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Insights into the mechanism of magnetic particle assisted gene delivery

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Abstract

In magnetic particle assisted gene delivery DNA is complexed with polymer-coated aggregated magnetic nanoparticles (AMNPs) to effect transfection. In vitro studies based on COS-7 cells were carried out using pEGFP-N1 and pMIR-REPORT-complexed, polyethylenimine (PEI)-coated iron oxide magnetic nanoparticles (MNPs). PEI-coated AMNPs (PEI-AMNPs) with average individual particle diameters of 8, 16 and 30 nm were synthesized. Normal, reverse and retention magnetic transfection experiments and cell wounding assays were performed. Our results show that the optimum magnetic field yields maximum transfection efficiency with good viability. The results of the normal, reverse and retention magnetic transfection experiments show that the highest transfection efficiency was achieved in normal magnetic transfection mode due to clustering of the PEI-AMNPs on the cells. Cell wounding assay results suggest that the mechanism of magnetic transfection is endocytosis rather than cell wounding.

Keywords

Gene delivery, In vitro test, Magnetic transfection, Cell wounding, Nanoparticles.

1. Introduction

Magnetic particle assisted gene delivery, also known as magnetic transfection, is a recently developed physical, non-viral delivery system which can enhance the transfection efficiency of synthetic gene carriers up to 1000-fold [1]. Efficient gene delivery occurs under the influence of a magnetic field acting on the nucleic acid–

magnetic particle complexes, resulting in rapid and substantial complex–target cell contact. Hence, magnetic transfection has the potential to be used for in vivo applications due to the very fast speed of the process [2]. Magnetic transfection also reduces the amount of nucleic acid required to effect similar levels of transfection compared with standard vectors such as Metafectene [3]. In vitro [1,4–14] and in vivo [1,3,4,7,9] magnetofection studies have been successfully conducted on many immortalized cell lines (e.g. CHO, COS-7, HeLa, HEK-293 and NIH-3T3), primary cells (e.g. HUVECs, neurons, aortic endothelial cells and nasal airway epithelium) and non-permissive cells such as tumor cells.

Since magnetic transfection is a physical method, transfection efficiency depends strongly on the properties of the magnetic particles and the applied magnetic field. Most of the magnetic transfection studies were performed using commercial magnetic particles with aggregate sizes between 200 and 1000 nm under a static magnetic field of 250 mT with an incubation time in the range 5–20 min [1,7,10,11,15,16]. Furlani and Ng modeled the magnetic transfection process and predicted that the accumulation time for 200 nm diameter particles was around 20 min [17]. Their analysis also indicated that the accumulation rate could be enhanced using larger particles. Kamau et al. and McBain et al. have used a pulsed magnetic field and an oscillating magnetic field, respectively, for magnetic transfection [10,18]. They reported that transfection efficiency was significantly enhanced.

Huth et al. examined magnetic transfection using polyethylenimine (PEI)-coated magnetic particles and standard PEI transfection by electron microscopy as well as by performing transfection in the presence of various endocytosis inhibitors [15]. They concluded that the uptake mechanism of nucleic acid–magnetic particle complexes was similar to that for PEI polyplexes, i.e. by endocytosis. The magnetic field itself does not alter the uptake mechanism of magnetic nanoparticles. The magnetic field causes rapid sedimentation of the complexes on the target cells such that the majority of the cells come into contact with the complexes.

A similar conclusion was drawn by Sauer et al., who studied the cellular dynamics of magnetic lipoplexes in real time by fluorescence microscopy [16]. Magnetic lipoplexes show a three-phase behavior similar to polyplexes. In the first phase magnetic lipoplexes attached to the cell surface and showed slow transport behavior. In the next phase confined diffusion of magnetic lipoplexes took place inside the cell. The final phase consisted of active transport along microtubules inside the cell. Magnetic lipoplexes and PEI polyplexes were internalized via endocytosis and both vectors show the same internalization timescale. Their results suggested that the higher transfection efficiency was due to the magnetic field, which induced an increased concentration of magnetic complexes on the cell surface.

The past literature has demonstrated the advantages of magnetic fields on transfection efficiency, however, no study has been conducted to examine the dependence of transfection efficiency on physical and magnetic parameters such as particle size, magnetic field strength and frequency. In this work we report for the first

time the effect of magnetic field strength on transfection efficiency and viability. We also examined whether the magnetic complexes entered the cell by cell wounding.

Iron oxide magnetic nanoparticles (MNPs) of 8, 16 and 30 nm diameter were synthesized for this study. These MNPs were subsequently coated with 25 kDa branched PEI. In vitro studies on COS-7 cells were performed using two plasmids which express luciferase and green fluorescence protein (GFP). The effects of a static magnetic field (in the range 0–250 mT) on transfection efficiency and viability were examined. Quantification of transfection efficiency and cell viability was performed using the luciferase assay and crystal violet staining, respectively. In this work maximum transfection efficiency was achieved with an optimum magnetic field of 130 mT. To determine the mechanism of magnetic transfection modified forms of “normal” magnetic transfection were performed, i.e. reverse magnetic transfection and retention magnetic transfection (Fig. 1). This study, together with cell wounding assays, suggests that the mechanism of magnetic transfection is endocytosis rather than cell wounding.

2. Materials and methods

2.1. Materials

pEGFP-N1 (Clontech Laboratories) which codes for green fluorescence protein (GFP) under the control of a cytomegalovirus (CMV) promoter, pMIR-REPORT which contains a firefly luciferase reporter gene under the control of a CMV promoter and COS-7 cells (kindly provided by Profs. M.S. Featherstone and A. Law of the School of Biological Sciences, Nanyang Technological University, Singapore), Roswell Park Memorial Institute 1640 medium (HyClone), fetal bovine serum (HyClone), penicillin/streptomycin (HyClone) and L-glutamine (Gibco) were used in the experiments. Crystal violet (Merck), sodium dodecyl sulfate (Hoefer), 25 kDa PEI, branched (Sigma–Aldrich), ZONYL FSA (Sigma–Aldrich), Spectra/Por 6 50 kDa cut-off dialysis membrane (Spectrum Laboratories), PolyMAG (Chemicell), a MagnetoFACTOR plate (arrays of 96 cylindrical magnets in the same geometry as a 96-well plate) (Chemicell), trypsin (Promega) and a luciferase assay kit (Promega) were also utilized. PolyMAG is a commercial magnetic particle suspension, mixed in a one-step procedure with the nucleic acid to be transfected. It has been successfully used with plasmid DNA, antisense oligonucleotides and siRNA [19]. The cell impermeant tracer fluorescein isothiocyanate (FITC)–dextran (molecular weight 10,000) and cytosolic fluorescent marker Green Cell Tracker were purchased from Molecular Probes. Nano-pure water ($18.2 \text{ M } \Omega \text{ cm}^{-1}$) was obtained from Milli-Q Synthesis (Millipore).

2.2. Nomenclature

The nomenclature of the magnetic nanoparticles used in this work is as follows.

MNPs: magnetic nanoparticles.

AMNPs: aggregate of magnetic nanoparticles. For example, the aggregates of magnetic nanoparticles of 8 nm diameter (referred to as A8MNP) have a hydrodynamic diameter of 323 nm. Aggregates of magnetic nanoparticles of 16 and 30 nm in diameter are referred to as A16MNP and A30MNP, respectively.

PEI-AMNPs: PEI-coated aggregated magnetic nanoparticles. For example, PEI-coated aggregates of magnetic nanoparticles of 8 nm in diameter (referred to as PEI-A8MNP) has a hydrodynamic diameter of 400 nm. PEI-coated aggregates of magnetic nanoparticles of 16 and 30 nm diameter are referred to as PEI-A16MNP and PEI-A30MNP, respectively.

2.3. Synthesis and characterization of MNPs

Magnetite nanoparticles were synthesized as described below using adaptations of the alkaline co-precipitation technique [20–22] and subsequently coated with 25 kDa branched PEI. They were characterized by transmission electron microscopy (JEOL JEM 2010), vibrating sample magnetometry (Lakeshore 7400), X-ray diffractometry (Shimadzu 6000) and photon correlation spectroscopy methods (Malvern Zetasizer Nano ZS) [23]. The PEI-coated AMNPs were then diluted in nano-pure water and used for subsequent transfections.

2.3.1. *Preparation of 8 nm magnetite nanoparticles*

Eight nanometer MNPs were synthesized by adapting the technique of Kim et al. [20]. In a typical experiment an iron source consisting of 1.28 M $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 0.64 M $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ and 0.4 M HCl was prepared by dissolving the chemicals in nano-pure water. The iron source was added to a 1.5 M NaOH solution under bubbling N_2 gas and vigorously stirred at room temperature for 30 min. The precipitated iron oxide was isolated using a permanent magnet (remanence 1.2 T) and the supernatant discarded. After washing the precipitate several times, it was dispersed in nano-pure water, the total volume being made up to 100 ml.

2.3.2. *Preparation of 16 nm magnetite nanoparticles*

Sixteen nanometer MNPs were synthesized by the technique of Nisho et al. [21]. An iron source consisting of 0.1 M $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was prepared by dissolving the chemical in nano-pure water. This iron source was added to an alkaline solution (made up of 0.02 M NaOH and 8.8 mM NaNO_3) under bubbling N_2 gas and vigorously stirred at 14 °C for 30 min. The resulting suspension was kept at 4 °C for 24 h, after which the AMNPs were isolated using the method described in Section 2.3.1.

2.3.3. *Preparation of 30 nm magnetite nanoparticles*

The synthesis of 30 nm MNPs was adapted from Nedkov et al. [22]. An iron source consisting of 0.03 M $\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$ was prepared by dissolving the chemical in nano-pure water. This iron source was added to 0.3 M NaOH solution under bubbling N_2 gas and

vigorously stirred at room temperature for 30 min. The AMNPs were isolated from the suspension as described in Section 2.3.1.

2.3.4. *Coating of MNPs with PEI*

The coating procedure followed that of Mykhaylyk et al. [24]. The coating solution, consisting of 1 g PEI and 0.5 ml ZONYL FSA in 18.5 ml nano-pure water, was added to the iron oxide suspension and stirred for 2 h. The product was sonicated for 10 min (60 s sonication/30 s break) to disperse the AMNPs and then dialyzed against nano-pure water over 2 days using a Spectra/Por 6 50 kDa cut-off dialysis membrane to neutralize the suspension and remove excess unbound coating material. Finally, the PEI-coated AMNP suspension was diluted to $0.05 \mu\text{g Fe } \mu\text{l}^{-1}$ and kept in aliquots at 4°C until further use.

2.4. Cell culture

COS-7 monkey kidney cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin (complete RPMI 1640) [25]. Cells were incubated at 37°C in a humidified 95% air and 5% CO_2 atmosphere, and subcultured before they reached 90% confluency.

2.5. Transfection experiments

To determine the mechanism of magnetic transfection, modified forms of magnetic transfection were performed, i.e. reverse magnetic transfection and retention magnetic transfection. Therefore, we denote the standard magnetic transfection normal magnetic transfection. In normal magnetic transfection cells were seeded 1 day before transfection. In reverse and retention magnetic transfection magnetic particle–plasmid DNA (pDNA) complexes were added and settled before adding the cells. In retention magnetic transfection a magnetoFACTOR plate was placed beneath the 96-well plate until luciferase and viability assays were conducted (Fig. 1). If endocytosis occurs the transfection efficiency and viability of reverse magnetic transfection would be expected to be significantly different from that of retention magnetic transfection, since the magnetic field would have prevented endocytosis of magnetic particle–pDNA complexes.

2.5.1. *Preparation of gene vector complexes*

pDNA, polyMAG and PEI-coated AMNPs were diluted in autoclaved distilled water. $0.5 \mu\text{g}$ pDNA per well was complexed with $0.5 \mu\text{l}$ of polyMAG and optimized amounts of PEI–AMNPs [23]. The pDNA solutions were added to the gene vector solutions and thoroughly mixed by pipetting up and down 10 times. Twenty five microliters of gene vector complexes per well were prepared in triplicate and the resulting complexes were incubated for 30 min at room temperature before use.

2.5.2. Normal magnetic transfection

0.5×10^4 cells per well were seeded in 96-well plates 1 day before transfection and grown in complete RPMI 1640 medium. At a confluency of about 50–60% the cells were washed with phosphate-buffered saline (PBS) and 125 μ l of incomplete RPMI 1640 (without FBS, L-glutamine and penicillin/streptomycin) per well was added.

2.5.2.1. Normal magnetic transfection under different static magnetic fields

Twenty five microliters of magnetic particle–pDNA complexes were added to each well, making the total volume in each well equal to 150 μ l. The 96-well plate was then placed on top of a magnetoFACTOR plate for 20 min incubation at room temperature. The magnetic flux density perpendicular to the well plate in the area of the cells depends on the separation between the cells and the magnetoFACTOR plate (Table 1). The transfection medium was replaced with 200 μ l of complete RPMI 1640 after 20 min. The magnetoFACTOR plate was removed and incubation was continued for 24 h at 37 °C in a humidified 95% air and 5% CO₂ atmosphere before the next medium change.

2.5.3. Reverse magnetic transfection

In reverse magnetic transfection magnetic particle–pDNA complexes were added and settled for 20 min before adding the cells. Twenty five microliters of magnetic particle–pDNA complexes were added into each well containing 125 μ l of incomplete RPMI 1640. The 96-well plate was then placed on top of a magnetoFACTOR plate for 20 min incubation at room temperature. The incomplete RPMI 1640 was replaced with 200 μ l of complete RPMI 1640 containing 1×10^4 cells after 20 min. The magnetoFACTOR plate was removed and incubation was continued for 24 h at 37 °C in a humidified 95% air and 5% CO₂ atmosphere before the next medium change.

2.5.4. Retention magnetic transfection

The methodology for retention magnetic transfection is the same as for reverse magnetic transfection. The medium change occurred with the magnetoFACTOR plate in place. The magnetoFACTOR plate was placed beneath the 96-well plate until luciferase and viability assays were conducted.

2.6. Luciferase activity measurement

After 48 h of post-transfection incubation gene expression was determined. Luciferase assays were performed in triplicate. For luciferase expression [26] the cells were washed with PBS and 20 μ l of luciferase cell culture lysis reagent was added to each well. Cells were incubated for at least 15 min at room temperature for complete lysis. Luciferase assay reagent (100 μ l for each assay) was kept at room temperature before performing the luciferase assay. Twenty microliters of cell lysate was added to a luminometer tube containing 100 μ l of luciferase assay reagent, and mixed by pipetting five times. Light emission was measured over 10 s by a Turner Biosystems 20/20ⁿ luminometer.

2.7. Cell viability

Cytotoxicity of the gene vectors was assessed in triplicate by crystal violet staining assay [27] 48 h post-transfection. Cells were washed with PBS and 50 μ l of 0.5% crystal violet solution in methanol was added to each well. The cells were incubated for 10 min at room temperature. The staining solution was discarded and the 96-well plate washed gently with tap water. The plate was placed upside down on paper towels to drain any remaining water. One hundred microliters of 1% SDS solution was added to each well to solubilize the stain. The plate was agitated until a uniform color was obtained. Absorbance at 570 nm was measured with a micro-plate spectrophotometer (Bio-Rad Benchmark Plus). Cell viability (%) was computed as $(a/b) \times 100$, where a is the OD₅₇₀ value derived from wells containing gene vector–pDNA complexes and b is the mean OD₅₇₀ value derived from control wells (i.e., wells containing only cells and no gene vector–pDNA complexes).

2.8. Cell wounding assays

2.8.1. *Cell impermeant tracer FITC–dextran*

To determine whether the plasma membrane is disrupted during magnetic transfection, cell wounding and membrane repair assays were conducted [28,29]. Wounded cells are recognized by the presence of the tracer FITC–dextran in the cytosol. Openings in the plasma membrane due to wounding permit the tracer to enter and reseal, trapping the tracer inside the cell. RPMI 1640 was removed from the wells and 125 μ l of FITC–dextran (0.5 mg ml⁻¹) per well was added to the cells. Magnetic transfection was subsequently performed as described in Section 2.5.2. Cells were washed three times with incomplete RPMI 1640. A fluorescence microscope (Olympus IX71) was used to detect wounded cells 30 min after magnetic transfection. For positive controls the cells were wounded by scratching 30 times in a grid pattern with a sterile hypodermic needle.

2.8.2. *Cytosolic fluorescent marker Green Cell Tracker*

Cell wounding could also be determined by the leakage of cytosolic material into the external medium [29]. Cells were incubated with 100 μ l of Green Cell Tracker (10 μ M) for 1 h at 37 °C in a humidified 95% air and 5% CO₂ atmosphere. The cells were washed three times with incomplete RPMI 1640. Magnetic transfection was subsequently performed. A microplate reader (Tecan Infinite M200) was used to measure the fluorescence at an excitation wavelength of 485 nm and emission wavelength of 518 nm within 30 min after magnetic transfection. Wells containing 150 μ l of incomplete RPMI 1640 served as negative controls and wells containing cells which were scratched 30 times with a syringe needle served as positive controls.

2.9. Statistical analysis

All biological data are expressed as means $\pm$ standard deviation. Luciferase activity, transfection efficiency and viability were analyzed by the single factor ANOVA test. A P value <0.05 was considered significant.

3. Results

Table 2 shows the properties of the synthesized PEI-AMNPs obtained in a previous work [23].

3.1. Effect of different static magnetic fields on transfection efficiency and viability

The magnetic force experienced by the MNPs is dependent on the magnetic field intensity. Fig. 2 shows the luciferase activity and viability for PEI-A8MNP, PEI-A16MNP and PEI-A30MNP as a function of magnetic field strength. It can be observed that with no external magnetic field the transfection efficiency was lowest and viability highest for all three sizes of MNPs. Transfection efficiency increased with magnetic field, reaching a maximum at 130 mT, and decreased thereafter. The lowest viability occurred at the highest magnetic field. Application of an optimum field of 130 mT resulted in a 4- to 5-fold increase in transfection efficiency compared with cells which were not exposed to a magnetic field.

3.2. Comparison between normal, reverse and retention magnetic transfection

To determine the mechanism of magnetic transfection, a comparison was made between normal, reverse and retention magnetic transfection. Fig. 3 shows the luciferase activity and viability for different types of magnetic transfection under a magnetic field strength of 130 mT for PEI-A8MNP (Fig. 3a), PEI-A16MNP (Fig. 3b), PEI-A30MNP (Fig. 3a and c) and polyMAG (Fig. 3d). Normal magnetic transfection yields the highest transfection efficiency for all cases. The transfection efficiency for reverse and retention magnetic transfections were similar, between 20% and 40% that of normal magnetic transfection. The transfection efficiency ratio of reverse to normal magnetic transfection for polyMAG was about half of that for the synthesized MNPs. Viabilities for the reverse and retention magnetic transfections were 50–100% higher than for normal magnetic transfection.

Fig. 4 shows typical optical micrographs of the PEI-AMNPs distributions after normal (Fig. 4a) and reverse magnetic transfection (Fig. 4c). PEI-A8MNP, which has a hydrodynamic diameter of 400 nm, is shown in these micrographs. The magnetic field used was 130 mT. Fig. 4b and d shows the distribution of PEI-AMNP aggregate size for normal and reverse magnetic transfection, respectively. PEI-AMNP aggregates were observed to cluster onto cells in case (a), but were uniformly distributed throughout the well in case (c). Image analysis was performed using ImageJ [30]. The average aggregate size for normal magnetic transfection ($7.3 \mu\text{m}^2$) was larger than that for reverse magnetic transfection ($3.7 \mu\text{m}^2$). The bigger PEI-AMNP aggregate size was due to clustering on the cell.

3.3. Wounding assays for cells subjected to maximum static magnetic field

Cell wounding assays were performed to ascertain if cells were wounded during magnetic transfection. Fig. 5 shows the result of the Green Cell Tracker cell wounding assay for the maximum static magnetic field (250 mT). There was no significant difference between samples and the negative control (cells in incomplete RPMI 1640), indicating no cell wounding.

Fig. 6 shows the result of the FITC–dextran cell wounding assay. The sample was PEI–A8MNP and the magnetic field strength used was 250 mT in normal transfection mode. Fig. 6a and b shows a bright field image and fluorescence image of the positive control (scratch), respectively. Fig. 6c and d shows a typical bright field image and fluorescence image of the sample, respectively. Wounded cells (white) could be seen in the positive control (Fig. 6b), indicating cell wounding. However, no wounded cells were detected in the other samples (Fig. 6d), showing that cell wounding did not occur.

4. Discussion

This study aimed to examine the effect of magnetic field strength on transfection efficiency and viability, and also to determine whether the magnetic complexes entered the cell by cell wounding. Our results show that magnetofection of COS-7 cells does not proceed by direct penetration. We now discuss the specific results obtained in the context of previous works.

For the MNPs sizes studied transfection efficiency initially increased with magnetic field, reaching a maximum at 130 mT. Since the MNPs consist of PEI, which is toxic, increased internalization means greater cell death, hence viability decreased with increasing magnetic field. The greater cell death at 250 mT resulted in a lower overall transfection efficiency. Therefore, these results suggest there is an optimum magnetic field to achieve maximum transfection efficiency. Previous studies [15,16] have suggested that a higher transfection efficiency was due to an increased concentration of magnetic complexes on the cell surface. Thus, to increase the concentration of magnetic complexes one could either use a higher magnetic field or a longer incubation time. In this work the optimum magnetic field was found to be 130 mT for an incubation time of 20 min. Generally, the magnetic field reported in the literature [1,3,10,11,15,16] is 250 mT for an incubation of between 5 and 15 min. It is therefore recommended that either the magnetic field or incubation time are optimized to achieve the maximum transfection efficiency.

To gain an insight into the mechanism of magnetic transfection, modified forms of the standard magnetic transfection protocol were performed, namely reverse and retention magnetic transfection. Cell wounding assays were also performed. The transfection efficiency for reverse magnetic transfection was 20–40% that of normal magnetic transfection and the viability for reverse magnetic transfection was 30–40% higher than normal magnetic transfection (Fig. 3). It was observed that MNPs clustered onto cells in normal magnetic transfection whereas MNPs were uniformly distributed in reverse magnetic transfection (Fig. 4). The difference in distribution of magnetic particles between

normal and reverse magnetic transfection was due to interaction between the cells and magnetic particles. In the normal method the magnetic particles were added after the cells. The plasma membrane of the cells is negatively charged, which attracts the magnetic particles containing positively charged PEI. In the reverse method the magnetic particles were dispersed in the absence of cells, thus resulting in an even distribution of particles. The clustering of MNPs onto cells means that the concentration of MNPs per cell is higher in normal magnetic transfection than in reverse magnetic transfection. This observation agrees with previous studies [15,16] that suggested that a higher transfection efficiency was due to an increased concentration of magnetic complexes on the cell surface. Having a higher concentration of magnetic complexes could also interfere with proper functioning of the plasma membrane and, hence, result in cell death. Another reason for the higher transfection efficiency of normal magnetic transfection could be that normal magnetic transfection may occur by cell wounding whereas endocytosis is the mechanism in reverse magnetic transfection.

The transfection efficiency and viability of reverse and retention magnetic transfection were similar, indicating that similar amount of DNA entered the nucleus after 48 h. This suggests a similar mechanism occurs in reverse and retention magnetofection. A likely possibility is that in retention magnetofection DNA or PEI–DNA became detached from the magnetic complex and entered the cell by endocytosis, resulting in a similar transfection efficiency to reverse magnetic transfection.

The results from both cell wounding assays show that cells were not wounded, even when they were subjected to the maximum static magnetic field (250 mT). Previous studies on the mechanism of magnetofection used a similar magnetic field strength [15,16] and also concluded that cellular uptake of magnetic complexes proceeds by endocytosis. Thus cell wounding could not be responsible for the higher cell death at the strongest magnetic field strength of 250 mT. The higher cell death is likely due to a higher concentration of particles being attracted to the cells under a stronger magnetic field.

5. Conclusions

In vitro magnetic transfection studies of transfection efficiency in COS-7 cells were carried out with pEGFP-N1 and pMIR-Report-complexed, polyethylenimine (PEI)-coated, iron oxide-based magnetic nanoparticles under various static magnetic fields. This work, combining normal, reverse and retention magnetic transfection and cell wounding assays, has shown that: for PEI-A8MNP and PEI-A30MNP, the optimum magnetic field to achieve maximum transfection efficiency is 130 mT for an incubation time of 20 min; a higher number of complexes per cell resulted in a higher transfection efficiency and lower viability. The mechanism of magnetic transfection is not cell wounding.

Appendix A. Figures with essential colour discrimination

Certain figures in this article, particularly Figures 1 and 5, are difficult to interpret in black and white. The full colour images can be found in the on-line version, at [doi:10.1016/j.actbio.2010.09.037](https://doi.org/10.1016/j.actbio.2010.09.037).

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- Table 1. Magnetic flux density perpendicular to the well plate at different positions above the magnet.
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- Fig. 1 Schematic of normal, reverse and retention magnetic transfection. In normal magnetic transfection cells were seeded 1 day earlier. In reverse and retention magnetic transfection PEI-AMNP-DNA complexes were added and settled before adding the cells. In retention magnetic transfection the magnetoFACTOR plate was placed beneath the 96-well plate until luciferase and viability assays were conducted.
- Fig. 2 Luciferase activity and viability for (a) PEI-A8MNP, (b) PEI-A16MNP and (c) PEI-A30MNP as a function of magnetic field strength. The transfection efficiency was lowest and viability highest with no external magnetic field for all three sizes of MNPs. Transfection efficiency increased with magnetic field, reaching a maximum at 130 mT, and decreased thereafter. The lowest viability occurred at the highest magnetic field. The luciferase activities for PEI-A8MNP and PEI-A30MNP under magnetic fields were significantly different ($P < 0.05$), whereas for PEI-A16MNP the luciferase activities were comparable ($P > 0.5$).
- Fig. 3 Luciferase activity and viability for different types of magnetic transfection under a magnetic field strength of 130 mT for (a) PEI-A8MNP, (b) PEI-A16MNP, (c) PEI-A30MNP and (d) polyMAG. Normal magnetic transfection yields the highest transfection efficiency for all cases. The transfection efficiencies for reverse and retention magnetic transfections were similar, ranging between 20% and 40% that of normal magnetic transfection. Viabilities for reverse and retention magnetic transfection was 50–100% higher than for normal magnetic transfection.
- Fig. 4 Typical optical micrographs showing the PEI-AMNP distribution under (a) normal and (c) reverse magnetic transfection. PEI-A8MNP, which has a hydrodynamic diameter of 400 nm, is shown in these micrographs. The magnetic field used was 130 mT. (b and d) The distribution of PEI-AMNP aggregate size for normal and reverse magnetic transfection, respectively. PEI-AMNP aggregates can be observed to cluster onto cells in (a), but are uniformly distributed throughout the well in (c). The PEI-AMNP aggregate size for normal magnetic transfection was larger than that for reverse magnetic transfection.
- Fig. 5 Green Cell Tracker cell wounding assay for the maximum static magnetic field (250 mT). There was no significant difference between the samples and negative control (cells in iRPMI), indicating that cell wounding was absent.
- Fig. 6 FITC-dextran cell wounding assay for PEI-A8MNP under a magnetic field strength of 250 mT in normal transfection mode. (a and b) Bright field image and fluorescence image of positive control (scratch), respectively. (c and d) Typical bright field image and fluorescence image

of sample. Wounded cells could be seen in the positive control (b), indicating signs of cell wounding. However, there were no wounded cells detected in any of the other samples (d), signifying that no cell wounding occurs.

Position z (mm)	Magnetic flux density (mT)
2	250
4	130
8	35

Table 1

Sample	D_{TEM} (nm)	M_s (emu g ⁻¹)	χ	Hydrodynamic diameter (nm)	
				Uncoated AMNPs	PEI-AMNPs
A8MNP	8 ± 2	41 ± 1	7 ± 1	323 ± 10	400 ± 75
A16MNP	16 ± 4	27 ± 1	6 ± 1	513 ± 31	618 ± 62
A30MNP	30 ± 10	75 ± 2	25 ± 2	2419 ± 235	1260 ± 88

D_{TEM} , diameter of uncoated MNP; M_s , saturation magnetization of PEI-AMNPs; χ , volume susceptibility of PEI-AMNPs.

Table 2

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Fig. 6