nikon microscope at a magnification of ×400 with an excitation filter. Pictures were taken with a Nikon digital camera. The tests were done in triplicate counting a minimum of 100 total cells.

Results

Device Construction and Methodology

For the creation of a dynamic magnetic field, the rotating property of an orbital shaker was used. Instead of a platform shaker, the magnetic system of cylindrical permanent magnets with a saturation induction of 1.33 T arranged in a checkerboard pattern unipolarly was inserted as shown in Fig. 1. A 24-well plate was located on a nonmagnetic metal surface over a magnet system. The distance from the bottom of the plates to the upper surface of the magnets was fixed and was 3.5 mm. Electronics provided a programmable rotation of the magnetic system on the orbit at a given speed of 1–150 rpm for a fixed time of 1 to 999 minutes. In addition, the direction of rotation was reversed after 30 seconds. The magnets were inserted in such a way that in view of their movement, it was possible to reach a magnetic field of 0.35 T and normal and tangential gradients of 3×10^{7} A/m^{2} according to the computer modeling of magnetic vector force. Fig. 2 demonstrates that during the magnet rotation, every magnetic particle crosses the magnet edges twice.

Optimal Conditions for Magnetofection in a Dynamic Gradient Magnetic Field

To determine the optimal exposure time to a dynamic gradient magnetic field, the plasmid-Lipofectamine2000-CombiMag complexes were incubated on the "DynaFECTOR" for 5, 10, and 20 minutes with a magnet rotation frequency of 5 rpm. Exposure to a dynamic gradient magnetic field for 5, 10, and 20 minutes resulted in a transfection efficiency of 77.7%±1.5%, 56.0%±2.6%, and 43.0%±3.6%, respectively (Fig. 3). The 5-minute exposure, which demonstrated the highest transfection efficiency, was further used to study the magnetically driven DNA delivery using various magnet rotation frequencies. The exposure of PC3 cells to the permanent magnetic field for 5, 10, and 20 minutes gave a transfection efficiency of 56.7%±4.0%, 47.2%±4.0%, and 39.1%±5.0%, respectively (data not shown). The highest transfection efficiency was also obtained by using 5-minute exposure to a permanent magnetic field.

In order to find out an optimal magnet rotation frequency for a dynamic gradient magnetic field, the plasmid-Lipofectamine2000-CombiMag complexes were incubated on the "DynaFECTOR" for 5 minutes with a magnet rotation frequency of 5, 25, 50, and 100 rpm. The variation of magnet rotation frequency resulted in the following transfection efficiency: 77.7%±1.5% (5 rpm), 44.7%±1.5% (25 rpm), 38.7%±1.5% (50 rpm), and 51.7%±1.5% (100 rpm) (Fig. 4).

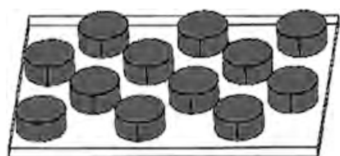

Fig. 1. Magnetic system of cylindrical permanent magnets arranged in a checkerboard pattern

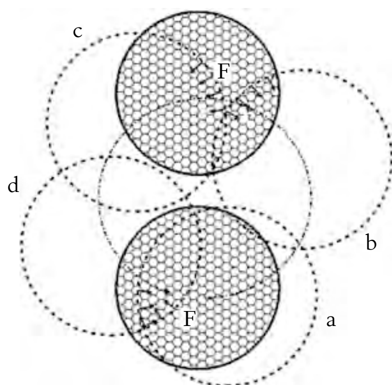

Fig. 2. Schematic illustration of the part of magnetic system for the magnetofection and magnetic force direction under the dynamic gradient magnetic field

F, tangential component of magnetic field acting on magnetoplexes in a direction indicated by the arrow. a, b, c, and d, sequential positions of the magnet at different time points.

Enhancment of Transfection Efficiency by a Dynamic Gradient Magnetic Field

The obtained results from PC3 cells exposed to a dynamic gradient and permanent magnetic field, and cells not exposed to a magnetic field, e.g., transfected by lipofection, are presented in Fig. 5. The highest transfection efficiency reaching 79% was achieved using a dynamic gradient magnetic field with the optimal parameters. It was greater by 21% and 42% as compared with liposomal magnetofection and lipofection, respectively.

Application of Dynamic Gradient Magnetic Field is Less Cytotoxic to Cells

To estimate the effect of a dynamic gradient magnetic field on cell viability, AO/EB staining of PC3 cells following liposomal magnetofection under permanent and dynamic gradient magnetic fields and lipofection was carried out. As shown in Fig. 6, the viability of PC3 cells magnetofected under a dynamic gradient magnetic field was higher (93%) than that of cells under a permanent magnetic field (83%) and traditional lipofection (89%). Therefore, the pDNA delivery into PC3 cells using a dynamic gradient magnetic field not only enhanced transfection efficiency, but also reduced cytotoxicity.

Discussion

In this study, an improved plasmid DNA delivery method based on the application of dynamic gra-

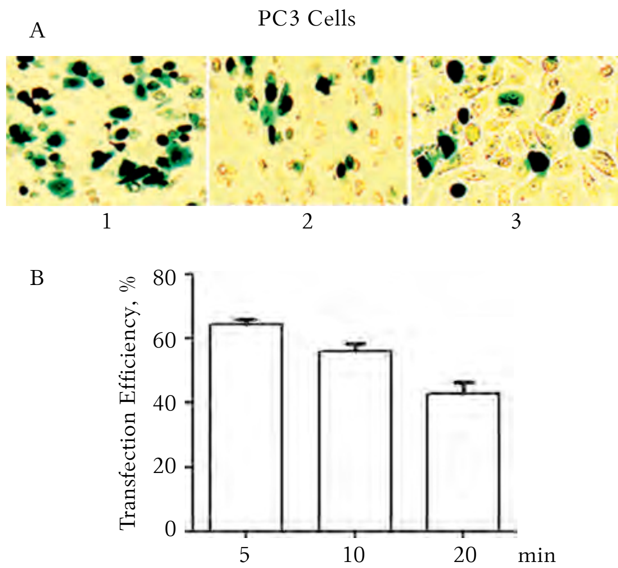

Fig. 3. Dependence of liposomal magnetofection on the duration of exposure to a dynamic gradient magnetic field

A, representative data show β-galactosidase expression in PC3 cells 24 hours after liposomal magnetofection using 5- (1), 10- (2), and 20-minute (3) exposure to a dynamic magnetic field; B, bar chart shows the proportion of transfected cells 24 hours after 5-, 10-, and 20-minute exposure to a dynamic gradient magnetic field. Data represent the mean values of 3 independent experiments.

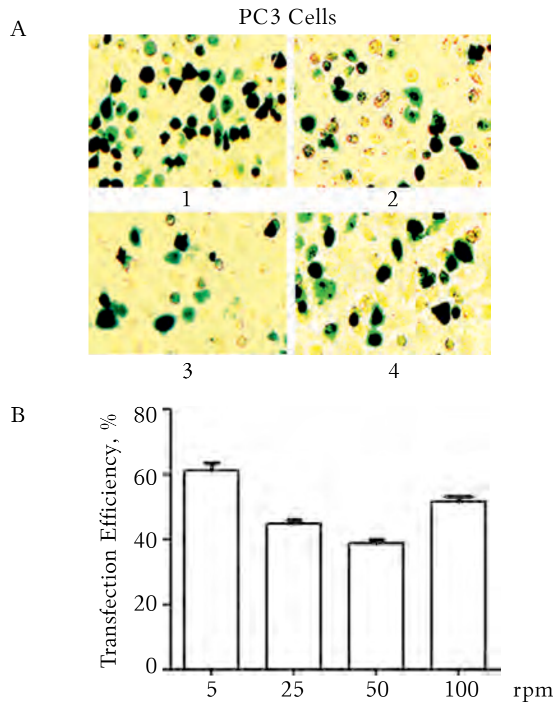

Fig. 4. The effect of magnet rotation frequency on the expression of β-galactosidase

A, representative data show β-galactosidase expression in PC3 cells 24 hours after liposomal magnetofection under a dynamic magnetic field with a magnet rotation frequency of 5 (1), 25 (2), 50 (3), and 100 (4) rpm; B, bar chart shows the proportion of transfected cells 24 hours after liposomal magnetofection under a dynamic magnetic field with a magnet rotation frequency of 5, 25, 50, and 100 rpm. Data represent the mean values of 3 independent experiments.

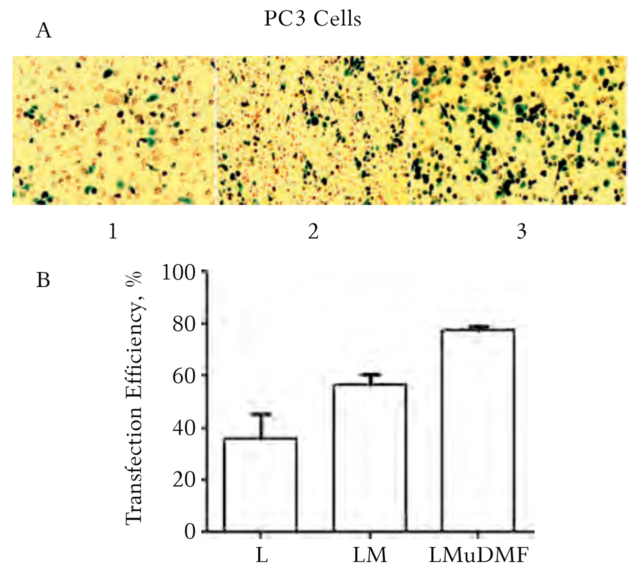

Fig. 5. The effect of different gene delivery methods on the transfection efficiency of PC3 cells by pcDNA3.1LacZ plasmid

A, representative data show β-galactosidase expression in PC3 cells 24 hours after lipofection (1), liposomal magnetofection (2), and liposomal magnetofection under a dynamic magnetic field (3); B, bar chart shows the proportion of transfected cells 24 hours after lipofection (L), liposomal magnetofection (LM), and liposomal magnetofection under a dynamic magnetic field (LMuDMF). Data represent the mean values of 3 independent experiments.

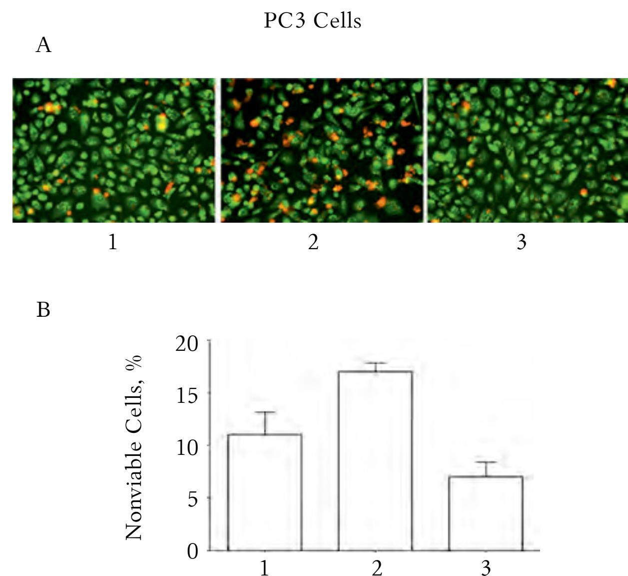

Fig. 6. Cytotoxicity of different gene delivery methods

A, representative data of AO/EB staining of PC3 cells 24 hours following lipofection (1), liposomal magnetofection (2), and liposomal magnetofection under a dynamic magnetic field (3); B, cytotoxicity (cell viability) following lipofection (1), liposomal magnetofection (2), and liposomal magnetofection under a dynamic magnetic field (3).

dient magnetic field, which enhances the nonviral gene delivery to prostate cancer cells, is described. The presence of a permanent magnetic field for 5 minutes is sufficient to significantly increase transfection efficiency over lipofection.

Our findings are consistent with the results of previous studies, which demonstrated that the permanent magnetic field enhanced transfection efficiency (13, 19). However, despite the promising results showing a high gene transfer rate, the permanent magnetic field has one disadvantage associated with a nonuniform distribution of complexes over the cell surface. To overcome this limitation, the device "DynaFECTOR" with an orbital rotation of cylindrical permanent magnets was constructed. The part of magnetic system consisting of 1 cylindrical permanent magnet and 2 wells of plate as shown in Fig. 2 illustrates the principle of our device. When the moving magnet is in the position (b) as shown in the same figure, the tangential component of magnetic force (F) directed to the center of the magnet starts attracting of magnetoplexes to the edge of the well. After the magnet reaches the position (c), the magnetoplexes are attracted to the opposite edge of the well doing displacements of magnetoplexes in parallel to the bottom of the well. Under prolonged rotation, magnetoplexes are pulled down to the bottom of the wells and make multiple displacements along the cell surface.

We hypothesize that the dynamic gradient magnetic field provides a more uniform distribution of complexes as well as a more efficient cellular uptake of cationic lipid/nucleic acid complexes with magnetic nanoparticles. This magnetic field forces cationic lipid/nucleic acid complexes with magnetic nanoparticles oscillate not only perpendicularly, concentrating the complexes on the surface of cells, but also parallel to the cell surface that leads to a slow rolling of the complexes over the surface of cells, facilitating the cellular uptake. Our results indicate that the efficiency of liposomal magnetofection in the presence of a dynamic magnetic field depends on the exposure time and magnet rotation frequency. Time dependency of transfection efficiency using a magnetic field is also reported previously by Kamau et al. (13). For human prostate cancer cells, the highest transfection efficiency exceeding that of standard magnetofection has been achieved in the presence of a dynamic magnetic field for 5 minutes with a magnet rotation frequency of 5 rpm.

The cytotoxicity of a dynamic magnetic field is lower than that of other delivery methods applied in our study. Because of orbital magnet rotation, the force of a magnetic field during the 5-minute incubation all the time is changed. We suppose that this alteration of magnetic force prevents from large aggregates and chain-like structure formation onto cell membranes decreasing cell damage and, consequently, cytotoxicity by this mean.

Magnetofection of reporter genes in vivo was performed in several studies using rats, mice, and pigs, and the feasibility of this technology has been demonstrated (14, 25). The same approach has also been used in few clinical trials (14, 24, 25). The technology uses an externally positioned magnet to create a localized magnetic field within the body, and the physical force created by the magnetic field draws the therapeutic genes/drugs through the arteriole into the targeted area. We think that using the same technology, the dynamic magnetic field also could be applicable for targeted delivery of a gene/drug in vivo, and further studies in an animal model are planned.

In summary, the obtained results strongly support the use of a dynamic gradient magnetic field as a perspective tool for nonviral gene delivery into malignant cells.

Conclusions

The magnetofection efficiency obtained by using "DynaFECTOR" was higher than that of lipofection and standard magnetofection in all performed experiments. The dynamic gradient magnetic field was found to be able to enhance the delivery of nucleic acids into prostate cancer cells with a reduced cytotoxicity. To confirm a universality of this approach, further studies using cell lines of various origins are required. In the future, the use of "DynaFECTOR" for such a bioapplication as shRNA coding plasmid screening for the drug therapy can undoubtedly have the advantage for successful results.

Acknowledgments

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Statement of Conflict of Interest

The authors state no conflict of interest.

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