New Device for Enhancement of Liposomal Magnetofection Efficiency of Cancer Cells

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

Abstract—Liposomal magnetofection is the most powerful nonviral method for the nucleic acid delivery into the cultured cancer cells and widely used for in vitro applications. Use of the static magnetic field condition may result in non-uniform distribution of aggregate complexes on the surface of cultured cells. To prevent this, we developed the new device which allows to concentrate aggregate complexes under dynamic magnetic field, assisting more contact of these complexes with cellular membrane and, possibly, stimulating endocytosis. Newly developed device for magnetofection under dynamic gradient magnetic field, "DynaFECTOR", was used to compare transfection efficiency of human liver hepatocellular carcinoma cell line HepG2 with that obtained by lipofection and magnetofection. The effect of two parameters on transfection efficiency, incubation time under dynamic magnetic field and rotation frequency of magnet, was estimated. Liposomal magnetofection under dynamic gradient magnetic field showed the highest transfection efficiency for HepG2 cells.

Keywords—Dynamic magnetic field, Lipofection, Magnetofection

I. INTRODUCTION

MAGNETIC field assisted cells transfection, known as magnetofection, is the most powerful nonviral method for the nucleic acid delivery into the cultured cancer cells and widely used for in vitro applications. This method comprises: the association of self-assembling complexes of cationic lipid: nucleic acid with magnetic nanoparticle and concentration of resulting aggregate complexes on the cell surface by applying permanent magnetic field [1]. Efficiency of magnetofection, as a physical method, depends on the magnetic carrier's formulation and the mode of applied magnetic field. Usually, magnetofection is performed with commercial superparamagnetic iron oxide nanoparticles under static magnetic field of 130-250 mT with an incubation time in the range 5-20 min [2]–[6]. According to Furlani and Ng modeled magnetofection, an accumulation time for 200 nm hydrodynamic diameter particles is predicted to be around 20 min and the rate of sedimentation magnetic polyplexes is enhanced with larger size complexes [7]. Despite the high efficiency of magnetofection method which can increase the cellular uptake for up to several-hundred –fold, Kamau et al. and McBain et al. reported that magnetofection efficiency was significantly increased by applying a pulsed magnetic field and oscillating magnetic field, respectively [8], [9]. Use of

the static magnetic field condition may result in non-uniform distribution of aggregate complexes on the surface of cultured cells and decrease contact between magnetic lipoplexes or polyplexes and cells. To prevent this, we developed the new device which allows concentrating aggregate complexes under dynamic magnetic field, assisting more contact of these complexes with cellular membrane and, possibly, stimulating endocytosis. For the creation of dynamic property of the magnetic field, rotating platform of orbital shaker was used. Magnets have been inserted into platform in such way that in view of their movement it was possible to reach the value of magnetic field 0.35 Tl and a level of normal and tangential gradients 3×10^7 a/m^2 according to computer modeling of the magnetic vector force. In this study newly developed device for magnetofection under dynamic gradient magnetic field, "DynaFECTOR", was used to compare transfection efficiency of human liver hepatocellular carcinoma cell line HepG2 with that obtained by lipofection and magnetofection. The effect of two parameters, incubation time under dynamic magnetic field and rotation frequency of magnet, on transfection efficiency was estimated.

II. MATERIALS AND METHODS

A. Materials

The CombiMag magnetic nanoparticles were obtained from Chemicell GmbH. They are composed from magnetite (Fe_3O_4) and have mean hydrodynamic diameter 100 nm. Cylindrical rare-earth neodymium–iron–boron (NdFeB) N38 grade 1.26 Tl induction magnets were purchased from Semicom Ltd. Attractene was obtained from (QIAGEN, Germany).

B. Cells

Human liver hepatocellular carcinoma cell line HepG2 was grown in Eagle's Minimum Essential 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% CO_2 humidified air atmosphere.

C. Plasmid

The plasmid pcDNA3.1LacZ encoding for ß-galactosidase protein under the control of a cytomegalovirus (CMV) promoter was purchased from (Invitrogen, CA, USA. pcDNA3.1LacZ was propagated in *E. coli* and purified with plasmid purification Midi kit (QIAGEN, Germany) according to the

M. Baryshev, D. Vainauska, S. Kozireva and A. Karpovs is with the August Kirchenstein Institute of Microbiology and Virology, Riga Stradins University, Latvia, Ratsupites Street 5, LV-1067 (phone: +371 26098711; fax:67428036; e-mail: mbarisevs@latnet.lv).

World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:5, No:10, 2011

manufacturer's protocol. The purified plasmid was predominantly in supercoiled form $\geq$ 95% ccc.

C. Magnetofection and Transfection

The transfection procedure was optimized and 1.2-1.5 x 10^5 cells were seeded in 24-well plates, one day before transfection to obtain 70-80% confluence. All incubations were done at 37°C and 5% CO_2. Before transfection, the medium was removed and 440 μl of Opti-MEM reduced serum medium (Invitrogen, CA, USA) was added.

For transfection 0.6 μg of pcDNA3.1LacZ DNA and an equal volume (0.6 μl) of CombiMag magnetic nanoparticles were diluted separately in 30 μl of Opti-MEM reduced serum medium and combined. After mixing 1.5 μl of Attractene reagent was added to the DNA/CombiMag mixture. The final CombiMag/DNA/Attractene mixture was incubated at room temperature for 10 min and subsequently added to each well. The cell culture plates were placed on magnet for 5 min or on dynamic magnetic field generator „DynaFECTOR" for 5 min, using magnets' rotation frequency - 5, 25, 50 and 100 rpm.

D. Reporter Gene Expression Analysis

After 24 hr of incubation, LacZ gene expression was detected using ß-Gal staining kit (Invitrogen, CA, USA) according to manufacturer's protocol. The transfection efficiency was determined by counting stained and unstained cells under a microscope and calculating the percentage of stained cells in the total population. Untransfected cells were included to account for background.

III. RESULTS AND DISCUSSION

A. Device Construction And Methodology

For the creation of dynamic magnetic field, rotating property of orbital shaker was used. Instead a platform shaker, the magnetic system of cylindrical permanent magnets with saturation induction 1.33 Tl arranged in a checkerboard pattern unipolar was inserted as shown in the Fig. 1. 24-well plate is located on a non-magnetic metal surface over a magnet system. The distance from the bottom of the plates up to the upper surface of the magnets is fixed and is 3.5 mm.
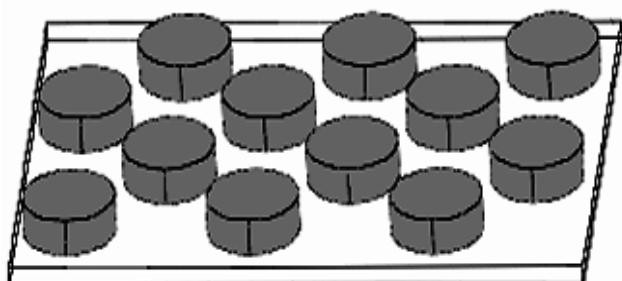

Fig. 1 Magnetic system of cylindrical permanent magnets arranged in a checkerboard pattern.

Electronics provides programmable rotation of the magnetic system on the orbit at a given speed 1-150 rpm for a fixed time of 1 to 999 minute. In addition, a change of rotation is reversed after 30 seconds. Magnets have been inserted in such way that in view of their movement it was possible to reach the value of magnetic field 0.35 Tl and a level of normal and tangential gradients $3 \cdot 10^7$ a/m² according to computer modeling of the magnetic vector force. Diameter of the magnets and their trajectories are chosen to maximize the uniform magnetic field action on wells. In our work, the Brownian motion is not to be taken into account. The motion of spherical magnetic particle in viscous carrier fluid under applied magnetic field is described by classical Newtonian dynamics

$$ m_p \frac{\partial^2 r}{\partial t^2} = F_m + F_g + F_f \tag{1} $$

where m_p is the mass of particle, r and $\frac{\partial^2 r}{\partial t^2}$ are the coordinate and acceleration of particle, and $F_m + F_g + F_f$ are the magnetic, gravitational and fluidic forces, respectively. The fluidic force is a Stokes force and described by:

$$ F_f = 6\pi\eta R_h u \;, \tag{2} $$

Where η is a viscosity of fluid, R_h is a effective hydrodynamical radius, u is a relative speed of particle in fluid.

The gravitational and inertial forces for particle in fluid are typically an order of magnitude smaller then the applied magnetic field [10]. Thus, we considered that the magnetic particle motion is determined by the magnetic field force,

$$ F_m = (m \nabla) H \tag{3} $$

where m is the induced magnetic moment in the particle due to the applied magnetic field, and H is the applied magnetic field strength. The magnetic system used in our device gives both the magnetic field magnetizing the particle and the field gradient that act together to create the magnetic force on the particle.

Magnetic field from cylindrical magnet in plane of well was calculated by method of finite elements. Magnetic particles behavior on plane defined mainly from tangential component of magnetic fields gradient reaching maximums at the magnet edges Fig 2. Besides that, the tangential component of magnetic field acts with direction to the center on magnet.
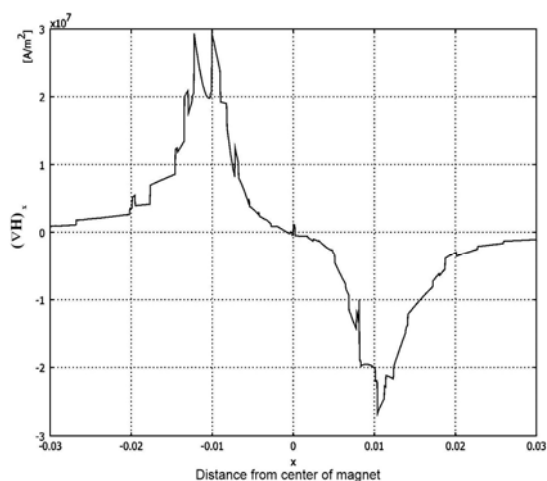
World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:5, No:10, 2011

Fig. 2 Magnetic field tangencial component gradient destribution above a cylindrical magnet

During the magnet rotation every magnetic particle cross twice magnet edges as shown in Fig 3. Depending on the frequency of the magnet, the magnetic force acting on the magnetic poliplexes, as shown in Fig. 3, to concentrate them on the cell surface forcing the oscillates. During the magnetofection with our device, the magnetic force alters the amplitude and the direction. This can lead to the displacement of magnetic lipoplexes along the cell surface, contributing to a greater number of physical contacts. Building a path of magnetic particles on the well surface due to the dynamic gradient magnetic field is still quite challenging because it requires taking into account many factors and can be a task of the future studies.
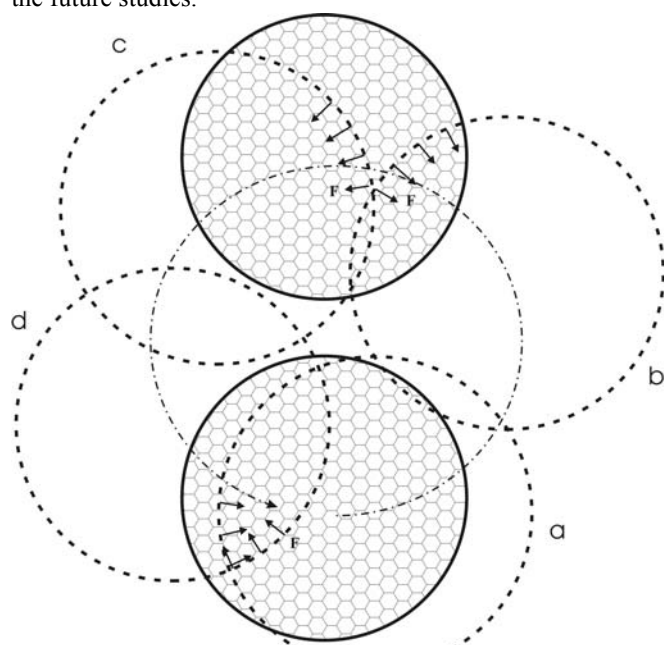

Fig. 3 Schematic illustration of the part magnetic system for the magnetofection and magnetic force direction under the dynamic magnetic field. (a), (b), (c), (d) The magnet sequential positions in a different time

B. Comparison Between Transfection And Magnetofection

To estimate the effect of the dynamic magnetic field on magnetofection efficiency we performed the transfection of hard-to-trasfect HepG2 cell line under dynamic magnetic field to compare with that obtained by liposomal and standard magnetofection under static magnetic field. All cells transfections were done in optimized conditions. As can be seen in Fig. 5, the obtained magnetofection efficiency by using the dynamic magnetic field is higher than that in compared methods. The data are shown as a mean of three independent experiments.
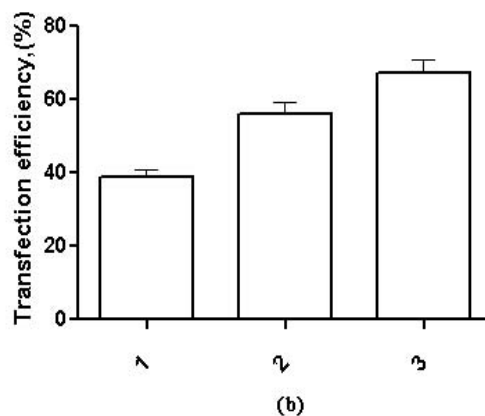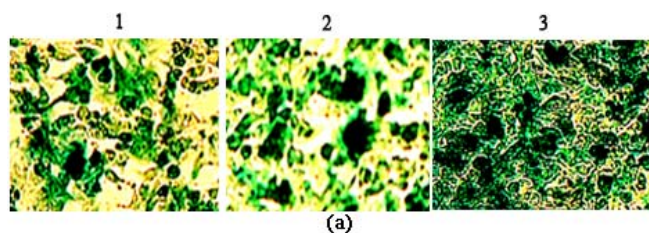

Fig.4 Effect of dynamic gradient magnetic field on transfection efficiency of pcDNA3.1LacZ plasmid. (a) Representative data showing ß-galactosidase expression in HepG2 cells at 24 h folowing lipofection (1) liposomal magnetofection (2) and liposomal magnetofection under dynamic magnetic field (3). (b) Bar chart showing the proportion of transfected cells at 24 h following lipofection (1), liposomal magnetofection (2) and liposomal magnetofection under dynamic magnetic field (3). Data represent the mean values of three independent experiments

C. Effect Of Magnet Rotation Frequency

To determinate the influence of the magnet rotation frequency on magnetofection efficiency 5, 25, 50 and 100 rpm were used. Optimal incubation time under the dynamic magnetic field was 5 min. (data not shown). Fig. 6 shows that 50 and 100 rpm have pronounced effect on magnetofection efficiency and yields the highest results.

On the whole, our results allow us hypothesize that the increase of magnetofection efficiency under dynamic magnetic field is achieved by the concentration of magnetic polyplexes on the cell surface forcing them oscillates and due to the contribution to a greater number of physical contacts which result in the stimulation of the process of endocytosis.
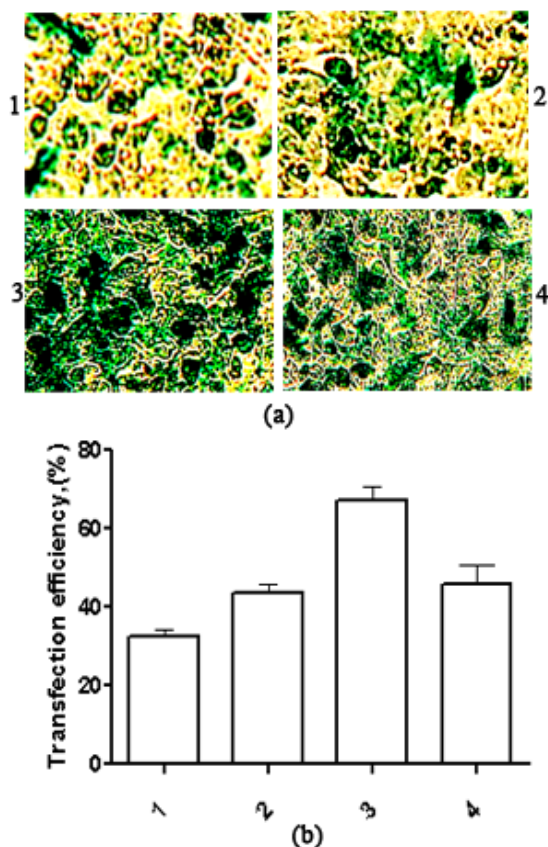
World Academy of Science, Engineering and Technology
International Journal of Biomedical and Biological Engineering
Vol:5, No:10, 2011

Fig. 5 Effect of magnets' rotation frequencies on expression of ß-galactosidase. (a) Representative data showing ß-galactosidase expression in HepG2 cells at 24 h folowing 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 (1), 25 (2), 50 (3) and 100 (4) rpm. Data represent the mean values of three independent experiments.

IV. Conclusion

We have described the construction and principle of work of the new device for the enhancement of liposomal magnetofection efficiency under dynamic magnetic field, named by authors the **"**DynaFECTOR",. We have also considered the forces governing particle motion under influence of applied field of dynafector and suggested possible mechanism of the increase of the magnetofection efficiency. *In vitro* magnetofection studies in hard-to-transfect HepG2 cell line carried out with gene reporter plasmid pcDNA3.1/LacZ on the "DynaFECTOR" device showed that the magnetofection efficiency was the incubation time and the magnet rotation frequency depended. The magnetofection efficiency obtained by using "DynaFECTOR", was higher than that of lipofection and standard magnetofection in all performed experiments. More cell lines should be tested to show the universality of use of the "DynaFECTOR" for the enhancement of magnetofection efficiency. In the future, the use of "DynaFECTOR" for such bioapplication as shRNA coding plasmid screening for the drug therapy can undoubtedly have the advantage for successful results.

References

[1] F. Scherer, M Anton, U Schillinger, J Henke1, C Bergemann, A Kruger, B Gansbacher and C Plank, *Gene Therapy* (2002) 9, 102–109.
[2] F. Krötz, C. De Wit, H.Y. Sohn, Zahler S, Gloe T, Pohl U, et al., Magnetofection – a highly efficient tool for antisense oligonucleotide delivery in vitro and in vivo. *Mol Ther* 2003;7:700–10.
[3] S. Huth, J. Lausier, S.W. Gersting, C.Rudolph, C.Plank, U. Welsch et al. Insights into the mechanism of magnetofection using PEI-based magnetofectins for gene transfer. *J Gene Med* 2004;6:923–36.
[4] A. M. Sauer, K. G. de Bruin , N. Ruthardt, O. Mykhaylyk, C. Plank, C. Bräuchle. Dynamics of magnetic lipoplexes studied by single particle tracking in living cells. *J Control Release* 2009;137:136–45.
[5] C. Plank, M. Anton, C. Rudolph, J. Rosenecker, F. Krotz. Enhancing and targeting nucleic acid delivery by magnetic force. *Expert Opin Biol Ther* 2003;3:1–14..
[6] N. Morishita., H. Nakagami, R. Morishita, S. Takeda, F. Mishima, B. Terazono, et al. Magnetic nanoparticles with surface modification enhanced gene delivery of HVJ-E vector. *Biochem Biophys Res Commun* 2005;334:1121–6.
[7] E.P. Furlani, K.C. Ng. Nanoscale magnetic biotransport with application to magnetofection. *Phys Rev* E 2008;77:1–8.
[8] S.W. Kamau, P.O. Hassa, B. Steitz, A. P. Fink, H. Hofmann, M. Hofmann-Amtenbrink, et al. Enhancement of the efficiency of non-viral gene delivery by application of pulsed magnetic field. *Nucleic Acids Res* 2006;34:e40.
[9] S.C. McBain, U. Griesenbach, S. Xenariou, A. Keramane, C.D. Batich, E..W.F.W, Alton, et al. Magnetic nanoparticles as gene delivery agents: enhanced transfection in the presence of oscillating magnet arrays. *Nanotechnology* 2008;19:405102.
[10] E.P. Furlani, Magnetic biotransport: analysis and aplplication. *Materials* 2010, 3, 2412-2446.