Development of Magnetic Force-Assisted New Gene Transfer System Using Biopolymer-Coated Ferromagnetic Nanoparticles

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Shin-Ichi Takeda, Fumihito Mishima, Bungo Terazono, Yoshinobu Izumi, and Shigehiro Nishijima

***Abstract*—Development of a simple method for converting the lipid envelope of an inactivated virus to a gene transfer vector was achieved a couple years ago in the medical school of Osaka University. Hemagglutinating virus of Japan (HVJ; Sendai virus) envelope (HVJ-E) vector was constructed by incorporating plasmid DNA into inactivated HVJ particles. This HVJ envelope vector introduced plasmid DNA efficiently and rapidly into various cell lines, including cancer cells and several types of primary cell culture. In the present study, efficiency of gene transfer was found to be greatly enhanced by application of a magnetic field. Therefore, we developed a new type of magnet for magnetically enhancing and targeting gene transfection system by using vectors associated with ferromagnetic particles coated with positively/negatively charged biopolymers, which can help to enhance and target gene delivery with higher efficiency. For the transfection experiment *in vitro*, the HVJ-E vector was mixed with ferromagnetic particles coated with biopolymer and this mixture was added to cultured cells which were set up under the permanent magnet. The effect of the dose of the ferromagnetic particles on the transfection efficiency was discussed. In order to clarify the effect of magnetic field gradient on the accumulation possibility of the magnetic particles and the accuracy of the targeted site in the blood vessels, calculation of the applied magnetic force for the ferromagnetic particles inside the blood vessel was also performed.**

***Index Terms*—Drug delivery system, gene therapy, HVJ-E, magnetic field effects, magnetic nanoparticle, magnetic targeting.**

I. Introduction

PROGRESS in gene therapy requires a novel drug delivery system (DDS). To enhance the transfection efficiency, we developed a hybrid vector utilizing the envelop of HVJ (hemagglutinating virus of Japan). The HVJ-E vector for rapidly targeting is still insufficient for rapid and specific accumulation of active vectors in target tissues. One solution is to engineer the surface proteins of viral vectors or to couple targeting ligands to viral as well as nonviral vectors, which might further improve tissue selectivity [1]–[4]. Thus, we examined modification of the membrane surface of HVJ to achieve an improvement. In this study, we focused on magnetic nanoparticles, such as maghemite, with an average size of 30nm, which can attach to vectors incorporating plasmid DNA, ligonucleotide or protein and transfer into cells by cell fusion [5]. These prepared magnetic vectors can be positionally regulated by a magnetic force. Magnetic nanoparticles basically consist of oxidized Fe, which is commonly used as a supplement for the treatment of anemia. We hypothesized that the association of magnetite and HVJ-E technology could allow the rapid attachment of HVJ-E and cells by application of a magnetic force, leading to enhanced transfection efficiency. Moreover, magnetic nanoparticles can be modified with several chemical compounds to allow modification of the charge, size, and affinity. In addition, there is increasing interest in using magnetic resonance imaging (MRI) to monitor the *in vivo* behavior of proteins labeled with magnetic nanoparticles [6], [7]. Indeed, magnetic nanoparticles were recently used for gene transfection, because magnetic targeting exploits paramagnetic particles as drug carriers, guiding their accumulation in target tissues with strong local magnetic fields [8], [9]. Here, the present study demonstrated that modification of magnetic nanoparticles with protamine sulfate enhanced the transfection efficiency associated with HVJ-E system in cultured cells *in vitro*.

Calculation of the applied magnetic force for the ferromagnetic particles inside the blood vessel was also performed using a model system of blood vessels in order to clarify the effect of magnetic field gradient on the accumulation possibility of the magnetic particles and design the practical magnet with high accuracy of the targeted site in the blood vessels.

II. Materials and Methods

A. Preparation of HVJ-E Vector and Plasmid DNA

HVJ-E vector was obtained from Ishihara (Osaka, Japan). Basically, aliquots of the inactivated virus (6 AU) were suspended in 40 μl TE solution (10 mM Tris—Cl, pH 8.0, 1 mM EDTA) and mixed with plasmid DNA (30 μg), and 0.3% Triton X. The mixture was centrifuged at 18 500 g for 15 min at 4 degrees Celsius. After washing the pellet with 1 ml balanced salt solution (BSS; 10 mM Tris—Cl, pH 7.5, 137 mM NaCl, and 5.4 mM KCl) to remove the detergent and unincorporated DNA, the envelop vector was suspended in 300 μl BSS. The titer of the inactivated virus was modified according to each experiment. pEGFP-C1 was purchased from Clontech (CA, USA). pCMV-luciferase-GL2 was constructed by cloning the luciferase gene from the pGL2-promoter vector (Promega, Madison, WI, USA) into pcDNA3 (5.4 kb) (Invitrogen, San Diego, CA, USA). Plasmids were purified with a Qiagen

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plasmid isolation kit (Hilden, Germany). FITC-oligodeoxynucleotides (ODN) (random sequence) were purchased from Gene Design (Osaka, Japan).

B. Preparation of Magnetic HVJ-E Vector

Commercially available maghemite particle (NanoTek γ-Fe2O3, C.I. Kasei) was used for the preparation of magnetic HVJ-E vector in the present study. The averaged primary size of maghemite particle used in this study was 29 nm. The particles were mixed with protamine sulfate or heparin solution to prepare magnetic nanoparticles modified with biopolymers, which consist of cationic or anionic polymer adsorbing onto ferromagnetic nanosized particles. The prepared magnetic nanoparticles were then mixed with HVJ-E vector to provide ferromagnetism to the HVJ-E vector.

C. Measurement of Zeta Potential of Maghemite Particles

Zeta potential is an important parameter in understanding electrostatic colloidal dispersion stability. Zeta potential is the charge that a particle acquires in a particular medium. It is dependent upon the pH, ionic strength and concentration of a particular component. The mobility of particles undergoing electrophoresis is measured by the technique of laser Doppler electrophoresis. This measured electrophoretic mobility is then converted to zeta potential using established theories. In order to know the surface property of the magnetic vector, the zeta potential of the particles with and without surface modification was determined (Zetasizer Nano ZS, Malvern, U.K.). Mixtures of magnetite particles with and without surface modification with protamine sulfate or heparin and the prepared magnetic HVJ-E vector were used.

D. Gene Transfer in vitro

For in vitro transfection, 5×10^5 cells were prepared in 6-well culture dishes 1 day before transfection experiment. HVJ-E vector (1 or 0.2 AU) containing luciferase plasmid (GL2: 5 or 1 μg) or FITC-ODN (2 or 0.4 μg) was mixed with various concentrations of maghemite and DMEM containing 10% FCS, and added to cells cultured in DMEM supplemented with 10% FBS on a magnetic sheet.

E. Magnetic Sheet and Cultured Dish

The magnetic sheet was placed under the 6-well culture dish. The magnetic field gradient is one of the parameters affecting the strength of the magnetic force applied to the magnetic vectors to sediment to the cell surface. Magnetic field measurements were performed by Hall detector to calculate the magnetic field gradient. A 3-axis Hall effect teslameter (MetroLab THM 7025, METROLAB Instruments SA, Switzerland) was used to measure the magnetic field. The averaged value of the measured magnetic field at the sheet surface was approximately 1.2 mT and the calculated value of the field gradient was 0.17 T/m. After 10 min of incubation at 37 degrees Celsius under 5% CO_2, the medium was replaced and the cells were cultured overnight before examination of gene expression. GFP expression or FITC ODN transfected cells were observed under a fluorescence microscope.

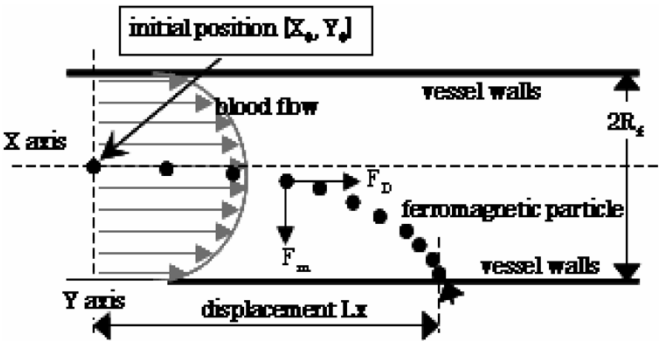

Fig. 1. Schematic of the controlling particle system utilized for modeling the targeting of magnetic particle inside the blood vessel using by the magnetic force. $2\mathrm{R_f}$ is a diameter of the blood vessel. The final position $[\mathrm{X_n}, \mathrm{Y_n}]$ is calculated based on the equations of drug force $(\mathrm{F_D})$ and magnetic force $(\mathrm{F_m})$ using a recursive formulae.

F. Luciferase Activity and LDH Release

Luciferase activity was measured following the manufacturer's instructions (Promega Madison) [10]. Twenty-four hours after transfection, cells were washed once with PBS and then incubated with lysis buffer. The protein concentration was determined using the Bio-Rad protein assay adapted for use in a 96-well plate. After 20 μl cell extract was mixed with 100 μl luciferase assay reagent (Promega, Madison), the light produced was measured for 2 s using a luminometer. The extent of cell death was assessed using a commercially available kit (Wako, Osaka) to measure released LDH activity from dead cells according to previous reports [11], [12], because loss of cell membrane integrity was observed in both necrotic and apoptotic cells. The percentage of LDH release was calculated relative to the maximum cell death of cells treated with 1% Triton X-100 for 10 min.

G. Calculation of the Magnetically Targeted Ferromagnetic Particle in Blood Vessel

A 2-D schematic of particle control system utilized for modeling the targeting of magnetic particle by the magnetic force arising from the magnet placed outside the blood vessel is represented in Fig. 1. Forces that act on the magnetic particle are shown in this figure. The particle feels viscous drag force when it begins to move. On the other hand, magnetic force acts on floating ferromagnetic particles when the particles in the blood vessel are placed under the magnetic field.

When the magnetic particle is assumed to be spherical, energy U arising from magnetic force is as follows:

$$\mathrm{U} = -\frac{1}{2}\mathrm{m\,H} = -\frac{2}{3}\pi \mathrm{b}\mu_0 \mathrm{H}^2(\mathrm{x_p} - \mathrm{x_f}) \tag{1}$$

where χ_p and χ_f are magnetic susceptibilities of the particle and fluid, respectively. And b is a radius of the magnetic particle. Therefore, magnetic force $\mathrm{F_m}$ acts the magnetic particle as follows:

$$\mathrm{F_M} = -\Delta \mathrm{U} = \frac{4}{3}\pi \mathrm{b}^3 \mu_0 (\mathrm{x}_p - \mathrm{x_f})\mathrm{H}\nabla\mathrm{H} \tag{2}$$

Magnetic force is product of magnetic field strength and magnetic gradient.

When velocity v_p of the magnetic particle is different from the blood flow velocity v_f, the particle is subjected to the drag force FD from the fluid, and thus Stokes's expression can be used on the magnetic particle

$$F_D = 6\pi\eta b(v_f - v_p) \tag{3}$$

where η is a viscous coefficient of the blood.

The vessel walls are assumed to be two parallel planes also placed perpendicular to the plane of the figure, as shown. The blood accesses the vessel wall from the initial position located in the left side in this figure with a velocity defined by a parabolic profile of initial velocity V_0 and it transports the magnetic particle to be captured by the wall. Finally, the magnetic particle is subjected to a homogeneous magnetic field H_o positioned perpendicular to the blood flow. The model accounts for magnetic force and hydrodynamic force while neglects the effect of gravity force as well as any effect due to the vessel walls. The fluid dynamics in the blood vessel is described by the equations of (2) and (3) mentioned above for an incompressible Newtonian fluid.

The trajectory of the magnetic particle is calculated by using (2) and (3). When we initially set the magnetic particle in the blood vessel with a velocity defined as an initial velocity $V_0 = V_f(S_0)$ at the location, $S_0[X_0, Y_0]$ shown in Fig. 1, a particle acceleration, $A_0 = F((S_0)$ at the $S_0[X_0, Y_0]$ is calculated using the following:

$$F = \frac{(F_D + F_m)}{m_p} \tag{4}$$

where m_p is a mass of the magnetic particle. The 2nd position, velocity, and acceleration are calculated using the following:

$$S_2 = S_1 + V_1 dt + \frac{1}{2} A_1 dt^2 \tag{5}$$

$$V_2 = V_1 + A_1 dt \tag{6}$$

$$A_2 = F(S_2) \tag{7}$$

Therefore, the nth position, velocity, and acceleration after nth infinitesimal time are represented by

$$S_n = S_{n-1} + V_{n-1} dt + \frac{1}{2} A_{n-1} dt^2 \tag{8}$$

$$V_n = V_{n-1} + A_{n-1} dt \tag{9}$$

$$A_n = F(S_n) \tag{10}$$

The calculation was made using recursive formulae for the particle with a diameter of 2 μm of maghemite under the various magnetic gradients from 1 T/m to 500 T/m in the blood flow (averaged flow velocity: 10 cm/s).

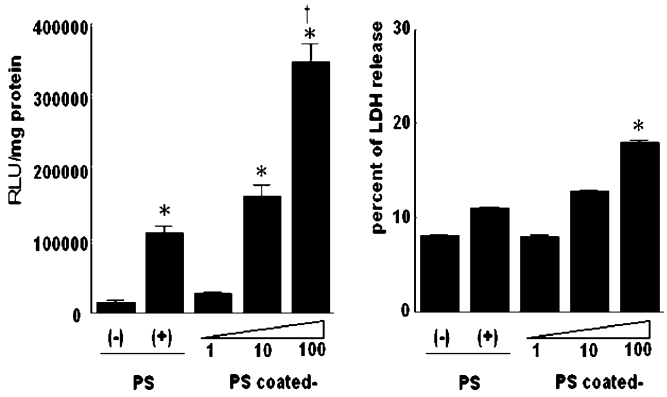

Fig. 2. Transfection efficiency and cell toxicity of maghemite-associated HVJ-E vector (1 HAU) containing the luciferase expression plasmid (5 μg) was transferred to BHK-21cells with various concentrations of protamine sulfate coated maghemite. The final concentrations of PS and PS-coated maghemite were as follows: 100 μg/ml PS, 1, 10, and 100 μg/ml PS-coated maghemite.

III. Results and Discussion

A. Magnetic Transfection Efficiency and Cell Toxicity

To evaluate the HVJ-E vector system mixed with maghemite, we measured luciferase activity to evaluate transfection activity and LDH release to evaluate cell toxicity after transfection. We mixed several doses of maghemite (from 1 mg/ml to 100 mg/ml) with HVJ-E vector infusing luciferase plasmid. However, unexpectedly, we did not find any improvement in transfection efficiency as assessed by luciferase activity in BHK-21 cells, whereas a high dose of maghemite induced cell death as assessed by LDH release (results not shown). From these results, we speculated that there might be a close association, such as electrostatic interaction, between HVJ-E and maghemite. Since we previously knew that protamine sulfate (PS), a low-molecular-weight naturally polycationic peptide (ca.4000 Da), enhanced the transfection efficiency based on the HVJ-E vector in an in vitro culture system [5], we coated the surface of maghemite with PS. After modification with PS, the zeta potential of maghemite was changed to 23.8 $\pm$ 1.8 mV from 17.5 $\pm$ 1.6 mV, which suggests that surface coating of maghemite enhanced its cationic charge. Interestingly, a mixture of PS-coated maghemite with HVJ-E vector significantly enhanced the transfection efficiency in a dose-dependent manner (Fig. 2, $P < 0.01$), which clearly indicated that the magnetic force can enhance the transfection efficiency. One reason of this effect is probably ascribed to enhancement of the rapidness of the magnetic vector approaching toward the BHK-21 cell surface. However, induction of cell death was still detected at a high dosage.

B. Calculations for Designing New Magnet for Gene Therapy

The calculated particle trajectory was shown in Fig. 3. This figure can allow us to realize how strong magnetic gradient is needed to accumulate the magnetic particle on the wall of the blood vessel. For example, when we apply the magnetic gradient of 500 T/m, a magnetic particle can be easily accumulated on the wall. Simultaneously, this graph can give us the information

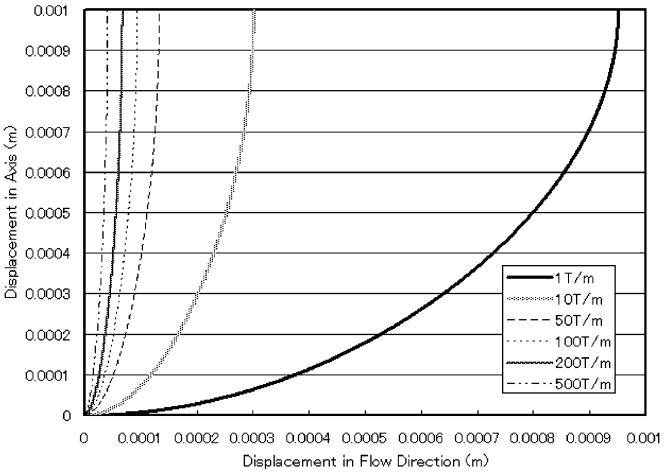

Fig. 3. Calculated particle trajectory under blood flow (flow velocity: 10 cm/s). Y-axis is a displacement from the center of the blood vessel to the wall. X-axis is a displacement in flow direction from the initial position to the final position.

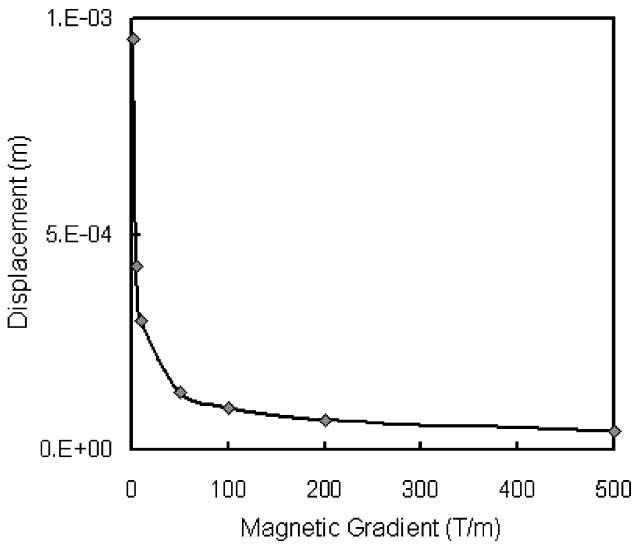

Fig. 4. Relation between a displacement of the magnetic particle in flow direction and magnetic gradient for 2 μm magnetic particle under flow velocity of 10 cm/s.

how long distance will be required to accumulate the particle on the wall of the blood vessel even in blood flow.

Fig. 4 clearly shows an effect of the magnetic gradient on the particle displacement in the blood flow. The displacement does not change so much in the range of magnetic gradient over 100 T/m.

IV. Conclusion

The present study demonstrated that modification of magnetic nanoparticles with protamine sulfate enhanced the transfection efficiency associated with HVJ-E system in cultured cells *in vitro*. This result can open the way for developing a novel gene therapy technique using magnet.

Calculation of the applied magnetic force for the ferromagnetic particles inside the blood vessel was also performed using a model system of blood vessels in order to clarify the effect of magnetic field gradient on the accumulation possibility of the magnetic particles and design the practical magnet with high accuracy of the targeted site in the blood vessels. The calculated results show the possibility of this technology and we can calculate the targeting site in the blood vessel.

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