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Magnetic nanoparticles as gene delivery agents: enhanced transfection in the presence of oscillating magnet arrays

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Abstract

Magnetic nanoparticle-based gene transfection has been shown to be effective in combination with both viral vectors and with non-viral agents. In these systems, therapeutic or reporter genes are attached to magnetic nanoparticles which are then focused to the target site/cells via high-field/high-gradient magnets. The technique has been shown to be efficient and rapid for *in vitro* transfection and compares well with cationic lipid-based reagents, producing good overall transfection levels with lower doses and shorter transfection times. In spite of its potential advantages (particularly for *in vivo* targeting), the overall transfection levels do not generally exceed those of other non-viral agents. In order to improve the overall transfection levels while maintaining the advantages inherent in this technique, we have developed a novel, oscillating magnet array system which adds lateral motion to the particle/gene complex in order to promote transfection. Experimental results indicate that the system significantly enhances overall *in vitro* transfection levels in human airway epithelial cells compared to both static field techniques ($p < 0.005$) and the cationic lipids ($p < 0.001$) tested. In addition, it has the previously demonstrated advantages of magnetofection—rapid transfection times and requiring lower levels of DNA than cationic lipid-based transfection agents. This method shows potential for non-viral gene delivery both *in vitro* and *in vivo*.

1. Introduction

Magnetic nanoparticles have recently generated significant interest as gene delivery agents. Mah *et al* [1, 2] first reported the technique in which they attached an rAAV vector encoding green fluorescent protein (GFP) to magnetic microparticles via a heparan sulfate linker. High-strength, high-gradient neodymium iron boron (NdFeB) magnets were used to target the vector to cells in culture and enhance GFP expression *in vitro*.

Following on from this initial work with viral vector/particle complexes, Plank, Rosenecker and others have elegantly demonstrated that *non-viral* magnetic nanoparticle-based transfection (termed magnetofection), using static magnetic fields, can result in very rapid transfection—on the order of minutes—in several cell types [3–5]. This is achieved through rapid sedimentation of the particle/gene complex due to the attraction of the particles along the magnetic field gradient—a concept similar to that first proposed for magnetic drug targeting [6, 7]. Another major advantage of this method is that the magnetic fields may be configured to target specific sites *in vivo* and increase the residence time of the vector at the target [2, 4, 8]. In addition to gene or

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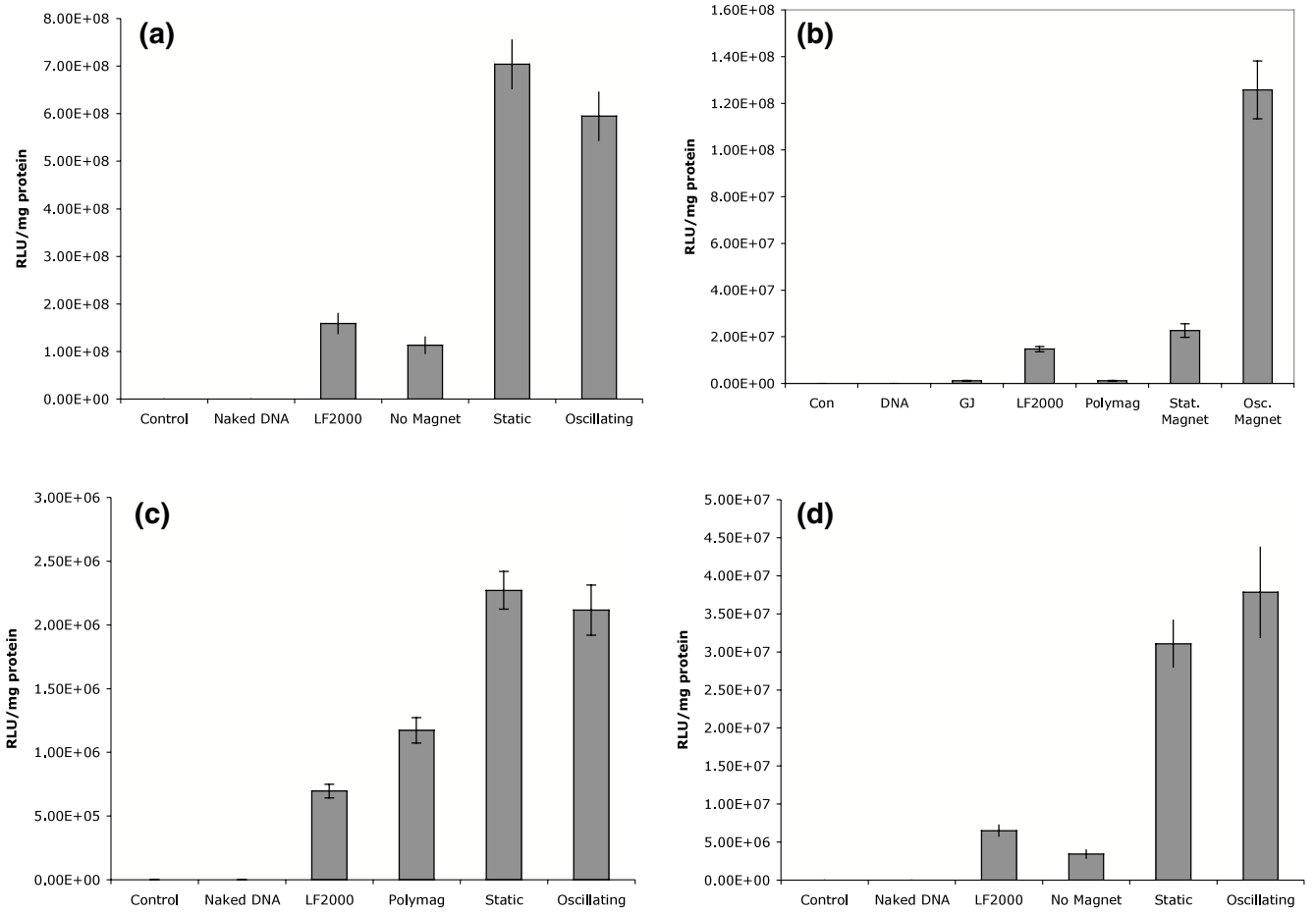

Figure 1. Luciferase activity in NCI-H292 human lung epithelial cells transfected with pCIKLux luciferase reporter construct using OzBiosciences Polymag® particles ('no magnet', 'static' and 'oscillating'), Lipofectamine2000 ('LF2000') and GeneJuice ('GJ') + naked DNA (control). Transfections were performed in 96-well tissue culture plates using 0.1 μg DNA/well with 2 h transfection time. Data shown as mean ± SEM ($n = 12$) for three oscillation amplitudes: (a) 10 μm/2 Hz, (b) 200 μm/2 Hz, (c) 100 nm/2 Hz and (d) 1 mm/2 Hz.

viral delivery, magnetofection has been also used to deliver antisense nucleic acids. Recent work by Krotz *et al* has demonstrated effective gene silencing using siRNA molecules associated with magnetic nanoparticles [9].

The primary mechanism underlying magnetofection is the attraction of the magnetic particle to the magnetic field source according to the equation

$$F_{\text{mag}} = (\chi_2 - \chi_1)V\frac{1}{\mu_0}B(\nabla B) \qquad (1)$$

where F_{mag} is the force on the magnetic particle, χ_2 is the volume magnetic susceptibility of the magnetic particle, χ_1 is the volume magnetic susceptibility of the surrounding medium, μ_0 is the magnetic permeability of free space, V is particle volume, and B is the magnetic flux density in tesla (T) [10]. This results in a translational force on the particle/gene complex in the direction of the magnetic field source, which is generally placed under the culture dish.

In this study, we have investigated the use of a horizontally oscillating magnet array which introduces a component of lateral motion as the particles are pulled towards the field source (figure 1). The extra energy and mechanical stimulation associated with this lateral motion of the particles should promote increased particle sedimentation and stimulate uptake, thus improving the transfection efficiency compared to static fields. Furthermore, *in vivo* the lateral 'wedging' motion of the particles may prove useful in aiding in the penetration of extracellular barriers (such as airway mucus) which can inhibit the ability of current agents to deliver therapeutic genes to the lungs in cystic fibrosis patients [11, 12].

In order to test this, the oscillating field transfection system (magnefect nano) was compared to static field magnetofection as well as the cationic lipid-based transfection agents GeneJuice and Lipofectamine2000 in NCI-H292 (human lung epithelial) cells.

2. Results and discussion

Of the frequencies and amplitudes tested, 200 μm/2 Hz produced the best results for the oscillating system. In these experiments, the oscillating magnet system was shown to enhance the overall transfection efficiency in comparison to static field systems and both cationic lipid-based agents (figure 1). Normalized data from six experiments at 200 μm/2 Hz ($N_{\text{total}} = 72$) showed that the overall transfection efficiency for the oscillating system was approximately four times greater than that of Lipofectamine2000 ($p < 0.01$),

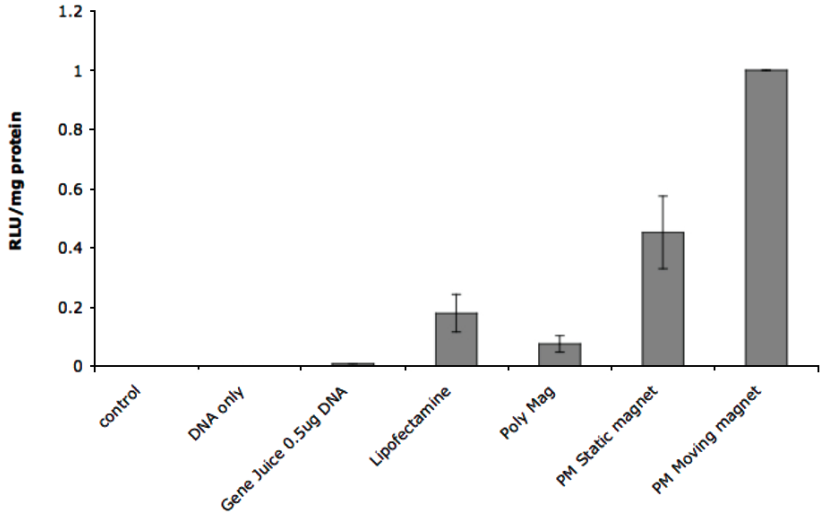

Figure 2. Normalized data from six experiments ($N_{\text{total}} = 72$) showing luciferase activity in NCI-H292 human lung epithelial cells transfected with pCIKLux luciferase reporter construct in response to static and oscillating magnetic fields at 200 μm amplitude and 2 Hz.

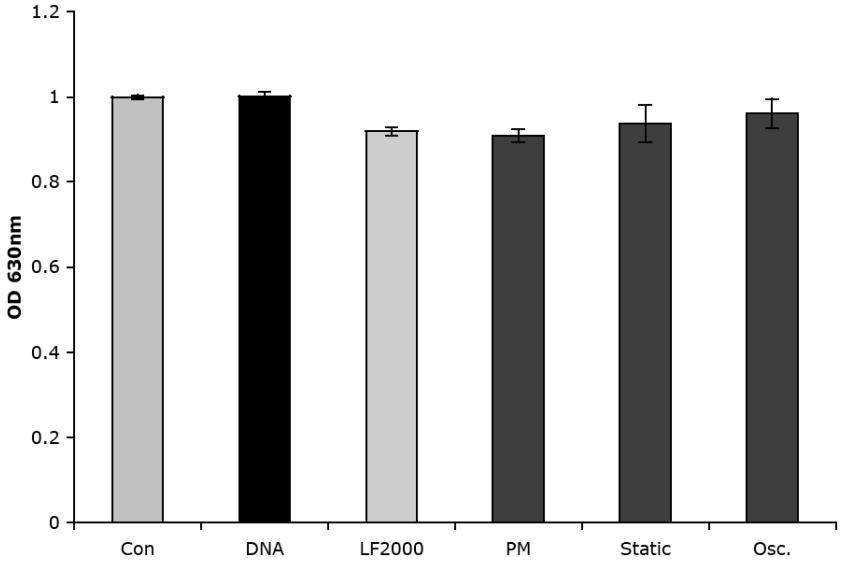

Figure 3. Normalized cell viability data ($N_{\text{total}} = 12$) for NCI-H292 human lung epithelial cells transfected with DNA alone ('DNA'), Lipofectamine2000 ('LF2000'), Polymag particles with no field ('PM'), static field with Polymag ('static') and oscillating field (200 μm/2 Hz) with Polymag ('osc.') at 48 h post transfection in comparison to untransfected control (con).

many times greater than that of GeneJuice ($p < 0.01$) and more than twice that of static magnetofection ($p < 0.01$) (figure 2). While some improvement in transfection was observed at 1 and 4 Hz and 200 μm, other frequencies and amplitudes were generally similar to those of the static systems (data not shown), indicating that perhaps the amplitude is more important than frequency, at least in the low frequency range. The optimal parameters for this technique require further investigation.

These studies also demonstrated that cell viability is not adversely affected using the oscillating system. Cell viability levels were broadly similar to controls after 48 h, indicating that the technique, though based on ballistic effects on the cells, does not appear to be harmful to them (figure 3). This is consistent with other magnetofection studies [13].

The results presented here are consistent with previous studies which have shown that magnetofection with static field systems generally reduces the amount of pDNA required, as well as the time of transfection. However, static field systems do not necessarily improve overall transfection levels when compared to lipid-based agents when used according to manufacturers' specifications. The experiments presented here were designed to test the potential for improvement in the overall transfection efficiency rather than the speed of transfection, which is now well established [3, 4].

To date, magnetofection protocols have exclusively employed static magnetic fields generated by rare earth (typically NdFeB) magnets. In the present work, we have demonstrated increased transfection efficiency in human airway epithelial cells in response to an oscillating magnet array. These levels should be further improved through ongoing optimization of the magnetic field geometry, amplitude and frequency parameters. Cell viability data demonstrate a significant advantage of this system in comparison to other

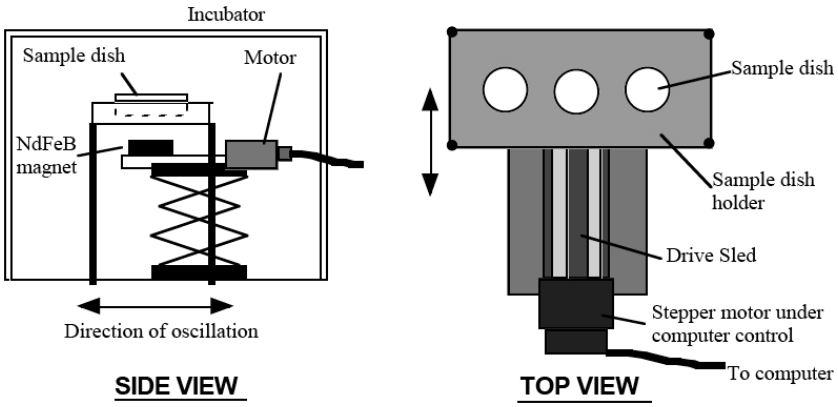

Figure 4. Schematic representation of the computer-controlled stepper motor drive and oscillating magnet array system. The system can be configured for 60 mm Petri dishes and multi-well plates.

physical-based transfection systems such as electroporation, where cell viability is seriously compromised. The oscillating array system used here appears to have little or no effect on cell viability.

Though work on oscillating magnetic fields in the x–y plane was first introduced by our group in 2005 [14, 15], Kamau and others presented compelling results from a similar system using electromagnets in 2006 [16]. They demonstrated that sinusoidal electromagnetic waves propagated in the x–y plane at frequencies of 50 and 0.75 Hz also enhanced overall transfection levels. However, the electromagnets used in that study produced significant heating and were applied at weaker field strengths and gradients in comparison to the system presented here. It is not clear what effect the heating may have on overall cell viability as data are not reported. The major advantages of using an array of oscillating permanent magnets are the increased field strength/gradient compared to that realistically achievable with electromagnets working in an incubator and the fact that they do not contribute additional heat to the cells.

Magnetofection has been shown to work via endocytosis of the particles after rapid, magnetically induced sedimentation [4]. While the mechanism for enhanced transfection in the presence of oscillating fields is not yet clear, Kamau *et al* state that the movements seem to 'enhance translocation of the particles across the cell membrane' [16]. We postulate that the mechanical stimulation of the cell membrane by the particles moving in the x–y plane promotes more efficient endocytosis and may also produce endosomal release. It is also possible that shear-induced transient membrane rupture may be a cause as large disruptions in mammalian cells are known to rapidly self-repair via endomembrane resealing [17]. However, viability data suggest the former as a more likely explanation.

Thus, through the use of oscillating magnet arrays, we were able to improve the transfection efficiency in comparison to the lipid-based agents tested and static magnetofection systems, while incorporating the significant advantages of magnetic vectors noted above. The system of oscillating permanent magnets has significant advantages over electromagnet-based systems.

3. Materials and methods

3.1. Magnet array and drive system

Magnet arrays were constructed using cylindrical stacks of NdFeB magnets (diameter = 6 mm). These stacks produced magnetic fields of ∼100 millitesla (mT) at the cell surface. Magnet stacks were arranged on an acrylic template to align with 12 wells of a 96-well plate with a spacing of at least 12 mm between the magnets to ensure that the fields did not interact. Magnetic fields were mapped using a Redcliffe Magtronics Ltd Magscan500. For static field experiments, the cell culture plates were placed directly above the magnet array. For oscillating exposures, the magnetic array was mounted on a computer-controlled stepper motor (Pacific Scientific, Inc., Newport Beach, California) interfaced to an Apple Macintosh G4 computer running National Instruments Labview 7. Cell culture plates were placed on a sample holder above the magnet array, where the magnet array generated a force on the particles approximately three orders of magnitude greater than the force of gravity (figure 4). Experiments were performed at oscillation frequencies of 1, 2 and 4 Hz and amplitudes of 100 nm, 10 μm, 200 μm and 1 mm. These amplitudes were chosen to provide a measure of effects on oscillations smaller than the cell size (100 nm), on the scale of a single cell (10 μm), larger than the cell size (200 μm) and much larger than the cell size (1 mm). Low frequencies were chosen as a starting point for these investigations as they are more physiologically relevant, and to prevent potential heating due to rapid particle oscillations. We are currently working on mathematical models to guide more specific investigations of amplitude and frequency variations.

3.2. Materials and reagents

The eukaryotic expression plasmid (pCIKLux) carrying a luciferase reporter gene under the control of a cytomegalovirus (CMV) immediate early promoter/enhancer was complexed with Polymag® particles (diameter 100 nm) which were purchased from OzBiosciences (Marseille, France). The pCI/pCIKLux plasmid DNA was kindly donated by the UK Cystic Fibrosis Gene Therapy Consortium. NdFeB magnets

were purchased from Magnet Sales (Swindon, UK). Luciferase assay and cell viability reagents were purchased from Promega (Southampton, UK). All cell culture reagents were supplied by Sigma-Aldrich (Dorset, UK).

3.3. Transfection conditions: OzBiosciences Polymag® particles and NCI-H292 cells

NCI-H292 (human lung epithelial) cells were maintained in RPMI 1640 culture medium supplemented with 10% foetal calf serum, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin, 0.25 μg ml^{-1} amphortericin B and 2 mM L-glutamine. Cells were seeded at 5×10^3 cells/well in 96-well tissue culture plates and incubated overnight at 37 °C 5% CO_2 to allow the cells to attach. Polymag® transfections (particle diameter = 100–200 nm) were performed in serum-free (SF) RPMI media using 0.1 μg DNA per well following the manufacturer's recommended protocol based upon 1 μl Polymag per μg DNA. Following the addition of reagents, the plates were transferred to an incubator at 37 °C 5% CO_2 and placed above static/oscillating magnetic fields for 2 h. At 2 h post transfection, the medium was replaced with an equal volume of RPMI 1640 culture medium supplemented with 10% foetal calf serum, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin, 0.25 μg ml^{-1} amphortericin B and 2 mM L-glutamine. At 48 h post transfection, the medium was removed from each well and the cells lysed by the addition of 30 μl of cell reporter lysis buffer (Roche). Samples were assayed for Luciferase activity using a Luciferase assay reagent (Promega, Madison, USA) and the total protein concentration determined using a BCA assay reagent (Pierce, Cramlington, UK).

3.4. Transfection conditions: GeneJuice, Lipofectamine and Lipofectamine2000 and NCI-H292

NCI-H292 cells were maintained as described above and seeded into 96-well tissue culture plates. GeneJuice™, Lipofectamine™ and Lipofectamine2000™ transfections were performed in SF RPMI medium using 0.1 μg DNA per well following the manufacturers' recommended protocol. Cells transfected with 0.1 μg DNA alone (DNA only) and cells exposed to SF medium alone (control) were included as controls. At 2 h post transfection the culture medium was replaced with 100 μl of RPMI 1640 medium supplemented with 10% foetal calf serum, 100 U ml^{-1} penicillin, 0.1 mg ml^{-1} streptomycin, 0.25 μg ml^{-1} amphortericin B and 2 mM L-glutamine. At 48 h post transfection, the medium was removed from each well and the cells lysed by the addition of 30 μl of cell reporter lysis buffer (Roche). Samples were assayed for Luciferase activity using a Luciferase assay reagent (Promega) and the total protein concentration determined using a BCA assay reagent (Pierce).

3.5. Cell viability assays and statistics

NCI-H292 (human lung epithelial) cells were seeded into 96-well tissue plates and transfected as described above. At 24 and 48 h post transfection the culture media was removed from each well and replaced with 100 μl of RPMI containing 25 μl CellTiter 96 Aqueous cell proliferation assay. The plates were incubated at 37 °C 5% CO_2 for 1 h and the optical density measured at 630 nm (OD 630 nm). One-way ANOVA with a Bonferroni correction was used to analyse normalized data from multiple experiments (figure 2). Data from individual experiments were normalized to the oscillating array group before statistical analysis, leading to no variation in that group. Error bars in the figures represent the standard error on the mean. Normalization was required in order to compare results from multiple experiments as variations in the activity of the luciferase substrate can lead to variations in fluorescence between experiments due to extra freeze–thaw cycles, age, etc.

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