Evaluation of the magnetic field requirements for nanomagnetic gene transfection

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Abstract
The objective of this work was to examine the effects of magnet distance (and by proxy, field strength) on nanomagnetic transfection efficiency.

Methods: Non-viral magnetic nanoparticle-based transfection was evaluated using both static and oscillating magnet arrays.

Results: Fluorescence intensity (firefly luciferase) of transfected H292 cells showed no increase using a 96-well NdFeB magnet array when the magnets were 5 mm from the cell culture plate or nearer. At 6 mm and higher, fluorescence intensity decreased systematically.

Conclusion: In all cases, fluorescence intensity was higher when using an oscillating array compared to a static array. For distances closer than 5 mm, the oscillating system also outperformed Lipofectamine 2000®.

Keywords: non-viral gene delivery; magnetic nanoparticle-based gene transfection; magnetic nanoparticles; magnetic field

Over the last decade, new magnetic technologies have been developed to improve the uptake and expression of DNA and siRNA in cells growing in culture (1, 2). The use of magnetic nanoparticles for DNA delivery was first exemplified by Mah et al. (3, 4). In those studies, adenovirus-associated viral vectors coding for the green fluorescent protein (GFP) were coupled to micron-sized magnetic nanoparticles and introduced into HeLa cell cultures. High field/high gradient NdFeB magnets were placed beneath the cell culture plate to concentrate the vectors at a specific location within the cell culture and this was shown to improve both targeting and speed of transfection.

Following on from that work, Scherer, Plank and others developed a non-viral alternative of this technology, known as magnetofection, in which DNA was coupled directly to magnetic nanoparticles via charge interactions (2, 5). In this technique, particle/DNA complexes are taken up via endocytosis mechanisms and, once inside the cell, proton pumps are activated that rupture the endosome and release the DNA (6, 7).

More recently, the use of a combination of translational magnetic forces acting on the particles along the z-axis and oscillation of the field or magnet arrays in the x-y plane has been shown to improve transfection efficiency when compared to both cationic lipid complexes and static magnetofection (8–11) (Fig. 1). This has recently been extended to the transport of magnetic-DNA carriers through viscous gels (12).

In all these cases, the force exerted on the particle is proportional to both the field strength and field gradient (13). As such, it is important to understand the effects of these parameters on transfection efficiency in order to improve both in vitro nanomagnetic transfection and, more importantly, to develop the technology for in vivo targeting and transfection. In order to understand the relationship between field strength/gradient and transfection efficiency, we have evaluated luciferase activity, a proxy of protein production, as a function of magnet distance in both static and oscillating systems and compared them to one of the leading cationic lipid transfection agents, Lipofectamine 2000®.
Results and discussion
In order to determine the optimal working distance between the magnet and the cell culture for the improvement of transfection efficiency in NCI-H292 cells, magnet arrays were rearranged in the following format: in column 1, magnet discs were positioned 3 mm beneath the cell surface of the 96-well plate. Following, in columns 3, 5, 7, 9 and 11, the magnet discs were rearranged so that the distance between the magnet and the cell surface (bottom of the culture plate) was at 3, 4, 5, 6, 7 and 8 mm correspondingly (Fig. 2).

This magnet array has been rearranged for the purposes of the experiment as described earlier, to gradually increase the distance between the magnet array

Fig. 1. Proposed mechanism of oscillating nanomagnetic transfection (After ref. 17). Plasmid DNA or siRNA is attached to magnet nanoparticles and incubated with cells in culture (left). An oscillating magnet array below the surface of the cell culture plate pulls the particle into contact with the cell membrane (i) and drags the particles from side-to-side across the cells (ii), mechanically stimulating endocytosis (iii). Once the particle/DNA complex is endocytosed, proton sponge effects rupture the endosome (iv) releasing the DNA (v), which then transcribes the target protein (vi).

Fig. 2. Redcliffe MagScan image of a nanoTherics Ltd. magnet array obtained at 3 mm distance between the scanning probe and the magnet surface.
and the cell culture between 3 and 8 mm. At the centre of the wells in column 1 (at 3 mm distance) the highest magnetic field was obtained, reaching 101 mT.

The same magnet array mapped in Fig. 2 was used in the next experiment to investigate the magnetofection levels of NCI-H292 cells when transfected with Chemicell Polymag nanoparticles at different magnet distances during 2-h exposure.

Transfections were performed in 96-well tissue culture plates using 0.1 μg of 0.2 mg/ml pCIKLux DNA/well for 2 h. The 96-well plates were placed above the magnets at a distance ranging between 3 and 8 mm. The data obtained were expressed as mean ± standard error of mean (SEM) relative light units (RLU)/milligram protein (N = 12) for 200 μm/2 Hz oscillation amplitude.

Results from the 2-h transfection of NCI-H292 cells presented in Fig. 3 show that, regardless of magnet distance, luciferase activity using the oscillating field was significantly higher than static magnetofection (P < 0.001). It was also observed that the highest level of activity was obtained at 3 mm distance between the magnet and the cell surface, though there was no statistically significant improvement when compared to 4, 5, 6 and 7 mm distances. At distances below 6 mm, the oscillating system outperformed Lipofectamine 2000™ at both 2- and 6-h transfection durations (P < 0.01).

The significant drop-off in luciferase activity between 7 and 8 mm was seen in both the oscillating and static systems. In Fig. 4, luciferase activity obtained by the transfection of NCI-H292 cells shown in Fig. 3 was compared with the corresponding magnetic field strength. This shows that protein production increases with increasing field strength up to a point. At fields higher than 40 mT, there does not appear to be a corresponding increase in luciferase activity. These observations give an indication of the field parameters required to translate the technology from multi-well plates to in vivo systems. At all field strengths (magnet distances) tested, luciferase expression was higher in the oscillating system compared to the static magnet array (Fig. 4).

Transfections were performed in 96-well tissue culture plates using 0.1 μg of 0.2 mg/ml pCIKLux DNA/well with a 2-h transfection time at 200 μm/2 Hz oscillation amplitude.

As part of the optimization of the magnetic nanoparticle-based transfection technique, it is necessary to understand the effects of magnetic field strength and distance on transfection efficiency and protein production. The effect of the gradually increased distance between the magnet array and the cell culture revealed that the highest luciferase expression levels of NCI-H292 cells were achieved at the closest spacing between the magnet and the cells (3 mm). However, the difference in luciferase expression was not statistically significant when compared to 4 and 5 mm distances, providing scope for potentially transfecting cultured tissue explants with this technique. These results demonstrate that there is a plateau effect beyond which transfection efficiency is not improved.

As the forces generated on the particle/plasmid complex are in the picoNewton range, effects on cell membrane integrity will be negligible. The primary mechanism for uptake is likely to be via increased endocytosis due to mechanical stimulation, as has been seen in previous studies of magnetic ion channel activation (14–16). Though this mechanical stimulation can potentially affect downstream protein production in mechanoresponsive cells, reporter constructs such as luciferase and GFP will be dependent on cell entry and

![Magnetofection at different magnet distance from the cell surface of NCI-H292 cells at 2 x10^4 cells/well](image)

**Fig. 3.** Luciferase activity in NCI-H292 human lung mucoepidermoid carcinoma cells transfected with pCIKLux luciferase reporter construct using Chemicell Polymag particles (‘no magnet’, ‘static field’ and ‘oscillating field’), Lipofectamine (LF2000) and DNA (control).
transcription rather than anomalous protein expression (14).

It should be noted here that luciferase activity is not directly related to the number/percentage of cells transfected, but rather is a proxy for protein/enzyme production. The technique was used here to provide a rapid and quantitative direct comparison between sample groups under the same experimental conditions at different magnet distances in order to determine the effects of field strength and gradient and, by proxy, magnetic force on protein/enzyme production. It was observed that the magnefect-nano™ oscillating field system showed improved luciferase expression when compared to both static field and Lipofectamine 2000™ (2 and 6 h). Such increases in luciferase activity as well as increases in transfection efficiency using GFP have been reported in earlier studies of oscillating magnetic systems (9-11). This is important as cationic lipid-mediated gene delivery, or lipofection, is the most widely used non-viral in vitro transfection method. These results provide evidence of threshold effects in nanomagnetic transfection that could be further investigated and exploited in the determination of field parameters for the translation of this technology from in vitro to in vivo studies.

Methods

Materials and reagents
The eukaryotic expression plasmid pCIKLux carrying a luciferase reporter gene was complexed with different Polymag particles (diameter 100 nm) that were purchased from Christian Plank (OZB composition – CP.X111.77; OzBiosciences, Marseille, France) and Chemicell (Berlin, Germany). 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 reagents were purchased from Promega (Southampton, UK). All cell culture reagents were supplied by Sigma-Aldrich (Dorset, UK) and Biosera (Sussex, UK).

Magnet arrays
Static and oscillating arrays of NdFeB magnets configured for 96-well plate transfections were supplied by nanoTherics Ltd. Magnetic fields were mapped using a Redcliffe Magtronics MagScan 500. For static field experiments, the cell culture plates were placed directly above the magnet array and both were transferred into an incubator for the duration of transfection. For oscillating field experiments, the cell culture plates were placed directly above the magnet array holder, mounted onto a computer-controlled slide assembly (magnefect-nano, nanoTherics Ltd.). The cell culture plate and magnefect-nano oscillating system were transferred into an incubator for the duration of transfection and were interfaced to control electronics outside the incubator. Oscillating array experiments were performed at 2 Hz oscillation frequency and 200 µm amplitude.

Mammalian cell treatment before transfection
Human lung mucoepidermoid carcinoma cells (NCI-H292) were maintained in RPMI 1640 culture medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.25 µg/ml amphotericin B and 2 mM l-glutamine. Before transfection, cells were seeded into 96-well tissue culture plates (from Iwaki) at a density of 2 × 10^4 cells per well and incubated at 37°C and 5.0% CO₂ for a period of 24 h to allow cells to adhere to the bottom of the wells.

Transfection conditions: magnet transfection
All transfections were performed in 100 µl of serum-free (SF) RPMI medium using 0.1 µg of 0.2 mg/ml pCIKLux DNA and 0.1 µl of Polymag per well. Following the addition of reagents, the cell culture plates were transferred to an incubator at 37°C, 5% CO₂, and placed above the static and oscillating magnetic fields for 2 h. At 2-h post-transfection, the medium was replaced with 100 µl of RPMI 1640 supplemented with 10% fetal calf serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.25 µg/ml amphotericin B and 2 mM l-glutamine and the cell culture plates were transferred back into the incubator for 48 h before analysis. DNA/particle concentrations were determined from DNA binding curves. The amount of bound and unbound DNA was measured using an Eppendorf BioPhotometer.

Transfection conditions: Lipofectamine 2000™
NCI-H292 cells were maintained as described above and seeded into 96-well tissue culture plates. Lipofectamine 2000™ transfections were performed in SF RPMI medium using 0.1 µg of 0.2 mg/ml pCIKLux DNA and 0.3 µg of Lipofectamine 2000™ per well following the manufacturer’s recommended protocol. Cells transfected with 0.1 µg of 0.2 mg/ml pCIKLux DNA (DNA only) and cells exposed to SF medium (medium only) were...
used as control samples. At 2-h post-transfection, the medium of all samples was replaced with 100 μl of RPMI 1640 medium supplemented with 10% fetal calf serum, 100 U/ml penicillin, 0.1 mg/ml streptomycin, 0.25 μg/ml amphotericin B and 2 mM l-glutamine and the cell culture plates were transferred back into the incubator for 48 h before analysis.

**Luciferase assay**

At 48-h post-transfection, the medium was removed from all samples and cells were lysed by the addition of 30 μl/ well of cell reporter lysis buffer (Promega). Samples were assayed for luciferase activity by mixing 10 μl of cell lysate with 30 μl of luciferase assay substrate (Promega) and by measuring the emitted light in relative light units (RLU) using a Lumat LB 9507 luminometer (Berthold Technologies).

The samples’ total protein concentration was assayed for the determination of relative light units per milligram of protein. The protein content of each sample was measured by using a BCA assay (Pierce, Rockford, IL) following the manufacturer’s recommended protocol. Absorbance was recorded at 630 nm using a Dynatech MR5000 plate reader and a standard curve was produced using serial dilutions of bovine serum albumin protein (0–2 mg/ml) to determine the protein content of each sample.

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**Conflict of interest and funding**

nanoTherics Limited is involved in the commercialization of nanomagnetic gene transfection.

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