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### Author(s)
Ang, Derrick; Tay, Chor Yong; Tan, L. P.; Preiser, Peter Rainer; Ramanujan, Raju V.

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In vitro studies of magnetically enhanced transfection in COS-7 cells

D. Ang\textsuperscript{a}, C.Y. Tay\textsuperscript{a}, L.P. Tan\textsuperscript{a}, P.R. Preiser\textsuperscript{b}, R.V. Ramanujan\textsuperscript{a,*}

\textsuperscript{a} School of Materials Science & Engineering, Nanyang Technological University, Singapore

\textsuperscript{b} School of Biological Sciences, Nanyang Technological University, Singapore

* Corresponding author. Tel.: +65 67904342; fax: +65 67909081.
E-mail address: Ramanujan@ntu.edu.sg (R.V. Ramanujan).

Abstract

In the magnetically enhanced gene delivery technique, DNA complexed with polymer coated aggregated magnetic nanoparticles (AMNPs) is used for effecting transfection. The aim of this study is to examine the relationship between transfection efficiency and the physical characteristics of the polymer coated AMNPs. In vitro studies of transfection efficiency in COS-7 cells were carried out using pEGFP-N1 and pMIR-REPORT complexed polyethylenimine (PEI) coated iron oxide magnetic nanoparticles. PEI coated AMNPs (PEI-AMNPs) with average individual particle diameters in the range of 8 nm to 30 nm were studied and characterized by transmission electron microscopy, vibrating sample magnetometry, X-ray diffractometry, thermal gravimetric analysis and photon correlation spectroscopy methods. PEI-A8MNP and PEI-A30MNP yielded higher transfection efficiency compared to commercial polyMAG particles as well as PEI of equivalent molar ratio of nitrogen/phosphorous (N/P ratio). The transfection efficiency was related to the physical characteristics of the PEI-AMNPs and its complexes: transfection efficiency was strongly positively correlated with saturation magnetization (Ms) and susceptibility (χ), strongly negatively correlated with N/P ratio, moderately positively correlated to zeta potential and moderately negatively correlated to hydrodynamic diameter of the complex. PEI-A8MNP and PEI-A30MNP possessing higher Ms, χ, lower N/P ratio and smaller complex size exhibited higher transfection efficiency compared to PEI-A16MNP which have weaker magnetic properties, higher N/P ratio and larger complex size. We have demonstrated that optimization of the physical properties of PEI-AMNPs is needed to maximize transfection efficiency.

Keywords: Gene delivery; Nonviral; \textit{In vitro} test; Magnetic transfection; Nanoparticles; COS-7

1. Introduction

Recent deaths, including those of a woman in an adeno-associated virus (AAV) gene therapy trial for arthritis [1] has provided an urgent impetus to improve non-viral gene delivery systems. Magnetically enhanced gene delivery is a recently developed non-viral gene delivery system which uses physical method to enhance gene transfection. The concept behind magnetically enhanced transfection is the use of an external magnetic field which rapidly pulls the DNA-AMNPs complexes toward the cells to be transfected.
This technique resulted in rapid kinetics and efficient gene delivery because most of the DNA-AMNPs complexes get in contact with the cells. Mah et al. first used magnetic particles for gene transfection [2], they transfected C125 cells in vitro and in vivo in mice using an AAV linked to magnetic microspheres via heparin. Other groups [3–15] have also studied the use of aggregated magnetic nanoparticles (AMNPs) for transfection and named this technique ‘magnetofection’.

The AMNPs used for magnetically enhanced transfection are commonly superparamagnetic iron oxides. Iron oxides are also used for in vivo applications, e.g., as contrast agents in magnetic resonance imaging [16]. The nucleic acid that the coated AMNP delivers is a polyanion consisting of phosphate group-repeated chains. Since the membrane of the target cells is also negatively-charged, due to electrostatic repulsion the nucleic acid cannot easily interact with the cell, hence a coating material is required. Polyethylenimine (PEI) is the preferred choice of coating material around the AMNPs, it is an excellent transfection vector due to the proton sponge effect which causes endosomal escape and hence avoids lysosomal degradation of nucleic acids [17–20].

The key limitations of non-viral gene delivery include impermeability of the cell membrane to nucleic acids and the diffusion limited delivery of nucleic acids which results in only a small percentage of nucleic acids reaching the target cells. By applying an external magnetic field to AMNPs complexed with nucleic acids, efficient delivery of nucleic acids to the target cells can be effected. Such magnetically targeted gene delivery can significantly increase transfection efficiency, reduce the amount of nucleic acids required and the time required for nucleic acids to reach the cells as compared to standard non-viral transfection [5].

Magnetically enhanced transfection has been successfully tested in vitro [3,4,6,8,9,12,14,21–23] and in vivo [4–6,12,23] on several immortalized cell lines as well as primary cells, e.g., HeLa, HEK 293, COS-7, NIH/3T3, porcine airway epithelium and human umbilical vein endothelial cells. Depending on the cell type, in vitro studies showed that cells incubated in a magnetic field displayed gene expression up to several hundred-fold greater than those not exposed to the magnet, the time required was reduced from ~4 h typically used in the standard transfection techniques to 5 min for magnetically enhanced transfection [5,7]. Magnetically enhanced transfection is also extremely useful for gene delivery of difficult-to-transfect or otherwise non-permissive cells such as human umbilical vein endothelial cells [3,6].

Huth et al. [9] showed that cellular uptake of magnetic nanoparticles proceeded by endocytosis, cellular uptake was similar to that for the case of PEI polyplexes, it was concluded that the magnetic field itself does not alter the uptake mechanism of magnetic nanoparticles. An in vivo study suggested that the magnetic force leads to accelerated sedimentation of AMNP-nucleic acid complexes on the cell surface and does not directly affect the endocytic uptake mechanism [13]. Pulsating magnetic fields [14,22] and oscillating magnet arrays [24] have been used to increase the efficiency of magnetically enhanced transfection. It was shown that in vitro efficiency was 10 times higher than in a static field, and both in vitro and in vivo gene transfer occurred within 5 min of exposure, this rapid uptake of nucleic acids is particularly useful for in vivo applications in order to minimize systemic clearance.
Most studies explored the feasibility of using the magnetically enhanced transfection technique in various cell types, however very few studies [25–27] have focused on the physical characteristics of the magnetic particles essential for high transfection efficiency. In particular, there is no literature on the effect of magnetic properties of AMNPs on transfection efficiency. Information on the magnetic properties of AMNPs is important since magnetically enhanced transfection relies on it to work effectively. Optimizing the magnetic properties would therefore enhance the transfection efficiency and could also potentially affect the mechanism of magnetically enhanced transfection. In this work, we report for the first time, a study of the effect of the magnetic and other physical properties of the coated AMNPs on the transfection efficiency.

Iron oxide magnetic nanoparticles of average particle sizes 8 nm, 16 nm and 30 nm were synthesized by modifications to the co-precipitation technique [28,29]. These particles were then coated with 25 kDa branched PEI. In vitro studies using COS-7 cells were performed with pEGFP-N1 (accession number: U55762) and pMIR-REPORT complexed PEI-coated magnetite particles. Plasmid pEGFP-N1 codes for green fluorescence protein (GFP) under the control of cytomegalovirus (CMV) promoter. Plasmid pMIR-REPORT contains a firefly luciferase reporter gene under the control of a CMV promoter. Quantification of transfection efficiency and cell viability were performed using luciferase assay and crystal violet staining assay, respectively. In this work, we found that AMNPs that resulted in higher transfection efficiency have higher Ms, χ and smaller complex size. The incubation time and the amount of PEI required for magnetically enhanced transfection was found to be much less than conventional transfection, thus magnetically enhanced transfection is a highly promising technique for gene delivery.

2. Materials and methods

2.1. Materials

pEGFP-N1 (Clontech Laboratories), pMIR-REPORT 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), phosphate-buffered saline (Gibco), fetal bovine serum (HyClone), penicillin/ streptomycin (PAA) and L-glutamine (Gibco) were used in the experiments. Crystal violet (Merck), sodium dodecyl sulfate (Hoefer), PEI 25 kDa, branched (Sigma-Aldrich), ZONYL FSA (Sigma-Aldrich), Spectra/Por 6 50 kDa cut-off dialysis membrane (Spectrum Laboratories), MagnetoFACTOR plate (Chemicell) (arrays of 96 cylindrical magnets, remanence Br = 1.1 T, in the same geometry as a 96-well plate), Lipofectamine 2000 transfection reagent (Invitrogen), trypsin (Gibco), luciferase assay kit (Promega), agarose gel (BioWhittaker Molecular Applications) and ethidium bromide (Sigma) were also utilized. PolyMAG, a commercial PEI-coated magnetic nanoparticles suspension, was obtained from Chemicell. Nano-pure water (18.2 MΩ/cm) was obtained from Milli-Q Synthesis (Millipore).

2.2. Nomenclature
The nomenclature of the synthesized magnetic nanoparticles used in this work is as follow:

MNPs: magnetic nanoparticles. AMNPs: aggregate of magnetic nanoparticles. For example, an individual magnetic nanoparticle is 8 nm in diameter, the aggregate of magnetic nanoparticles of 8 nm in diameter is referred to as A8MNP. Aggregates of magnetic nanoparticles of 16 nm and 30 nm in diameter are referred to as A16MNP and A30MNP, respectively. PEI-AMNPs: PEI coated aggregated magnetic nanoparticles. For example, PEI coated aggregate of magnetic nanoparticles of 8 nm in diameter (referred to as PEI-A8MNP) has a hydrodynamic diameter of 647 nm. PEI coated aggregate of magnetic nanoparticles of 16 nm and 30 nm in diameter are referred to as PEI-A16MNP and PEI-A30MNP, respectively.

2.3. Synthesis and characterization of MNPs

Iron oxide nanoparticles were synthesized as described below using adaptations of the alkaline co-precipitation technique [28–30] and subsequently coated with 25 kDa branched PEI. Synthesis of the MNPs was adapted from three sources because the three sizes could not be obtained from any one of the synthesis method. The PEI-coated AMNPs were then diluted in nano-pure water and used for subsequent transfections.

2.3.1. Preparation of iron oxide nanoparticles

8 nm MNPs were synthesized by adapting the technique of Kim et al. [28]. In a typical experiment, an iron source consisting of 1.28 M FeCl₃·6H₂O, 0.64 M FeCl₂·4H₂O 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 N₂ gas and vigorously stirred at room temperature for 30 min. The precipitated iron oxide was isolated by a permanent magnet (1.2 T) and the supernatant was discarded. After washing the precipitate several times, it was dispersed in nano-pure water, the total volume was made up to 100 ml.

16 nm MNPs were synthesized by the technique of Nishio et al. [29]. An iron source consisting of 0.1 M FeCl₂·4H₂O was prepared by dissolving the chemical in nano-pure water and added into an alkaline solution (made up of 0.02 M NaOH and 8.8 nM NaNO₃), under bubbling N₂ 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 from the suspension as described in Section 2.3.1.

The synthesis of 30 nm MNPs was adapted from Nedkov et al. [30]. An iron source consisting of 0.03 M FeCl₂·4H₂O was prepared by dissolving the chemical in nano-pure water. This iron source was added into 0.3 M NaOH solution under bubbling N₂ gas and vigorously stirred at room temperature for 30 min. The resulting suspension was kept at 4 °C for 24 h after which the AMNPs were isolated from the suspension as described in Section 2.3.1. Table 1 summarizes the different synthesis conditions described above.

2.3.2. Coating of AMNPs with PEI

The coating procedure followed that of Mykhaylyk et al. [31]. The coating solution, consisting of 1 g PEI and 0.5 ml fluorinated surfactant (ZONYL FSA) [32,33] 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 interval) 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 to remove excess unbound coating material. Finally the PEI-coated AMNPs suspension was diluted to 0.05 μg Fe/μl and kept in aliquots at 4 °C until further use.

2.3.3. Characterization of MNPs

A transmission Electron Microscope (JEOL JEM 2010) operated at 200 kV was used to determine the particle morphology, size, distribution and phase. A X-ray diffractometer (Shimadzu 6000) operated at 50 kV and 50 mA with monochromatic Cu-Kα radiation (λ = 0.154 nm), scanning speed of 2°/min and scan range from 10 to 80° was used to determine the crystal structure of iron oxide. A vibrating sample magnetometer (Lakeshore 7400) operated at ± 10 kG at room temperature was used to determine magnetic properties such as saturation magnetization (Ms) and magnetic susceptibility (χ). Photon correlation spectroscopy (Malvern Zetasizer Nano ZS) was used to determine the aggregate size and zeta potential of the AMNPs dispersed in phosphate buffered saline (PBS), pH 7.4, at a concentration of 0.01 μg/μl. Measurements were performed at room temperature with 10 sub-run measurements per sample and Henrys Function F(Ka) equal to 1.5 (Smoluchowski approximation). The PEI content on the AMNPs was obtained by determining the weight loss from heating 20 mg of dehydrated PEI-coated AMNPs to 800 °C at a rate of 20 °C/min under air flow in a TA Instruments TGA Q500 thermo gravimetric analyzer. The PEI content was used to calculate the N/P ratio, the molar ratio of PEI nitrogen to pDNA phosphate. This N/P ratio is a measure of the ionic balance of the pDNA-PEI coated AMNP carriers. The positive charge is due to the nitrogen present in NHCH₂CH₂, which is the repeat unit of PEI with a molecular weight of 43 g/mol. The negative charge is due to the phosphate group present in the deoxyribose nucleotides. The average molecular weight of the nucleotides is 330 g/mol [34].

2.4. Cell culture

COS-7 monkey kidney cells were cultured in Roswell Park Memorial Institute 1640 medium, supplemented with 10% fetal bovine serum, 1% L-glutamine and 1% penicillin/streptomycin (complete RPMI) [35]. Cells were incubated at 37 °C in humidified 95% air and 5% CO₂ atmosphere, they were subcultured before they reach 90% confluency.

2.5. Transfection experiments

2.5.1. Preparation of gene vector complexes

Plasmid DNA (pDNA), Lipofectamine, polyMAG, PEI, uncoated and PEI-coated AMNPs were diluted in incomplete RPMI. For lipofection which was used as a positive control, optimized amount of 0.3 μg pDNA per well was complexed with 0.5 μl of Lipofectamine. For magnetically enhanced transfection, the positive control was polyMAG particles [36]. For transfection, optimized amount of 0.5 μg pDNA per well was
complexed with 0.5 μl of polyMAG, PEI, and the AMNPs. For PEI-A8MNP, PEI-A16MNP and PEI-A30MNP, the optimized AMNP to DNA weight ratio was determined in the range from 0 to 2 per well. PEI with N/P ratios ranging from 2 to 10 was used as another control. The pDNA solutions were added to the gene vector solutions and thoroughly mixed by pipetting up and down 10 times. 25 μl of gene vector complexes per well were prepared in triplicates and the resulting complexes were incubated for 30 min at room temperature before use.

2.5.2. Magnetic and non-magnetic transfection

0.5×10^4 cells per well were seeded in 96-well plates one day before transfection and grown in complete Roswell Park Memorial Institute (RPMI) medium. At a confluency of about 50% to 60%, cells were washed with phosphate-buffered saline (PBS) and 125 μl of incomplete RPMI (without FBS, L-glutamine and penicillin/streptomycin) per well was added.

25 μl of gene vectors/pDNA complexes were added into each well, making the total volume in each well equal to 150 μl. For magnetically enhanced transfection, the 96-well plate was placed on top of a magnetoFACTOR plate for 20 min of incubation at room temperature. The magnetic field strength and magnetic field gradient used in this study were 0.13 T and 50 T/m. For non-magnetic transfection, incubation was done at room temperature for 20 min (5 h for lipofection) under no magnetic field. The transfection medium was replaced with 200 μl of complete RPMI after incubation. Incubation was continued for 24 h at 37 °C in humidified 95% air and 5% CO2 atmosphere before the next medium change. In the incubation time study, incubation times of 5 min, 20 min, 40 min, 1 h, 3 h and 5 h were studied at 37 °C in humidified 95% air and 5% CO2 atmosphere.

2.6. Green fluorescent protein (GFP) expression and luciferase activity measurement

After 48 h of post transfection incubation, determination of gene expression was performed. For GFP expression, the cells were washed with PBS and replaced with fresh complete medium. Transfected cells expressing GFP were detected with a fluorescence microscope (Olympus IX71). Transfection efficiency was calculated as 100 times the number of green cells divided by the total number of cells. Ten fields were taken from the triplicates to determine the average.

For luciferase expression [37] the cells were washed with PBS and 20 μl of luciferase cell culture lysis reagent (CCLR) was added to each well. Cells were incubated for at least 15 min at room temperature for complete lysis. Luciferase assay reagent (100 μl for each assay) was kept at room temperature before performing luciferase assay. A luminometer (Turner Biosystems 20/20th) was programmed to perform a 10 s measurement read for luciferase activity. 20 μl of cell lysate was added to a luminometer tube containing 100 μl of luciferase assay reagent, and mixed by pipetting 5 times. Transfection efficiency was determined by light emission over 10 s, measured by the luminometer.

2.7. Cell viability
Cytotoxicity of the gene vectors was assessed in triplicates by crystal violet staining (CVS) assay [38] after 48 h post transfection. Cells were washed with PBS and 50 μl of 0.5% crystal violet solution in methanol was added to each well. 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. 100 μl 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 by a microplate spectrophotometer (Bio-Rad Benchmark Plus). Cell viability (%) was computed as (a/b)×100, where a is OD570 value derived from well 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. Agarose gel electrophoresis

Complexes for this assay were prepared for N/P ratios of 0, 0.25, 0.5, 0.75, 1, 1.25, 1.5, 1.75, 2, 2.25 and 2.5. 1 μl of diluted loading dye was added to each of the 50 μl solutions containing 1 μg of plasmid-DNA at the end of the 30 min incubation period. After brief vortex mixing, 20 μl was retrieved from each resultant nanoplex solution and loaded into the wells of 1% agarose gel pre-stained with 1 mg/ml ethidium bromide. Samples were left to run at 100 V for 30 min in 1X TAE buffer and visualized under UV illumination with a UV trans-illuminator (UVP).

2.9. Statistical analysis

All biological data are expressed as mean ± standard deviation. Luciferase activity, transfection efficiency and viability were analyzed by the single-factor ANOVA test. A p-value (p) less than 0.05 was considered to be significant.

3. Results

3.1. Characterization of MNPs

Fig. 2 shows the TEM micrographs and size distribution of uncoated average sized 8 nm, 16 nm and 30 nm MNPs, the MNPs were predominantly equiaxed. The average particle size was calculated by determining the median size from a sample size of 75 to 165 particles.

Fig. 3 shows the XRD spectra of the uncoated, average sized 8 nm, 16 nm and 30 nm MNPs. The positions of the peaks and relative intensities indicate that the MNPs could be either magnetite (Fe₃O₄) or maghemite (γ-Fe₂O₃), hence Rietveld refinement analysis was performed. Fig. 4 shows a typical Rietveld refinement for 30 nm MNPs, it was found that both 8 nm and 30 nm MNPs were magnetite as the fit was better for magnetite compared to maghemite. For 16 nm MNPs, the fit was equally good for maghemite and magnetite (Fig. 5), implying a higher volume fraction of maghemite phase in 16 nm MNPs compared to the 8 nm and 30 nm MNPs. The crystallite size of the MNPs was also determined from Rietveld refinement (Table 1). The calculated crystallite size from XRD (DₓRxD) agrees well with the particle size from TEM (DTEM).
Significant peak broadening was observed as the particles decrease in size (Fig. 3). The peak broadening effect can be explained by the Scherrer formula [39]: \( B_t = \frac{(0.9 \lambda)}{t \cos \theta} \), where \( t \) is the crystalline size, \( \lambda \) is the wavelength of the X-ray, \( \theta \) is the Bragg angle and \( B_t \) is peak width (in radians) of the peaks, i.e., the crystalline size is inversely proportional to peak width, hence a decrease in particle size will cause the peak to broaden.

Fig. 6 shows the magnetization-field (M-H) loops of the uncoated MNPs measured at room temperature. 8 nm and 16 nm MNPs show superparamagnetic behavior, i.e. zero remanence. There is a small hysteresis loop for 30 nm MNPs due to the small volume fraction of particles larger than 30 nm, possessing non-zero remanence. The Ms values for 8 nm, 16 nm and 30 nm MNPs were 43, 31 and 79 emu/g respectively, and were similar to the values reported by Kim [28], Mikhaylova [40] and Nishio [29]. The Ms value for 30 nm MNPs were close to those reported for bulk magnetite (82 emu/g) [41].

The Ms values of coated AMNPs were also high (27 to 75 emu/g), only 3 to 4 emu/g lower than the counterpart values of the uncoated particles. Susceptibility is defined by the relationship \( M = \chi H \), where \( M \) is the magnetization of the material and \( H \) is the magnetic field strength. The susceptibility for coated AMNPs was comparable to earlier values reported in the literature for uncoated AMNPs [28,42–44]. The Ms and susceptibility (\( \chi \)) values of PEI-AMNPs are tabulated in Table 1.

The hydrodynamic diameter and zeta potential of the transfection vectors and their respective complexes are presented in Table 2. The hydrodynamic diameters of PEI-AMNPs/DNA complexes are in the range of 798 nm to 1436 nm, the zeta potentials of the complexes are about +16 mV.

As described later, transfection efficiency and viability of the coated AMNPs were experimentally determined. The MNP/DNA ratio and N/P ratio for highest transfection efficiency and viability comparable to or better than polyMAG were determined, coated particles prepared using such MNP/DNA ratio and N/P ratio are called “optimized” particles. Table 3 shows the values of the MNP/DNA ratio and N/P ratio of the optimized PEI-A8MNP, PEI-A16MNP and PEI-A30MNP, the MNP/DNA ratios (w/w) varies from 0.9 to 1.5 and the corresponding N/P ratio varies in the range of 0.8 to 2.2. No particular trend of N/P ratio with increasing particle size was observed. At the optimized ratio, there was complete complexation between the vector and DNA (Fig. 7).

3.2. Magnetically enhanced transfection

3.2.1. Optimization of PEI-A8MNP—luciferase activity assay and CVS assay

Fig. 8 shows a plot of the luciferase activity and viability for PEI-A8MNP as a function of MNP/DNA ratios in the range from 0 to 2.1 for a fixed incubation time of 20 min. The luciferase activity of naked DNA was close to the background level (cells only) and the luciferase activity for low MNP/DNA ratio (equal to 0.5) was comparable to that of naked DNA. With increased MNP/DNA ratio luciferase expression was enhanced. At MNP/DNA ratio equal to 1.1, luciferase activity increased sharply, reaching a maximum at MNP/DNA ratio equal to 1.5. Viability generally decreased with increasing
MNP/DNA ratio. Interestingly, the transfection efficiency and viability for MNP/DNA ratios in the range of 1.1 to 2.1 were higher than the corresponding values for the polyMAG particles. A ratio of MNP/DNA equal to 1.5 was chosen as the optimum ratio for further investigation as it showed highest transfection efficiency and viability comparable to that of polyMAG.

3.2.2. Effect of AMNPs

Fig. 9 shows the luciferase activity and viability for PEI with N/P ratios in the range from 0 to 10, as well as for optimized PEI-A8MNP. The luciferase activity for low N/P ratio equal to 2 was comparable to that of naked DNA. Luciferase activity increased with increasing N/P ratio, with N/P ratio equal to 7.5 having similar transfection efficiency as optimized PEI-A8MNP. Viability decreased with increasing N/P ratio. The viability at N/P ratio equal to 10 was about 70% that of optimized PEI-A8MNP.

The incubation times for AMNPs in the presence of the applied magnetic field gradient were 20 min and 5 h for DNA and PEI. The amount of PEI on the optimized PEI-A8MNP corresponds to a N/P ratio of 2.2 (Table 3). The transfection efficiency of the optimized PEI-A8MNP was 32 times that of pure PEI with N/P equal to 2. Significantly, only 20 min of incubation time was required, the significant increase in transfection efficiency, together with the much shorter incubation time confirmed the high efficacy of the magnetically enhanced transfection process.

3.2.3. Optimization of PEI-A16MNP and PEI-A30MNP

Optimization results for PEI-A16MNP and PEI-A30MNP are shown in Figs. 10 and 11 respectively. Fig. 10 shows the luciferase activity and viability for PEI-A16MNP as a function of MNP/DNA ratio in the range from 0 to 1.8 for a fixed incubation time of 20 min. Luciferase activity increased with increasing MNP/DNA ratio, reaching a maximum at MNP/DNA ratio equal to 0.9. Viability generally decreased with increasing MNP/DNA ratio, similar to what was observed for PEI-A8MNP.

Fig. 11 shows the luciferase activity and viability for PEI-A30MNP as a function of MNP/DNA ratios in the range from 0 to 1.6 for a fixed incubation time of 20 min. Luciferase activity for low MNP/DNA ratio equal to 0.4 was comparable to that of naked DNA. At MNP/DNA ratio equal to 0.8, luciferase activity increased sharply, reaching a maximum at MNP/DNA ratio equal to 1.1. As with PEI-A8MNP and PEI-A16MNP, viability generally decreased with increasing MNP/DNA ratio. A ratio of MNP/DNA equal to 1.1 was chosen as optimum as it showed highest transfection efficiency and viability comparable to that of polyMAG. The luciferase activity of the optimum MNP/DNA ratio was higher than that of polyMAG.

The optimized MNP/DNA ratios were found to be 0.9 and 1.1 for PEI-A16MNP and PEI-A30MNP respectively as compared to an optimum ratio of 1.5 for PEI-A8MNP; at these ratios, the highest transfection efficiency and viability comparable to or better than polyMAG were observed, both these sizes had comparable or better performance than polyMAG.

3.2.4. Particle size comparison study
Fig. 12 shows the luciferase activity and viability of naked DNA, optimized PEI-A8MNP, PEI-A16MNP and PEI-A30MNP, polyMAG and Lipofectamine. Magnetic vectors (PEI-AMNPs and polyMAG) under no magnetic field served as additional controls. The luciferase activity was normalized against that of Lipofectamine. Luciferase activities of magnetic vectors under magnetic field were 4 to 7 times higher than that of magnetic vectors under no magnetic field. Under magnetic field, the transfection efficiencies of PEI-A8MNP and PEI-A30MNP were found to be 45% higher than polyMAG, whereas the transfection efficiencies of PEI-A16MNP and polyMAG were comparable. The transfection efficiencies of the PEI-A8MNP and PEI-A30MNP were ~80% that of Lipofectamine. The viabilities of the PEI-AMNPs were about 2 times higher than that of Lipofectamine, indicating that the PEI-AMNPs were less toxic than Lipofectamine.

3.2.5. Plasmids comparison study

Luciferase activity was determined from the pooled average of transfected and untransfected cells, whereas GFP expression was obtained from the percentage of cells expressing the protein. The purpose of the plasmids comparison study was to determine whether the luciferase activity reflects the actual transfection efficiency. It was found that up to 36% of the cells were expressing GFP when Lipofectamine was used. Fig. 13 shows the transfection efficiency (normalized against Lipofectamine) and viability (normalized against control cells) of naked DNA, optimized PEI-A8MNP, PEI-A16MNP and PEI-A30MNP, Lipofectamine and polyMAG using luciferase and GFP expressing plasmids. The transfection efficiencies for each vector using different plasmids were comparable, particularly for optimized PEI-A8MNP, PEI-A16MNP and PEI-A30MNP. The viability was also similar in both plasmids. Generally, they showed similar trend as Fig. 12, i.e. luciferase activity of naked DNA was close to the background level (cells only); luciferase activities of PEI-A8MNP and PEI-A30MNP were ~80% that of Lipofectamine; viabilities for the PEI-AMNPs were about two-folds higher than Lipofectamine.

3.2.6. Incubation time study

Fig. 14 shows the luciferase activity of naked DNA, optimized PEI-A8MNP, PEI-A16MNP and PEI-A30MNP, polyMAG and Lipofectamine for incubation times of 5 min, 20 min, 40 min, 1 h, 3 h and 5 h under magnetic field gradient. Luciferase activity of Lipofectamine increased with incubation time and reached a maximum after 5 h of incubation. Luciferase activities for PEI-AMNPs including polyMAG achieved near maximum activity after only 20 min of incubation, except for PEI-A16MNP which required 40 min. Luciferase activities obtained from PEI-A8MNP, PEI-A16MNP and PEI-A30MNP were higher than those of PolyMAG at longer incubation times of 1 h, 3 h and 5 h.

Qualitative comparison of the amount of particles was performed after incubation. The medium was replaced with complete RPMI and the cells were observed under optical microscope. Fig. 15 shows the optical micrographs of COS-7 cells transfected using PEI-A16MNP with incubation times of 20 min and 40 min. There were apparently more particles after 40 min of incubation (Fig. 15B) than 20 min of incubation (Fig. 15A). For
the same incubation times of 20 min and 40 min, the amount of particles for PEI-A8MNP, PEI-A30MNP and polyMAG were not discernible.

4. Discussion

Our results show that magnetically enhanced transfection of COS-7 cells is feasible using the magnetic particles developed in this study. We now discuss the specific results obtained in the context of previous work.

4.1. Characterization of MNPs

TEM and XRD analyses indicated that a narrow size distribution of iron oxide MNPs were obtained using the chemical synthesis techniques developed in this work. An average individual particle size of 8 nm, 16 nm and 30 nm were obtained. The saturation magnetization of these MNPs were comparable, for the corresponding size, to literature values [28,29]. Lee et al. reported that the saturation magnetization (Ms) value increases as the particle size increases [45]. Varanda et al. also reported a linear relationship between Ms and particle size [46]. This was attributed to surface modification, e.g., defects at particle surfaces or disordered crystal orientation which could lead to the formation of non-magnetic layer, this factor is more important for smaller particles less than 10 nm diameter since they have higher surface to volume ratio and larger surface curvature. As a result, there is a significant decrease in the Ms value of 8 nm MNPs compared to the 30 nm MNPs (Table 1). However, in this study, the Ms value does not increase with particle size, e.g., the Ms value of 16 nm MNPs is lower than that of 8 nm MNPs. This could be explained by the presence of higher volume of maghemite phase which has a lower Ms value in the 16 nm MNPs as indicated from Rietveld analysis refinement.

The Ms values of coated AMNPs were also high, only slightly lower than uncoated AMNPs, hence the coated AMNPs were useful for efficient transfection. The 30 nm MNPs have the highest susceptibility implying greater attraction by the external magnetic field.

The optimized MNP/DNA ratios (w/w) range from 0.9 to 1.5, in general agreement with earlier reports that the optimized ratio was in the range of 1 to 3 depending on the particle type and gene vector [47]. The charge on the PEI-AMNPs can be explained as follows: Magnetite, being an amphoteric solid, develops charges in the protonation (Fe-OH + H⁺ ⇌ Fe-OH₂⁺) and deprotonation (Fe-OH ⇌ Fe-O⁻ + H⁺) reactions of Fe-OH sites on its surface [48], PEI molecules are subsequently attached on the surface of AMNPs by physisorption through Van der Waals forces [25,26,49]. After coating the AMNPs with PEI, the charges of the AMNPs were positive (Table 2) due to protonation of the NH₂ group, the pKa of primary amines is around 5.5 [50]. After complexing with DNA, the hydrodynamic diameters of PEI-A8MNP and PEI-A30MNP increased by about 150 nm and 100 nm respectively, whereas the increase for PEI-A16MNP was about 650 nm. The amount of increasement could be attributed to the AMNP's N/P ratio (Table 3), i.e. the higher the N/P ratio the bigger the increase in hydrodynamic diameter.
It is interesting to note that PEI-A30MNP has completely complexed with DNA at lower N/P ratio than PEI-A8MNP and PEI-A16MNP even though PEI-A30MNP has lower PEI content than PEI-A8MNP and PEI-A16MNP. Higher PEI content should be completely complexed with DNA at lower N/P ratio. For this study in which the PEI is a shell surrounding the MNP core, the radius of the MNP core can affect the complexation behavior (Fig. 16). The PEI coating is thicker on A8MNP than on A30MNP. The thicker PEI coating on A8MNP enables shielding of the negative charges on the MNP surface, thus allowing negatively charged DNA to bond with PEI. This shielding effect is reduced for A30MNP due to thinner PEI coating, therefore lowering the amount of DNA that is able to bond with PEI-A30MNP.

4.2. Effectiveness of magnetically enhanced transfection

Transfection efficiency was increased ~32 times by the use of PEI-AMNPs under magnetic field compared to PEI of similar N/P ratio (Fig. 9). Transfection efficiency of magnetic vectors under magnetic field was 4 to 7 times higher than that of magnetic vectors under no magnetic field (Fig. 12). This enhancement was attributed to the physical concentration of DNA at the cell surface. Luo and Saltzman showed that the low DNA concentration at the cell surface was the barrier that limits transfection efficiency [51]. They used silica nanoparticles, which by themselves do not deliver DNA, to enhance DNA delivery mediated by other transfection reagents; the increased concentration of DNA was achieved by settling of DNA-vector-nanoparticle complexes under gravity. In our case, the increased concentration of DNA at the cell surface was achieved by the magnetic pull of the DNA-AMNPs toward the cells.

Significantly, the use of AMNPs also reduced the incubation time from 5 h to 20 min, it also considerably decreased the amount of PEI required for transfection. PEI with N/P ratio equal to 7.5 was needed to achieve transfection efficiency similar to that of PEI-AMNPs having N/P ratio equal to 2.2 (Fig. 9).

4.3. Effect of PEI-AMNP properties on transfection efficiency

It is acknowledged that it is not practical to determine precisely the relationship between transfection efficiency and each of the physical parameters as individual independent variables because the properties are interrelated. Hence, we chose to fix the MNP/DNA ratio equal to 1.1 which is the optimized value for PEI-A30MNP. This value was also used for PEI-A8MNP and PEI-A16MNP. The transfection efficiency of PEI-A8MNP, PEI-A16MNP and PEI-A30MNP with MNP/DNA ratio equal to 1.1 was normalized against the transfection efficiency of Lipofectamine. The data for PEI-A16MNP was interpolated from the values of MNP/DNA ratio of 0.9 and 1.3 in Fig. 10 and normalized against the transfection efficiency of Lipofectamine. Linear regression was performed to determine the correlation between transfection efficiency and each physical property. The transfection efficiency is strongly positively correlated to Ms and $\chi$, strongly negatively correlated with N/P ratio, moderately positively correlated to zeta potential and moderately negatively correlated to hydrodynamic diameter of the complex.

A strong linear trend between transfection efficiency and Ms ($R^2$ of 1), $\chi$ ($R^2$ of 0.92), and N/P ratio ($R^2$ of 0.93), and a moderate linear trend for zeta potential ($R^2$ of 0.79) and
hydrodynamic diameter ($R^2$ of 0.35) was observed. PEI-AMNPs with better magnetic properties have higher transfection efficiency since they can be easily captured by an external magnetic field, resulting in shorter magnetically enhanced transfection time as well as higher transfection efficiency since more DNA is concentrated at the cell surface. However, high Ms also results in larger agglomerates due to increased attractive forces between particles, these larger aggregates may be too large to be taken up by the cell.

PEI-A16MNP has the highest N/P ratio, i.e., a larger amount of PEI, thus more protons can be absorbed to cause both endosome destabilization (proton sponge effect) as well as to provide a larger physical barrier to degradative enzymes. A similar effect is observed if larger PEI molecules are used [52]. However, a larger amount of PEI could translate to higher cell death and thus lower transfection efficiency.

There is no universal optimum hydrodynamic diameter as cells vary in size and structure. Chorny et al. transfected rat aortic smooth muscle cells and bovine aortic endothelial cells and reported that 375 nm sized polylactide AMNP exhibited higher transfection rates compared with 185 nm and 240 nm sized AMNP [53]. Thorek and Tsourkas [54] studied the cellular uptake of superparamagnetic iron oxide (SPIO) in non-phagocytic T cells over a range of particle sizes ranging from 33 nm to 1.5 μm. They found efficient labeling of cells for particle sizes up to 300 nm, micron-sized particle uptake was limited. This could explain the lower transfection efficiency of PEI-A16MNP since the size of its complex is in the micron range.

The surface charge plays an important role in the formation of complexes and association with the cell membrane [55]. Sufficient positive surface charge is critical for DNA compaction, which serves to protect the DNA [17], however, too high a charge implies that DNA will not be released from the complexes easily. Foged et al. investigated the uptake of polystyrene particles by dendritic cells in a broad particle size range (0.04 μm to 15 μm) and variable surface properties, they reported that uptake of larger particles could be greatly enhanced with a positive surface charge [56]. In the present study, the complex of PEI-A16MNP is about twice the size of polyMAG/DNA complex, and their zeta potentials were similar. The positive surface charge on PEI-A16MNP/DNA complex could have enhanced the uptake of these larger particles which could explain its similar transfection efficiency with polyMAG.

**4.4. Plasmids comparison**

The normalized transfection efficiencies (against Lipofectamine) were similar for each vector (Fig. 13), indicating that the number of plasmids entering cell nucleus was the same for luciferase and GFP plasmids. We noticed that while viability is significantly higher in magnetically enhanced transfection, transfection efficiency is lower. This indicates that the amount of DNA taken up by each cell is lower, leading to a reduced signal per cell (based on data using GFP plasmid in Fig. 13). Our data indicates that a key limitation of magnetically enhanced transfection is the amount of DNA that gets into cells—if this can be improved it may be possible to get even higher transfection efficiencies without decreased viability. Also, increased viability would indicate that the cells are less stressed during magnetically enhanced transfection. This would be advantageous in applications where higher cell viability is required.
4.5. Incubation time study

Our results show that Lipofectamine requires at least 3 h of incubation to achieve near maximum transfection efficiency. For PEI-AMNPs including polyMAG, similar level was achieved after only 20 min of incubation, except for PEI-A16MNP which required 40 min. This is in agreement with the recommended incubation time of 20 min stated in the protocol for polyMAG [36].

For PEI-A16MNP, there was a much larger difference in transfection efficiency between 20 min and 40 min of incubation as compared to PEI-A8MNP, PEI-A30MNP and polyMAG. This could be explained by the significant difference in the amount of particles captured by the magnetic field gradient after 20 min and 40 min of incubation for PEI-A16MNP (Fig. 15). For PEI-A8MNP, PEI-A30MNP and polyMAG, the difference in the amount of particles were not discernible. This difference was attributed to the weaker magnetic properties of PEI-A16MNP (Table 1). Compared to PEI-A8MNP, PEI-A30MNP or polyMAG particles a longer time was therefore required to capture similar amount of particles. This further emphasizes the importance of magnetic properties in the magnetically enhanced transfection process.

The mechanism of magnetically enhanced transfection is this study is believed to be via endocytosis. The study by Rejman et al. showed that endocytosis of PEI polyplexes are clathrin and caveolae-mediated [57]. However, the size of the complex was not reported. Ogris et al. and Weecharangsan et al. did show that PEI larger than 500 nm and chitosan nanoparticles of 500 nm in diameter were able to successfully effect transfection, but unfortunately no mechanism was proposed [58]. Huth et al. used similar material system as this study and a complex size of about 200 nm showed that besides unspecific endocytosis, clathrin-dependent and caveolae-mediated endocytosis were also involved [9]. The magnetic field and field gradient are similar to the one used in our study, through their study, they found that magnetic forces do not lead to a direct cellular uptake mechanism. The magnetic gene vector complexes were taken into the cells by endocytosis followed by a similar mechanism as PEI-mediated transfection, whereby the proton sponge effect causes endosomal escape of the complex. In another work which used the same particles as the present study, cell wounding assays showed that the mechanism of magnetic transfection is through endocytosis rather than cell wounding [59]. Our previous study [59] focused on the mechanism of magnetically enhanced transfection. Normal, reverse, retention magnetic transfection experiments and cell wounding assays were performed. The results of the normal, reverse and retention magnetic transfection experiments show that highest transfection efficiency was achieved in the normal magnetic transfection mode due to a clustering of PEI-AMNPs on the cells. Cell wounding assays results suggest that the mechanism of magnetic transfection is through endocytosis rather than cell wounding. On the other hand, the current work examines the relationship between transfection efficiency and the physical characteristics of the PEI-AMNPs and its complexes. The physical properties of the PEI-AMNPs and its complexes were characterized by transmission electron microscopy, vibrating sample magnetometry, X-ray diffractometry, thermal gravimetric analysis and photon correlation spectroscopy methods. Transfection efficiency was found to be strongly positively correlated with saturation magnetization and susceptibility, strongly negatively correlated
with N/P ratio, moderately positively correlated to zeta potential and moderately negatively correlated to hydrodynamic diameter of the complex.

5. Conclusions

In vitro magnetically enhanced transfection studies of transfection efficiency in COS-7 cells were carried out with pEGFP and pMIR-Report complexed polyethylenimine (PEI) coated iron oxide based magnetic nanoparticles.

- It was found that PEI-A8MNP and PEI-A30MNP yielded higher transfection efficiency compared to polyMAG and PEI of equivalent N/P ratio.

- The transfection efficiency was related to the physical characteristics of the coated nanoparticles. Transfection efficiency was found to be strongly positively correlated to magnetic properties (Ms and χ) and strongly negatively correlated to N/P ratio. It was moderately positively correlated to zeta potential and negatively correlated to hydrodynamic diameter.

- PEI-AMNPs that yielded higher transfection efficiency have higher Ms and χ, lower N/P ratio and smaller complex size.
References


**List of Figures**

**Fig. 1.** Principle of magnetically enhanced transfection. (1) Polyelectrolyte-coated AMNPs are mixed with DNA. (2) These DNA-AMNPs complexes are added to cells in the culture dish. (3) The cell culture dish is placed on top of a magnet for 5 to 30 min of incubation. The magnetic field rapidly pulls the complexes onto the cells to be transfected, resulting in rapid kinetics and efficient gene delivery as most of the complexes get in contact with the cells.

**Fig. 2.** TEM micrographs and size distribution plots of uncoated, average sized (A) 8 nm (B) 16 nm and (C) 30 nm MNPs. The MNPs were predominantly equiaxed. The average particle size was calculated by determining the median size from a sample size of 75 to 165 particles.

**Fig. 3.** X-ray diffraction patterns of the uncoated, average sized 8 nm, 16 nm and 30 nm MNPs. The positions of the peaks and relative intensities indicate that the MNPs could be either magnetite (Fe$_3$O$_4$) or maghemite (γ-Fe$_2$O$_3$). Significant peak broadening was observed with decreasing particle size.

**Fig. 4.** Rietveld refinement of 30 nm MNPs using (A) γ-Fe$_2$O$_3$ and (B) Fe$_3$O$_4$ as the possible crystal structures. The fit was better for magnetite compared to maghemite.

**Fig. 5.** Rietveld refinements of 16 nm MNPs using (A) γ-Fe$_2$O$_3$ and (B) Fe$_3$O$_4$ as the crystallographic models. The fitting was equally good for maghemite and magnetite implying a higher volume fraction of maghemite phase in 16 nm MNPs compared to the 8 nm and 30 nm MNPs.

**Fig. 6.** Magnetization-field (M-H) plots of uncoated, average sized 8 nm, 16 nm and 30 nm MNPs at room temperature. 8 nm and 16 nm MNPs exhibit superparamagnetic behavior whereas there is a small hysteresis loop for 30 nm MNPs.

**Fig. 7.** Agarose gel electrophoresis for (A) PEI, (B) PEI-A8MNP, (C) PEI-A16MNP and (D) PEI-A30MNP at N/P ratio from 0 to 2.5. Full complexation occurs at N/P ratio of 2.25, 0.75, 1 and 0.25 for PEI, PEI-A8MNP, PEI-A16MNP and PEI-A30MNP respectively.

**Fig. 8.** Luciferase activity and viability for PEI-A8MNP as a function of MNP/DNA ratio for a fixed incubation time of 20 min. Transfection efficiency and viability for MNP/DNA ratios in the range of 1.1 to 2.1 were superior to the corresponding values of polyMAG particles. A ratio of MNP/DNA equal to 1.5 was chosen as optimum as it showed highest transfection efficiency and viability comparable to that of polyMAG. Luciferase activity and viability for optimum MNP/DNA ratio of PEI-A8MNP were significantly higher than PolyMAG, P<0.05 vs. PolyMAG.

**Fig. 9.** Luciferase activity and viability for PEI as a function of N/P ratio. Luciferase activity increased with increasing N/P ratio. Viability decreased with increasing N/P ratio. Optimized PEI-A8MNP with PEI content corresponding to a N/P ratio equal to 2.2 showed enhanced transfection compared to PEI with the same N/P ratio. Luciferase
activities for PEI with ratios 2 and 5 were significantly lower than PEI-A8MNP, P<0.05 vs. 8 nm PEI-AMNPs.

**Fig. 10.** Luciferase activity and viability for PEI-A16MNP as a function of MNP/DNA ratio for a fixed incubation time of 20 min. Viability generally decreased with increasing MNP/DNA ratio. A ratio of MNP/DNA equal to 0.9 was chosen as optimum as it showed highest transfection efficiency and viability better than that of polyMAG. At this ratio luciferase activity was comparable to polyMAG, with viability 100% better than that of polyMAG. Luciferase activity for optimum MNP/DNA ratio of PEI-A16MNP was not significantly different from PolyMAG, P>0.05 vs. PolyMAG.

**Fig. 11.** (A) Luciferase activity and (B) viability for PEI-A30MNP as a function of MNP/DNA ratio for a fixed incubation time of 20 min. A ratio of MNP/DNA equal to 1.5 was chosen as optimum as it showed highest transfection efficiency and viability comparable to that of polyMAG. The luciferase activity and viability at the optimum MNP/DNA ratio were higher than that of polyMAG. Luciferase activity for optimum MNP/DNA ratio of PEI-A30MNP was significantly higher than PolyMAG, P<0.05 vs. PolyMAG.

**Fig. 12.** (A) Normalized luciferase activity (against lipofectamine) and (B) viability for optimized PEI-A8MNP, PEI-A16MNP and PEI-A30MNP for a fixed incubation time of 20 min. Magnetic vectors under no magnetic field served as additional controls. Luciferase activities of magnetic vectors under no magnetic field were 4 to 7 times higher than that of magnetic vectors under no magnetic field. Under magnetic field, luciferase activities of PEI-A8MNP and PEI-A30MNP were 45% higher than polyMag and ~80% that of lipofectamine. Viabilities for the AMNPs under magnetic field were about 100% higher than lipofectamine. Luciferase activities for optimized PEI-A8MNP and PEI-A30MNP were significantly different from PolyMAG, P<0.05 vs. PolyMAG.

**Fig. 13.** (A) Transfection efficiency and (B) viability of optimized PEI-A8MNP, PEI-A16MNP and PEI-A30MNP for a fixed incubation time of 20 min using luciferase and GFP expressing plasmids. The transfection efficiencies and viabilities of each vector for different plasmids were comparable (P>0.05). This shows that luciferase activity is directly reflecting transfection efficiency.

**Fig. 14.** Luciferase activity of optimized PEI-A8MNP, PEI-A16MNP and PEI-A30MNP for varying incubation times ranging from 5 min to 5 h. Luciferase activity of lipofectamine increased with incubation time and reached a maximum after 5 h of incubation. Luciferase activities for PEI-AMNPs including polyMAG achieved near maximum activity after only 20 min of incubation, except for PEI-A16MNP which took 40 min. Luciferase activities of PEI-A8MNP, PEI-A16MNP and PEI-A30MNP were higher than PolyMAG (P<0.05 vs. PolyMAG) at longer incubation times of 1 h, 3 h and 5 h.

**Fig. 15.** Optical micrographs of COS-7 cells transfected using PEI-A16MNP with incubation times of (A) 20 min and (B) 40 min. There were apparently more particles after 40 min of incubation than 20 min.
**Fig. 16.** Schematic diagram of PEI coating on A8MNP and A30MNP. The radius of the MNP core can affect the complexation behavior. The PEI coating is thicker on A8MNP than on A30MNP. The thicker PEI coating on A8MNP enables shielding of the negative charges on the MNP surface, thus allowing negatively charged DNA to bond with PEI. This shielding effect is reduced for A30MNP due to thinner PEI coating, therefore lowering the amount of DNA that is able to bond with PEI-A30MNP.
List of Tables

Table 1  Synthesis conditions and properties of 8 nm, 16 nm and 30 nm MNPs.

Table 2  Hydrodynamic diameters (nm) and zeta potentials (mV) of transfection vectors and their respective complexes.
Fig. 2
Fig. 3
Fig. 4
Fig. 5
Fig. 6
Fig. 8
Fig. 9
Fig. 10
Fig. 11
Fig. 12
Fig. 13
Fig. 14
Fig. 15

A

B

MNPs

Cells covered with MNPs

200 μm

200 μm
<table>
<thead>
<tr>
<th>Sample</th>
<th>Volume ratio of NaNO₃/FeCl₃·6H₂O</th>
<th>Synthesis temperature (°C)</th>
<th>Aging conditions</th>
<th>Diameter of uncoated MNP (TEM), Dₘ (nm)</th>
<th>Diameter of uncoated MNP (XRD), Dₘ (nm)</th>
<th>Saturation magnetization of PEI-AMNPs, Ms (emu/g)</th>
<th>Volume susceptibility of PEI-AMNPs, χ (cm³/g)</th>
<th>PEI content of PEI-AMNPs (wt.%)</th>
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<tr>
<td>8 nm MNP</td>
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<td>25</td>
<td>–</td>
<td>8 ± 2</td>
<td>7</td>
<td>41 ± 1</td>
<td>7 ± 1</td>
<td>15</td>
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<tr>
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<td>24 h, 4 °C</td>
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<td>17</td>
<td>27 ± 1</td>
<td>6 ± 1</td>
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<tr>
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<td>31</td>
<td>75 ± 2</td>
<td>25 ± 2</td>
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Table 1
Table 2

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<th>Sample</th>
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<th>Zeta potential of vector (mV)</th>
<th>Hydrodynamic diameters of complex (nm)</th>
<th>Zeta potential of complex (mV)</th>
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<td>PEI-A16MNP</td>
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<tr>
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<tr>
<td>PEI</td>
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<td>+20 ± 1</td>
<td>639 ± 49</td>
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<tr>
<td>PolyMAG</td>
<td>516 ± 74</td>
<td>+21 ± 1</td>
<td>619 ± 91</td>
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Table 3

MNP/DNA ratios and corresponding N/P ratios of PEI-AMNPs.

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<th>N/P ratio</th>
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<td>PEI-A16MNP</td>
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<tr>
<td>PEI-A30MNP</td>
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<tr>
<td>PEI-A8MNP</td>
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<td>1.6</td>
</tr>
<tr>
<td>PEI-A16MNP</td>
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<td>2.4</td>
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</table>
Mr Derrick Ang, Derrick Ang is a graduate student in the School of Materials Science and Engineering in Nanyang Technological University. He received his Master degree in Advanced Materials for Micro and Nano Systems from Singapore-MIT Alliance, National University of Singapore in 2003, and his Bachelor degree in Mechanical Engineering from the same University in 2002. His PhD thesis is on magnetic nanoparticle assisted gene delivery.

Mr Tay Chor Yong, Tay Chor Yong is a graduate student in the School of Materials Science and Engineering in Nanyang Technological University. His current research is focused on developing and employing novel micro and nano scaled-material platforms to steer stem cell responses for reconstruction of functional tissue.

Prof. Tan Lay Poh, Prof. Tan Lay Poh earned her Ph.D. degree in materials science and engineering from Nanyang Technological University, Singapore. Her group's current research activities focus on biopolymers and material-cell interaction; specifically on cell-material interaction initiated stem cell differentiation.
Prof. Peter Rainer Preiser, Prof. Peter Preiser is currently in the School of Biological Sciences. He received his Bachelor degree in Biological Science from the University of Delaware, USA in 1985 and graduated with a Ph.D. degree in Biology from the same University in 1991. His research interests focus on the malaria parasite with particular emphasis on merozoite invasion of the erythrocyte and mechanisms of immune evasion. He has been often invited as an expert reviewer for numerous international funding agencies and is a regular reviewer for a number of international journals, including Science, Nature Medicine, Blood, PNAS, etc.

Prof. Raju V. Ramanujan, Prof. R. V. Ramanujan earned his Ph.D. degree in materials science and engineering from Carnegie Mellon University, U.S.A. His group's current research activities focus on the synthesis, characterization and property evaluation of magnetic nanoparticles for novel bioengineering and energy applications. Current areas of interest include development of functionalized magnetic nanoparticles for gene delivery, as well as magnetic drug targeting and controlled drug release for therapeutic applications. He is the Secretary of the Magnetic Materials Committee of TMS (USA). He is Associate Editor or Editorial board member of several internationals journals, including Nanomedicine: Nanotechnology, Biology and Medicine.