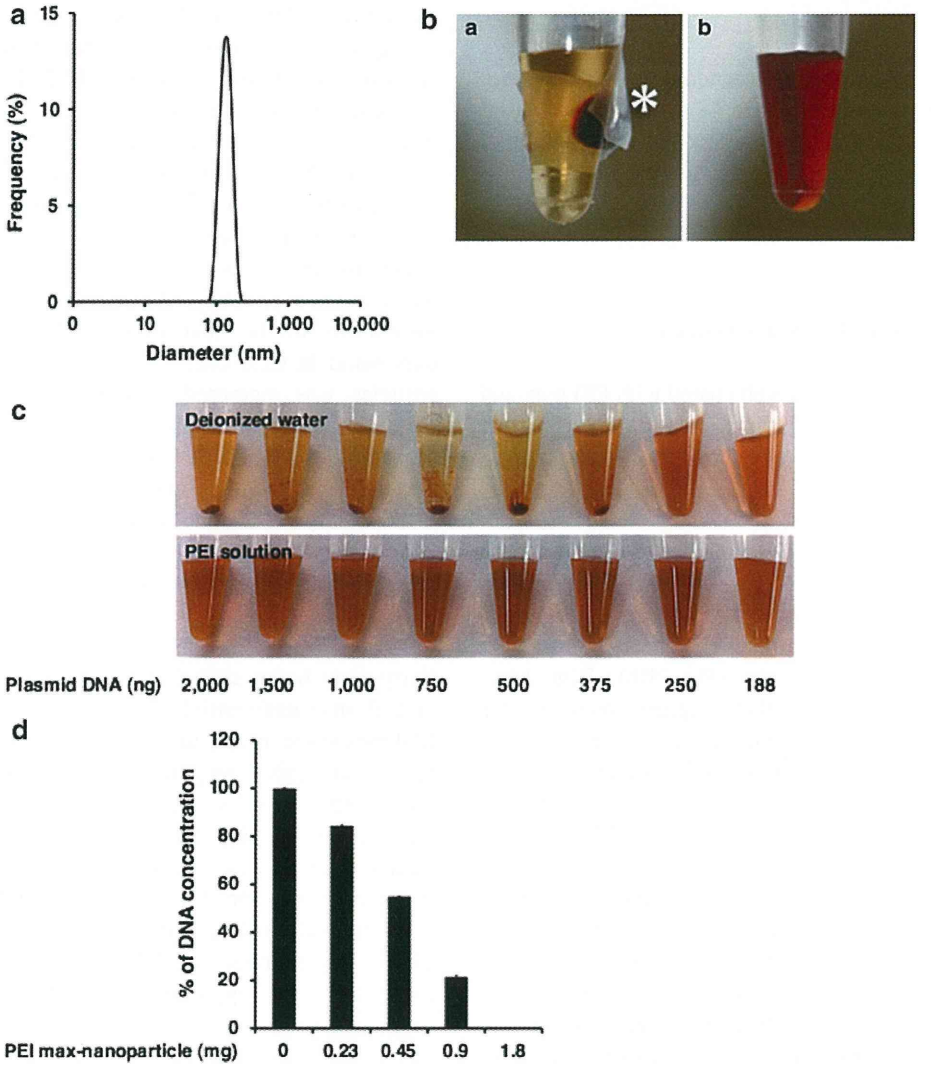

Fig. 2 Characteristics of the deacylated polyethylenimine (PEI max)-nanoparticle: **a** The size of the PEI max-nanoparticles was measured with a laser light-scattering method using a fiberoptics particle analyzer (FPAR-1000, Otsuka Electronics) at 37°C. Secondary particle size of the PEI max-nanoparticles was approximately 121.32 ± 27.36 nm. **b** PEI max-nanoparticles were induced to aggregate by a magnet (*a*) and were then dispersed (*b*). *Asterisk* indicates column-shaped neodymium magnet. **c** Cationic PEI max-nanoparticles (100 μg per tube) in deionized water or PEI max solution (1 mg/ml) were reacted with anionic plasmid [pCAGGS–enhanced green fluorescent protein (EGFP)] by an ionic bond. PEI max-nanoparticles in deionized water and plasmid aggregated more easily than that in PEI max solution and plasmid. **d** To evaluate whether plasmid DNA attached to PEI max-nanoparticles in deionized water, PEI max-nanoparticles were reacted with plasmid DNA for 15 min at room temperature. Measuring the concentration of plasmid DNA in the upper layer (hyaline layer), the weight of PEI max-nanoparticles was reduced in a dependent manner

because PEI max-nanoparticle and plasmid DNA complexes are taken in by endocytosis. Thus, it might be difficult to take the large complexes into the cytoplasm by endocytosis. Furthermore, the expression level of the *EGFP* gene was also reduced under transfection during a prolonged time on the magnetic sheet (24 h) (Fig. 4b). This result may demonstrate a causal relationship between the cell division cycle and time on the magnetic sheet. Plasmid DNAs in the cytoplasm were transported into the nucleus when the nuclear membrane disappeared on cell division [24]. Thus, plasmid DNAs and magnetic nanoparticle complexes might not be transported into the nucleus because they are drawn to the bottom of the cell by magnetic force.

We succeeded in producing PEI max-nanoparticles that enabled P19CL6 cells, which is derived from embryonic carcinoma transfected on a magnetic sheet. In addition, this method resulted in a highly efficient gene transduction compared with that of conventional transfection methods (Fig. 5a, c). This transfection method using PEI max-nanoparticles is a relatively low-cost and quick method of

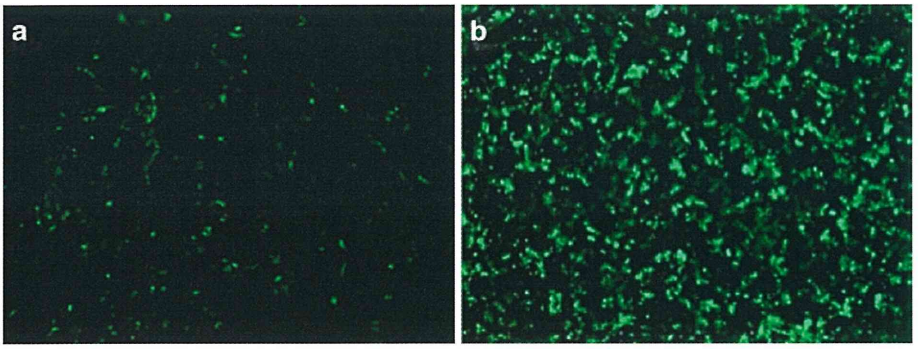

Fig. 3 Enhanced green fluorescent protein (EGFP) expression in CL6 cells using deacylated polyethylenimine (PEI max)-nanoparticle and magnetic field. Phase-contrast fluorescent micrograph of CL6 cells were transfected with pCAGGS-EGFP and PEI max as a control (**a**) and PEI max-nanoparticles (**b**). The numbers of EGFP-positive cells were further increased by PEI max-nanoparticles

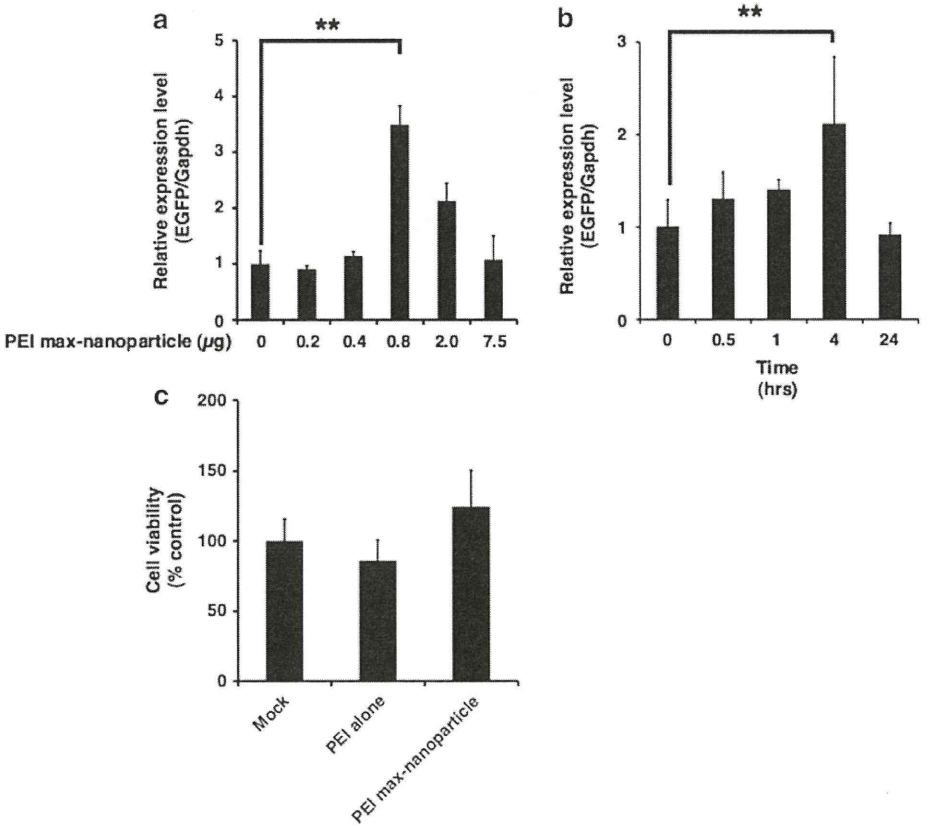

Fig. 4 Optimum condition for transfection of the deacylated polyethylenimine (PEI max)-nanoparticle. To optimize the transfection method, we examined PEI max-nanoparticles in terms of volume (**a**) and time (**b**) on the magnetic sheet. These results were evaluated by quantitative real-time reverse transcriptional polymerase chain reaction (RT-PCR). The expression level of the CL6 cells treated with PEI max alone is regarded as 1. The optimal conditions for transfection using PEI max-nanoparticles were when the CL6 cells were treated with 0.8 μg of PEI max-nanoparticles and 2.0 μg of pCAGGS-EGFP for 4 h on the magnetic sheet. The *double asterisks* indicate a significant difference ($P < 0.05$). Cytotoxicities of PEI max and PEI max-nanoparticles were evaluated by Alamar Blue assay (**c**). After 48 h of PEI max or PEI max-nanoparticle exposure, there were no significant differences in cell viability between CL6 cells treated with PEI max and those with PEI max-nanoparticles. *Mock* the CL6 cells treated without any treatment as a negative control. *PEI max alone* the CL6 cells treated with PEI max. *PEI max-nanoparticles* the CL6 cells treated with PEI max-nanoparticles (0.8 μg) for 4 h on the magnetic sheet. The relative absorbance of untreated CL6 cells is regarded as 100%

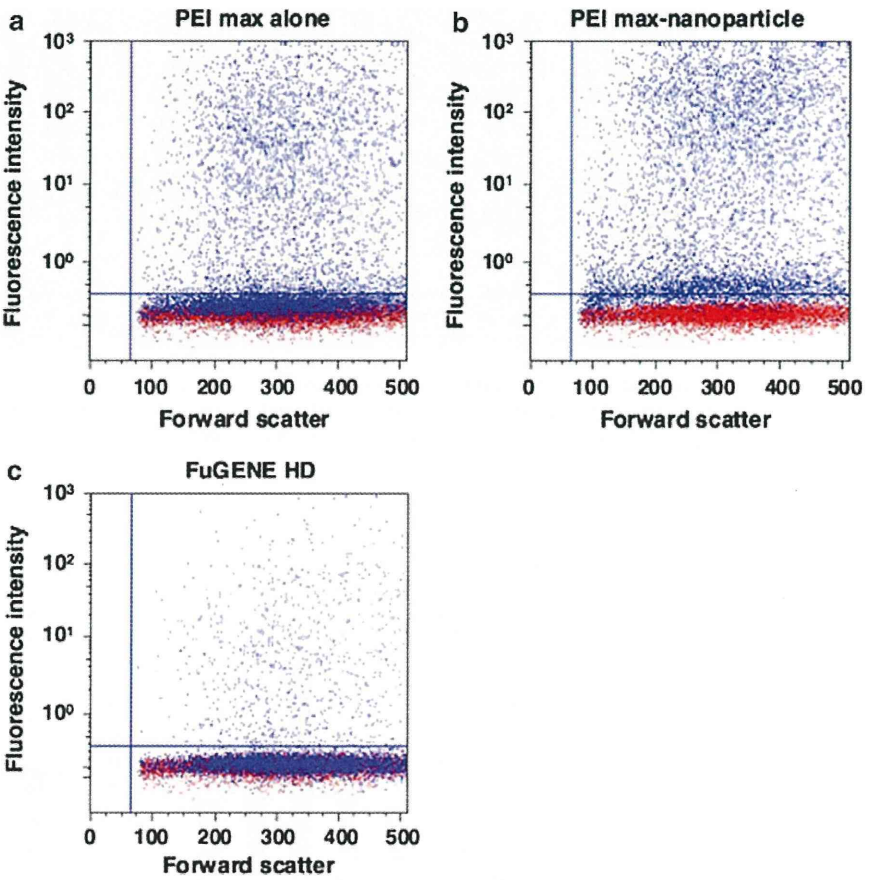

Fig. 5 Transfection efficiency of the deacylated polyethylenimine (PEI max)-nanoparticle. Comparison of scattering properties of the untreated CL6 cells (mock, *red dot*) and with PEI max alone (**a**, *blue dot*, 42.2 ± 8.5%), PEI max-nanoparticles (**b**, *blue dot*, 81.1 ± 4.0%), or FuGENE HD (**c**, *blue dot*, 13.9 ± 1.1%) by flow cytometry

Table 1 Comparison of transfection methods using the polyethylenimine and magnetic nanoparticles

Author	Year	Vector	Component	Cell	Transfection efficiency	Cell viability (% of control)	References
Kami	–	Plasmid	PEI max (MW 25k), MNP (γ-Fe2O3, 70 nm), MF (0.2 T)	P19CL6	80%[a]	100	This paper
Zhang	2010	Plasmid	Branched PEI (MW 25k), SPION (30 nm), MF (1.2 T)	NIH3T3	64%[a]	100	[14]
		siRNA	Branched PEI (MW 25k), SPION (30 nm), MF (1.2 T)	NIH3T3	77%[a]	100	
Kievit	2009	Plasmid	PEI (MW 25k), SPION (200 nm)	C6	90%[a]	10	[13]
		Plasmid	PEI (MW 25k), Chitosan, SPION (200 nm)	C6	45%[a]	100	
		Plasmid	PolyMag (commercial magnification reagent), MF (1.2 T)	C6	32%[a]	66	
Scherer	2002	Plasmid	PEI (MW 800k), SPION (200 nm), MF (1 T)	NIH3T3	5-fold[b]	–	[15]
		Adenovirus	PEI (MW 800k), SPION (200 nm), MF (1 T)	K562	100-fold[b]	–	
		Retrovirus	PEI (MW 800k), SPION (200 nm), MF (1 T)	NIH3T3	20%[a]	–	

Transfection efficiency indicates optimal transfection condition

PEI polyethylenimine, *PEI max* deacylated PEI, *MNP* magnetic nanoparticle, *SPION* superparamagnetic iron oxide nanoparticle, *MW* molecular weight, *MF* magnetic force, *T* tesla

[a] Flowcytometric analysis

[b] Luciferase activity assay

introducing plasmid into target cells with increased efficiency. Furthermore, a major advantage of this method is its tolerability among cells. Other methods might be limited either by possible cytotoxic effects of the lipidic transfection reagent (lipofection) or simply by the directly applied force on the cells (electroporation). In contrast, methods such as lipofection offer only a certain probability of hits between cargo and cells because of the three-dimensional motion of cells and transfection aggregates in a liquid suspension. Normally, transfection was inhibited

by serum using transfection reagent [25]. However, this method can also be performed in the presence of serum, which is a further benefit. Additionally, synergistic effects on transfection efficiency can arise from the possible combination of PEI max and nanoparticles. This technology might be an alternative to the currently used viral and nonviral vectors in gene therapy and gene transfer [26].

Our results suggest that PEI max-nanoparticles offer the ability to deliver various DNA formulations in addition to the traditional methods. Furthermore, gene transfer efficiency was not inhibited in the presence of serum in the cells. PEI max-nanoparticles may be a promising gene carrier with high transfection efficiency and low cytotoxicity.

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References

1. Kimura T, Iwai S, Moritan T, Nam K, Mutsuo S, Yoshizawa H, Okada M, Furuzono T, Fujisato T, Kishida A. Preparation of poly(vinyl alcohol)/DNA hydrogels via hydrogen bonds formed on ultra-high pressurization and controlled release of DNA from the hydrogels for gene delivery. J Artif Organs. 2007;10:104–8.
2. Moritake S, Taira S, Ichiyanagi Y, Morone N, Song SY, Hatanaka T, Yuasa S, Setou M. Functionalized nano-magnetic particles for an in vivo delivery system. J Nanosci Nanotechnol. 2007;7:937–44.
3. Tomitaka A, Koshi T, Hatsugai S, Yamada T, Takemura Y. Magnetic characterization of surface-coated magnetic nanoparticles for biomedical application. J Magn Magn Mater. 2010;323: 1396–1403.
4. Yokoyama M. Drug targeting with nano-sized carrier systems. J Artif Organs. 2005;8:77–84.
5. Lauterbur PC, et al. Image formation by induced local interactions. Examples employing nuclear magnetic resonance. Clin Orthop Relat Res. 1973;1989:3–6.
6. Nakamura H, Ito N, Kotake F, Mizokami Y, Matsuoka T. Tumor-detecting capacity and clinical usefulness of SPIO-MRI in patients with hepatocellular carcinoma. J Gastroenterol. 2000;35: 849–55.
7. Karlsson HL, Cronholm P, Gustafsson J, Moller L. Copper oxide nanoparticles are highly toxic: a comparison between metal oxide nanoparticles and carbon nanotubes. Chem Res Toxicol. 2008; 21:1726–32.
8. Karlsson HL, Gustafsson J, Cronholm P, Moller L. Size-dependent toxicity of metal oxide particles–a comparison between nano- and micrometer size. Toxicol Lett. 2009;188:112–8.
9. Boussif O, Lezoualc'h F, Zanta MA, Mergny MD, Scherman D, Demeneix B, Behr JP. A versatile vector for gene and oligonucleotide transfer into cells in culture and in vivo: polyethylenimine. Proc Natl Acad Sci USA. 1995;92:7297–301.
10. Wang J, Gao L. Adsorption of polyethylenimine on nanosized zirconia particles in aqueous suspensions. J Colloid Interface Sci. 1999;216:436–9.
11. Vancha AR, Govindaraju S, Parsa KV, Jasti M, Gonzalez-Garcia M, Ballestero RP. Use of polyethyleneimine polymer in cell culture as attachment factor and lipofection enhancer. BMC Biotechnol. 2004;4:23.
12. Thomas M, Lu JJ, Ge Q, Zhang C, Chen J, Klibanov AM. Full deacylation of polyethylenimine dramatically boosts its gene delivery efficiency and specificity to mouse lung. Proc Natl Acad Sci USA. 2005;102:5679–84.
13. Kievit FM, Veiseh O, Bhattarai N, Fang C, Gunn JW, Lee D, Ellenbogen RG, Olson JM, Zhang M. PEI-PEG-chitosan copolymer coated iron oxide nanoparticles for safe gene delivery: synthesis, complexation, and transfection. Adv Funct Mater. 2009;19:2244–51.
14. Zhang H, Lee MY, Hogg MG, Dordick JS, Sharfstein ST. Gene delivery in three-dimensional cell cultures by superparamagnetic nanoparticles. ACS Nano. 2010;4:4733–43.
15. Scherer F, Anton M, Schillinger U, Henke J, Bergemann C, Kruger A, Gansbacher B, Plank C. Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo. Gene Ther. 2002;9:102–9.
16. Bertram J. MATra—magnet assisted transfection: combining nanotechnology and magnetic forces to improve intracellular delivery of nucleic acids. Curr Pharm Biotechnol. 2006;7: 277–85.
17. Arsianti M, Lim M, Marquis CP, Amal R. Polyethylenimine based magnetic iron-oxide vector: the effect of vector component assembly on cellular entry mechanism, intracellular localization, and cellular viability. Biomacromolecules. 2010;11:2521–31.
18. Georgieva JV, Kalicharan D, Couraud PO, Romero IA, Weksler B, Hoekstra D, Zuhorn IS. Surface characteristics of nanoparticles determine their intracellular fate in and processing by human blood-brain barrier endothelial cells in vitro. Mol Ther. 2011; 19:318–25.
19. Longmire M, Choyke PL, Kobayashi H. Clearance properties of nano-sized particles and molecules as imaging agents: considerations and caveats. Nanomedicine (Lond). 2008;3:703–17.
20. Niwa H, Yamamura K, Miyazaki J. Efficient selection for high-expression transfectants with a novel eukaryotic vector. Gene. 1991;108:193–9.
21. Nakayama GR, Caton MC, Nova MP, Parandoosh Z. Assessment of the Alamar blue assay for cellular growth and viability in vitro. J Immunol Methods. 1997;204:205–8.
22. Namgung R, Singha K, Yu MK, Jon S, Kim YS, Ahn Y, Park IK, Kim WJ. Hybrid superparamagnetic iron oxide nanoparticle-branched polyethylenimine magnetoplexes for gene transfection of vascular endothelial cells. Biomaterials. 2010;31:4204–13.
23. Song HP, Yang JY, Lo SL, Wang Y, Fan WM, Tang XS, Xue JM, Wang S. Gene transfer using self-assembled ternary complexes of cationic magnetic nanoparticles, plasmid DNA and cell-penetrating Tat peptide. Biomaterials. 2010;31:769–78.
24. Coonrod A, Li FQ, Horwitz M. On the mechanism of DNA transfection: efficient gene transfer without viruses. Gene Ther. 1997;4:1313–21.
25. Purow BW, Sundaresan TK, Burdick MJ, Kefas BA, Comeau LD, Hawkinson MP, Su Q, Kotliarov Y, Lee J, Zhang W, Fine HA. Notch-1 regulates transcription of the epidermal growth factor receptor through p53. Carcinogenesis. 2008;29:918–25.
26. Davis ME. Non-viral gene delivery systems. Curr Opin Biotechnol. 2002;13:128–31.

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Review

Application of Magnetic Nanoparticles to Gene Delivery

Daisuke Kami [1,*], Shogo Takeda [2], Yoko Itakura [1], Satoshi Gojo [1], Masatoshi Watanabe [2] and Masashi Toyoda [1]

[1] Research Team for Vascular Medicine, Tokyo Metropolitan Institute of Gerontology, 35-2 Sakae-cho, Itabashi-ku, Tokyo 173-0015, Japan; E-Mails: yitakura@tmig.or.jp (Y.I.); satoshigojo-tky@umin.ac.jp (S.G.); mtoyoda@tmig.or.jp (M.T.)

[2] Laboratory for Medical Engineering, Division of Materials and Chemical Engineering, Yokohama National University, 79-1 Tokiwadai, Hodogaya-ku, Yokohama 240-8501, Japan; E-Mails: smilesnow1987@gmail.com (S.T.); mawata@ynu.ac.jp (M.W.)

* Author to whom correspondence should be addressed; E-Mail: dkami@tmig.or.jp; Tel.: +81-3-3964-3241; Fax: +81-3-3579-4776.

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Abstract: Nanoparticle technology is being incorporated into many areas of molecular science and biomedicine. Because nanoparticles are small enough to enter almost all areas of the body, including the circulatory system and cells, they have been and continue to be exploited for basic biomedical research as well as clinical diagnostic and therapeutic applications. For example, nanoparticles hold great promise for enabling gene therapy to reach its full potential by facilitating targeted delivery of DNA into tissues and cells. Substantial progress has been made in binding DNA to nanoparticles and controlling the behavior of these complexes. In this article, we review research on binding DNAs to nanoparticles as well as our latest study on non-viral gene delivery using polyethylenimine-coated magnetic nanoparticles.

Keywords: magnetic nanoparticles; Magnetofection; gene delivery; polyethylenimine

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1. Introduction

Nanotechnology describes the creation and utilization of materials, devices, and systems through the control of nanometer-sized materials and their application to physics, chemistry, biology, engineering, materials science, medicine, and other endeavors. In particular, intensive efforts are in progress to develop nanomaterials for medical use as agents that can be targeted to specific organs, tissues, and cells. For example, magnetic nanoparticles (MNPs) are being used clinically as contrast agents for magnetic resonance imaging (MRI) (Table 1). MRI is a noninvasive technique that can provide real-time high-resolution soft tissue information [1,2]. MRI image quality can be further improved by utilizing contrast agents that alter proton relaxation rates [3–8]. MNP-based drug delivery systems (DDS) [9–11], and treatments of hyperthermia [12–21], using MNPs have been studied for over a decade. Furthermore, researchers have reported that MNPs have been useful in hyperthermic treatment for various cancers *in vivo* [22–31]. Nanotechnology-based anti-cancer agent DDS have already been approved, such as pegylated liposomal doxorubicin (DOXIL) for ovarian cancer [32–37]. MNPs have been used effectively as transfection reagents for introducing nucleic acids (plasmids or siRNAs) [38–53], or viruses (retrovirus, or adenovirus) [44,54–56] into cells. Our own research is focused on MNP-mediated gene delivery systems (called as "Magnetofection").

Table 1. Biomedical Applications of Magnetic Nanoparticles (MNPs).

	Purpose	References
MRI	Diagnosis	[1–8,57–61]
DDS	Anti-cancer therapy, Enzyme therapy	[9–11,22–31]
Hyperthermia	Anti-cancer therapy	[12–18,33–37]
Gene Delivery	Anti-cancer therapy, Cell transplantation therapy	[38–55]

2. Gene Delivery

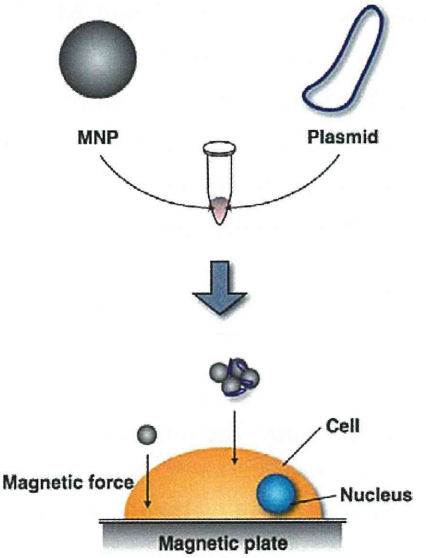
Gene delivery techniques efficiently introduce a gene of interest in order to express its encoded protein in a suitable host or host cell. Currently, there are three primary gene delivery systems that employ viral vectors (retroviruses and adenoviruses), nucleic acid electroporation, and nucleic acid transfection. These systems vary in efficacy (Table 2). Gene delivery by viral vectors can be highly efficient (80–90%) but may insert viral vector nucleic acid sequences into the host genome, potentially causing unwelcome effects, such as inappropriate expression of deleterious genes. Electroporation is also a highly efficient technique for introducing foreign genes into a host (50–70%); however, half of the recipient cells die due to the electrical stimulation. Transfection reagents do not efficiently deliver nucleic acids into cells (20–30%); however, cell viability is largely preserved and the method is safe enough for clinical use. Therefore, this method holds relatively more promise for medical applications, provided that its efficiency can be improved. MNPs are already in use by basic researchers to increase transfection efficiencies of cultured cells. Thus, MNP-nucleic acid complexes are added to cell culture media and then onto the cell surface by applying a magnetic force (Figure 1).

Table 2. Gene delivery systems.

	Expression Type	Efficiency (%)	Cell Viability (%)	Safety
Virus *	Stable, or Transient	80–90%	80–90%	Low
Electroporation	Transient	50–70%	40–50%	High
TF reagent **	Transient	20–30%	80–90%	High

* Virus including adenovirus (transient), retrovirus (stable), and lentivirus (stable); ** TF reagent, transfection reagents including PEI (Polysciences Inc.), FuGENE HD (Promega), and Lipofectamine 2000 (Invitrogen); All values are ours (unpublished experiments).

Figure 1. MNP gene delivery system (Magnetofection). Plasmids are bound to MNPs, which then move from the media to the cell surface by applying a magnetic force.



Oxide nanoparticles mixed with high magnetic moment compounds such as $CoFe_2O_4$, $NiFe_2O_4$, and $MnFe_2O_4$ exhibit superior performance compared to other magnetic materials [62,63]. However, these nanoparticles are highly toxic to cells, limiting their use for *in vivo*, and *in vitro* biomedical applications [64–67]. However, iron oxides such as magnetite (Fe_3O_4) and maghemite (γ-Fe_2O_3), in particular, possess high magnetic moments, are relatively safe, and currently in clinical use as MRI contrast agents [57–61]. These iron oxide based-magnetic materials are also suitable for biomedical applications. Fe^{3+} is widely dispersed in the human body so leaching of this metal ion from nanoparticles should not reach toxic concentrations [68,69]. As a result, maghemite is a popular choice for MNPs used biomedical applications. It is very important to modify the surface of MNPs so that they can be used for biomedical applications. Thus, MNPs are coated with compounds such as natural polymers (proteins and carbohydrates) [70–75], synthetic organic polymers (polyethylene glycol), polyvinyl alcohol, poly-L-lactic acid) [72,76–78], silica [79], and gold [80,81]. These surface coating agents prevent nanoparticle agglomeration, cytotoxicity, and add functionality. MNPs agglomerate readily in aqueous solutions around pH 7 [82], and it is difficult to control the properties and amounts of agglomerated MNPs. The greater toxicity of MNPs compared to those of microparticles can be attributed to their high surface to volume ratio [83]. Coating agents prevent the leaching of potentially toxic components from MNPs. In fact, the cytotoxicity of uncoated $NiFeO_4$ MNPs is dramatically

decreased by coating with cationic polymer, polyethylenimine (PEI) [84–86]. PEI, a cationic polymer, is widely used for nucleic acid transfection [87–89] and also serves as a nanoparticle dispersant [90]. PEI-coated MNPs enhance transfection efficiency [38,41,42,44–46,48,49,51,54,55].

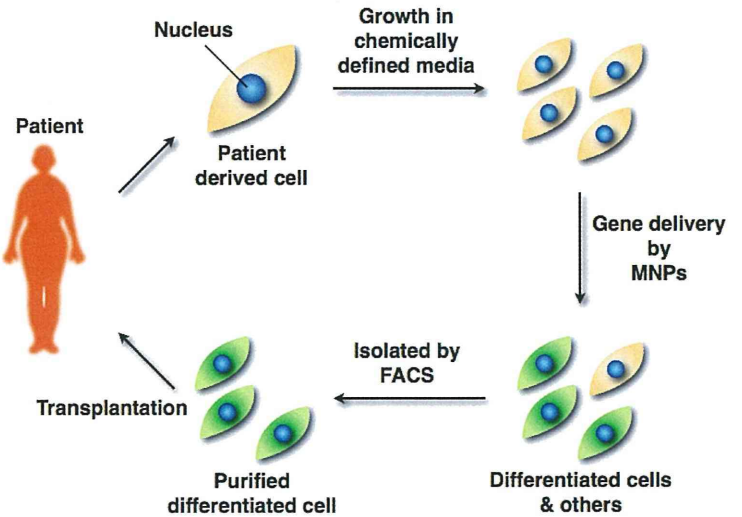
3. Cell Transplantation Therapy Using MNPs

Autologous cell transplantation has been widely used in the clinic for decades. Delivering therapeutic genes to patients using their own cells avoids using immunosuppressive drugs. We reasoned, therefore, that a non-viral gene delivery system using iron oxide-based MNPs could provide a powerful tool for next-generation therapies. Gene delivery using MNPs has been successful for delivering nucleic acids into living cells with high efficiency and low cytotoxicity [38,41,42,44–46,48,49,51,54,55]. Currently, there are several methods for inducing cellular differentiation.

One of these methods, termed direct reprogramming, or direct conversion, has successfully yielded induced cardiomyocytes, induced neurons, reprogrammed pancreatic β cells, and induced pluripotent stem cells (iPSCs) [91–95]. Direct reprogramming represents a more straightforward strategy to treat diseases involving loss of function by specific cell populations compared to approaches requiring an intermediate embryonic stem cell. Thus, patient-derived differentiated cells by gene transfer are suitable for autologous cell transplantation, potentially resulting in faster patient recoveries. The scheme is classified into *ex vivo* gene therapy. The steps involved in this technique are as follows: (1) Patient-derived cells (such as fibroblasts) are cultured in chemically defined media *in vitro*; (2) These cells are transfected by MNPs, and differentiated into functional cells; (3) Differentiated cells are isolated by fluorescence-activated cell sorting (FACS); (4) FACS-purified differentiated cells are transplanted into the patient's target tissue (Figure 2).

Here we briefly describe the magnetofection [96], and our latest study concerning non-viral gene delivery using deacylated polyethylenimine coated MNPs.

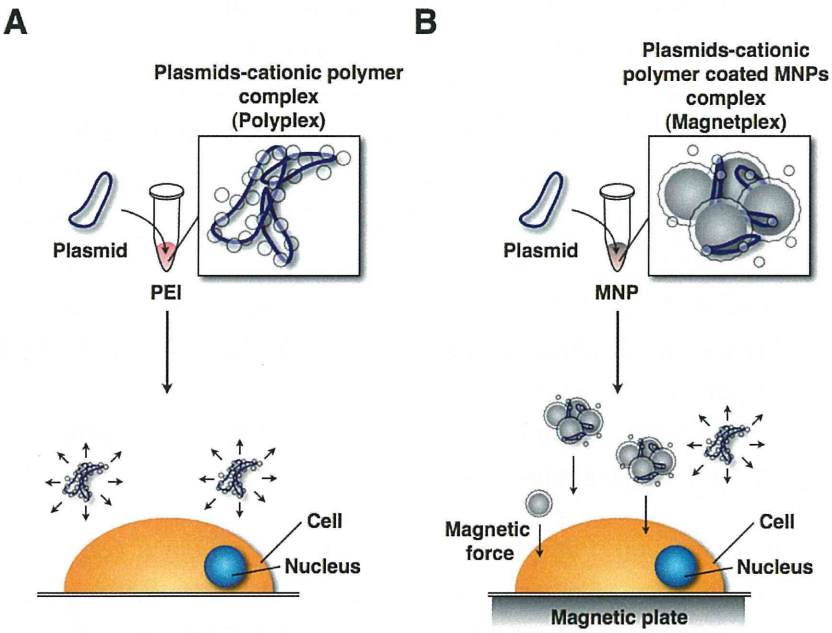
Figure 2. Strategy for cell transplantation therapy. A patient's cells are cultured in chemically defined media. MNP-transfected cells by the introduced gene are isolated by FACS. FACS-purified differentiated cells are transplanted into the patient.



4. Gene Delivery Using MNPs and Magnetic Force

The mechanism of magnetofection is similar to using transfection reagents (Lipofectamine 2000, FuGENE HD, and PEI). The only difference is that the plasmids form complexes with cationic polymer-coated MNPs (called as "Magnetoplex") [42,48,97–99] (Figure 3). Figure 3 shows the two difference techniques. The behavior of magnetoplex is readily controlled by magnetic force. Upon binding to the cell surface they are taken up by endocytosis [51,100,101]. Thus, the transfection efficiency was increased.

Figure 3. Gene delivery systems using a transfection reagent (cationic polymer) and MNPs: (**A**) Gene delivery system using transfection reagent. The polyplex moves randomly in culture medium; (**B**) Magnetofection system. The magnetoplex only moves to the cell surface.



Many researchers have described magnetofection methods (Table 3). They modified the surface of iron oxide-based MNPs to increase transfection efficiency and reduce cytotoxicity. To achieve this, some investigators selected coating agents such as anionic surfactants (oleic acid, lauroyl sarcosinate) [42,50,102], a non-ionic water-soluble surfactant (Pluronic F-127) [42], fluorinated surfactant (lithium 3-[2-(perfluoroalkyl) ethylthio]propionate) [54], a polymer (polyethylene glycol, poly-L-lysine, poly(propyleneimine) dendrimers) [40,103,104], carbohydrates (Chitosan, Heparan sulfate) [41,47], silica particles (MCM48) [49], proteins (serum albumin, streptavidin) [40,55], hydroxyapatite [105], phospholipids [49,50], a cationic cell penetrating peptide (TAT peptide) [43], non-activated virus envelope (HVJ-E) [47], a transfection reagent (Lipofectamine 2000) [53], and viruses (adenovirus, retrovirus) [44,54–56]. These coating agents are often used in conjunction with PEI. PEI is a well-known cationic gene carrier with high transfection efficiency. However, the high toxicity, depended on its molecular weight, has limited its use as a potential gene carrier. Thus, the PEI was modified to increase transfection efficiency, and decrease cytotoxicity [88,106]. To enhance transfection

efficiency, most researchers used the PEI, or the modified PEI to coat the nanoparticle surface [38,41,42,44–46,48,49,51,54,55,102,107]. PEI-coated MNPs are stable in water, bind nucleic acids, and control MNP behavior by magnetic force. In addition, linear PEI possesses low cytotoxicity compared with branched PEI *in vivo* and *in vitro* [108,109] The highest transfection efficiencies have been achieved using 25,000 molecular weight linear PEI [89]. However, PEI cytotoxicity due to its acyl groups has been described [88]. Therefore, our group focused on commercial deacylated PEI (Polyethylenimine "Max" (PEI "Max"), Polysciences Inc.) as an MNP (γ-Fe_2O_3, d = 70 nm, CIK NanoTek) coating agent.

Deacylated polyethylenimine (linear, 25,000 molecular weight) is built from the same polymer backbone as the popular linear polyethylenimine, and possesses high cationic reactivity. PEI "Max"-coated MNPs (PEI max-MNPs) are stable in deionized water, and positively charged. Thus, PEI max-MNPs electrostatically bind to plasmids. We attempted to introduce the green fluorescent protein (GFP) gene into a mouse embryonic carcinoma cell line, P19CL6 using PEI max-MNPs, and succeeded in establishing a highly efficient and low cytotoxic gene delivery system [107]. Furthermore, we applied this system to human fetal lung-derived fibroblasts (TIG-1 cells) using six-well plates. Using MNPs, the transfected gene's expression level increased 2- to 4-fold under optimum conditions (Figure 4, unpublished data). Furthermore, to assess whether the multiple plasmids were expressed in a single cell, we attempt to induce the expression of three fluorescent proteins GFP, cyan fluorescent protein (CFP), and yellow fluorescent protein (YFP). Most cells expressed these three proteins (Figure 5, unpublished data) indicating that gene delivery using MNPs could introduce and allow expression of multiple genes in a single cell.

Figure 4. Optimum conditions for PEI max-MNPs magnetofection. To optimize conditions, we varied volume (**A**) and time on the magnetic plate (**B**). These results were evaluated by quantitative real-time RT-PCR. The relative expression level (*GFP/GAPDH*) in the human fetal lung-derived fibroblasts (TIG-1 cells) treated with PEI max alone (A), and in the absence of magnetic force (0 h) (B) was defined as 1. Optimal transfection conditions were established when TIG-1 cells were treated with 0.8 μg PEI max-MNPs and 2.0 μg pCAG-GFP for 8 h on the magnetic plate in either a six-well plate or a 35 mm dish. The asterisk (*) indicates a significant difference ($P < 0.05$).

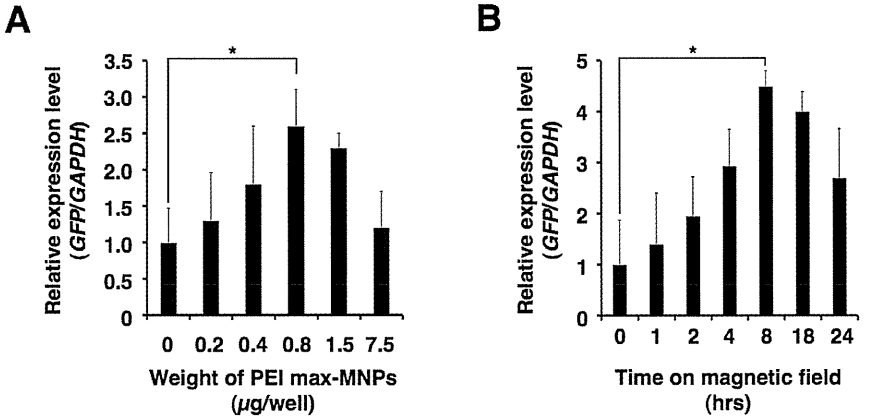


Table 3. Summary of magnetofection literature.

Author	Year	Vector	Magnetic Nanoparticles	Modifying Agent	Targeting Cell, or Tissue	TF Efficiency	Cell Viability (% of Control)	Reference
Kami D	2011	Plasmid	Iron oxide (γ-Fe_2O_3)	PEI max (MW: 25 k)	P19CL6	* 82%	100%	[107]
Pickard MR	2011	Plasmid	NeuroMag	-	Neural precursor cell	* 30%	70%	[39]
Hashimoto M	2011	Adenovirus, Biotin	SPION	PEI, Streptoavidin	HeLa	** 4-fold	-	[55]
		Adenovirus, Biotin	SPION	PEI, Streptoavidin	NIH3T3	** 10-fold	-	
		Adenovirus, Biotin	SPION	PEI, Streptoavidin	Mouse embryonic brain	-	-	
Biswas S	2011	Plasmid	Iron oxide (Fe_3O_4)	Aminooxy, Oxime ether	MCF-7	** 1425-fold	89%	[110]
B González	2011	Plasmid	SPION	Poly(propyleneimine) dendrimers	Saos-2 osteoblasts	* 12%	75%	[104]
Zhang H	2010	Plasmid	SPION	Branch PEI (MW: 25 k)	NIT3T3	* 64%	100%	[38]
		siRNA	SPION	Branch PEI (MW: 25 k)	NIT3T3	* 77%	100%	
Song HP	2010	Plasmid	PolyMag	Tat peptide	U251	* 60%	80%	[43]
		Plasmid	PolyMag	Tat peptide	Rat spinal cord	** 2-fold	-	
Arsianti M	2010	Plasmid	Iron oxide	Branch PEI (MW: 25 k)	BHK-21	-	60–90%	[51]
Shi Y	2010	Plasmid	Magnetite	Hyperbranch PEI (MW: 10 k)	COS-7	** 13-fold	-	[45]
Ang D	2010	Plasmid	Magnetite	Branch PEI (MW: 25 k)	COS-7	** 6-fold	70%	[46]
Tresilwised N	2010	Adenovirus	Iron oxide (Fe_2O_3, Fe_3O_4)	Branch PEI (MW: 25 k), Zonyl FSA fluorosurfactant	EPP85-181RDB	** 10-fold	-	[54]
Namgung R	2010	Plasmid	SPION	PEG, Branch PEI (MW: 25 k)	HUVEC	** 12-fold	80%	[48]
Yiu HH	2010	Plasmid	Iron oxide (Fe_3O_4)	PEI (MW: 25 k), MCM48 (Silica particle)	NCI-H292	** 4-fold	-	[49]
HC Wu	2010	Plasmid	Magnetite	Hydroxyapatite	Rat marrow stromal cells	* 60–70%	100%	[105]
Namiki Y	2009	Plasmid	Magnetite	Oleic acid, Phospholipid	HSC45	** 8-fold	-	[50]
		siRNA	Magnetite	Oleic acid, Phospholipid	Tissue sample from gastric cancer	-	-	
Kim TS	2009	Plasmid	PolyMag	-	Boar spermatozoa	-	-	[52]
Kievit FM	2009	Plasmid	SPION	PEI (MW: 25 k)	C6	* 90%	10%	[41]
		Plasmid	SPION	PEI (MW: 25 k), Chitosan	C6	* 45%	100%	
		Plasmid	PolyMag	-	C6	* 32%	66%	

Table 3. *Cont.*

Author	Year	Vector	Magnetic Nanoparticles	Modifying Agent	Targeting Cell, or Tissue	TF Efficiency	Cell Viability (% of Control)	Reference
Lee JH	2009	siRNA	MnMEIO	Serum albumin, PEG-RGD	MDA-MB-435-GFP	* 30%	-	[40]
Li Z	2009	Plasmid	Iron oxide	Poly-L-lysine	Lung tissue	*** 60%	-	[103]
Yang SY	2008	Plasmid	Iron oxide (Fe_3O_4)	Lipofectamine 2000	He99	-	-	[53]
		Plasmid	Iron oxide (Fe_3O_4)	DOTAP:DOPE	He99	-	-	
Pan X	2008	Plasmid	Magnetite	Oleic acid, Branch PEI (MW: 25 k), Transferrin	KB	** 300-fold	92%	[102]
Mykhaylyk O	2007	Plasmid	Iron oxide (Fe_2O_3, Fe_3O_4)	Branch PEI (MW: 25 k)	H441	* 49%	-	[42]
		Plasmid	Iron oxide (Fe_2O_3, Fe_3O_4)	Pluronic F-127	H441	* 37%	-	
		Plasmid	Iron oxide (Fe_2O_3, Fe_3O_4)	Lauroyl sarcosinate	H441	-	-	
		Plasmid	Iron oxide (Fe_2O_3, Fe_3O_4)	Branch PEI (MW: 25 k), Lauroyl sarcosinate	H441	-	-	
Morishita N	2005	Plasmid	Iron oxide (γFe_2O_3)	HVJ-E, protamine sulfate	BHK-21	** 4-fold	-	[47]
		Plasmid	Iron oxide (γ-Fe_2O_3)	HVJ-E, heparin sulfate	Liver, BALB/c mice (8 weeks age)	** 3-fold	-	
Scherer F	2002	Plasmid	SPION	PEI (MW: 800 k)	NIH3T3	** 5-fold	-	[44]
		Adenovirus	SPION	PEI (MW: 800 k)	K562	** 100-fold	-	
		Retrovirus	SPION	PEI (MW: 800 k)	NIH3T3	* 20%	-	
Mah C	2002	Adenovirus	Avidinylated magnetite	Biotunylated heparan sulfate	C12S	* 75%	-	[56]
		Adenovirus	Avidinylated magnetite	Biotunylated heparan sulfate	Adult 129/SvJ mice	-	-	

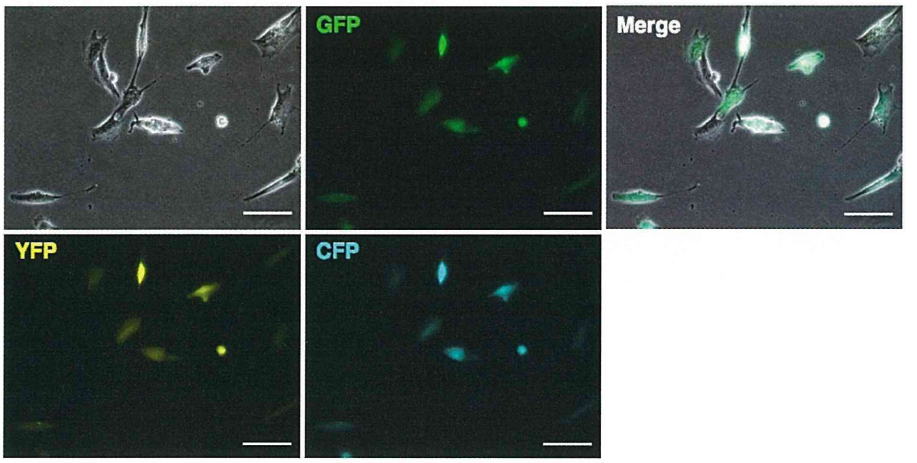
* indicates % of fluorescent positive cells analyzed by flow cytometric analysis.

** indicates analysis by luciferase activity assay compared with control. Transfection efficiency was indicated optimal transfection condition.

*** indicates transfection without magnetic force.

PEI: Polyethylenimine; PEI max: Deacaylated PEI; MNP: Magnetic nanoparticle; SPION: Superparamagnetic iron oxide nanoparticle; MW: Molecular weight; TF: transfection; PolyMag: Commercial Magnetofection reagent), NeuroMag (Commercial Magnetofection reagent); HVJ-E: hemagglutinating virus of Japan-envelope; DOTAP: 1,2-dioleoyl- 3-trimethylammonium-propane; DOPE: 1,2-dioleoyl-3-sn- phosphatidyl-ethanolamine; Tat peptide: cationic cell penetrating peptide; MeMEIO: Manganese-doped magnetism-engineered iron oxide; PEG: polyethylene glycol, Zonyl FSA fluorosurfactant: Lithium 3-[2-(perfluoroalkyl)ethylthio]propionate).

Figure 5. Transfection of TIG-1 cells with multiple genes using PEI max-MNPs. TIG-1 cells were simultaneously transfected with GFP, CFP, and YFP expression vector plasmids. TIG-1 cells were treated with 0.8 μg of PEI max-MNPs and 0.7 μg each of pCAG-GFP (GFP, provided by Dr. Nishino), pPhi-Yellow-N (YFP, Evrogen), and pAmCyan1-C1 (CFP, Clonetech) for 8 h on the magnetic plate in a six-well plate or a 35 mm dish. White bar indicates 200 μm.



5. Conclusions

The great promise of gene therapy for treating devastating, incurable diseases has yet to be realized. Less toxic and more efficient systems will be required, and robust research efforts in this regard are currently underway. Rapid advances have been made in adapting nanoparticle technology for basic biomedical and clinical research. Nanoparticles are already being used clinically to enhance MRI imaging, and drug delivery for cancer patients. Our own research has focused on gene delivery systems for autologous cell transplantation therapy, in which the patient's own cells are transfected with the gene required to correct their condition. In particular, our laboratory and those of others have aimed to optimize magnetofection by developing better nanoparticle coating agents [38,40–51,53–55]. Nanoparticle size is another important parameter but there were few reports addressing this subject [111]. Since cells endocytose MNPs [51,100,101], MNP size has significant implications for transfection efficiency. PEI-MNPs forms magnetoplex, which increased its influence on the magnetic force. Furthermore, MNP size influences cytotoxicity [112], and more studies on this aspect of MNP technology will be crucial for enhancing transfection efficiencies.

The two research groups reported the important developments in the field of magnetofection. The first is the influence of the oscillating magnetic force on transfection [113,114]. The second is the use of MNP-heating, and -transfection [15,16]. The purpose of these studies have increased the efficiency of transfection, and/or induced a fever by oscillating MNPs for hyperthermia. The latter, a combination of MNP-heating and -transfection, was expected to research the efficacy of both hyperthermia and gene delivery. In the future, the studies of magnetofection using the oscillating MNPs could be developed as a novel methodology.

We found that PEI is an excellent cationic polymer for dispersing MNPs and that its water solubility, stability, and low toxicity contribute to enhancing transfection efficiency [95,115–119].

Derivation of iPSCs with the use of non-viral gene delivery using PEI max MNPs should provide a powerful tool for treating diseases such as Alzheimer's, Huntington's, and Parkinson's by autologous cell transplantation. Reprogramming cells requires the action of multiple transcription factors. Our studies demonstrate that MNP-mediated transfection efficiently introduces at least three genes in a single cell. This indicates the feasibility of our system for one-step reprogramming.

References

1. Chouly, C.; Pouliquen, D.; Lucet, I.; Jeune, J.J.; Jallet, P. Development of superparamagnetic nanoparticles for MRI: Effect of particle size, charge and surface nature on biodistribution. *J. Microencapsul.* **1996**, *13*, 245–255.
2. Schlorf, T.; Meincke, M.; Kossel, E.; Gluer, C.C.; Jansen, O.; Mentlein, R. Biological properties of iron oxide nanoparticles for cellular and molecular magnetic resonance imaging. *Int. J. Mol. Sci.* **2010**, *12*, 12–23.
3. Yoo, B.; Pagel, M.D. An overview of responsive MRI contrast agents for molecular imaging. *Front. Biosci.* **2008**, *13*, 1733–1752.
4. Sun, C.; Fang, C.; Stephen, Z.; Veiseh, O.; Hansen, S.; Lee, D.; Ellenbogen, R.G.; Olson, J.; Zhang, M. Tumor-targeted drug delivery and MRI contrast enhancement by chlorotoxin-conjugated iron oxide nanoparticles. *Nanomedicine (Lond. UK)* **2008**, *3*, 495–505.
5. Medarova, Z.; Rashkovetsky, L.; Pantazopoulos, P.; Moore, A. Multiparametric monitoring of tumor response to chemotherapy by noninvasive imaging. *Cancer Res.* **2009**, *69*, 1182–1189.
6. Wu, Y.L.; Ye, Q.; Sato, K.; Foley, L.M.; Hitchens, T.K.; Ho, C. Noninvasive evaluation of cardiac allograft rejection by cellular and functional cardiac magnetic resonance. *JACC Cardiovasc. Imaging* **2009**, *2*, 731–741.
7. Wu, Y.L.; Ye, Q.; Foley, L.M.; Hitchens, T.K.; Sato, K.; Williams, J.B.; Ho, C. *In situ* labeling of immune cells with iron oxide particles: An approach to detect organ rejection by cellular MRI. *Proc. Natl. Acad. Sci. USA* **2006**, *103*, 1852–1857.
8. Chen, C.L.; Zhang, H.; Ye, Q.; Hsieh, W.Y.; Hitchens, T.K.; Shen, H.H.; Liu, L.; Wu, Y.J.; Foley, L.M.; Wang, S.J.; *et al.* A New Nano-sized Iron Oxide Particle with High Sensitivity for Cellular Magnetic Resonance Imaging. *Mol. Imaging Biol.* **2010**, doi:10.1007/s11307-010-0430-x.
9. Matsumura, Y.; Maeda, H. A new concept for macromolecular therapeutics in cancer chemotherapy: Mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. *Cancer Res.* **1986**, *46*, 6387–6392.
10. Maeda, H.; Matsumura, Y. Tumoritropic and lymphotropic principles of macromolecular drugs. *Crit. Rev. Ther. Drug Carrier Syst.* **1989**, *6*, 193–210.
11. Oh, K.T.; Baik, H.J.; Lee, A.H.; Oh, Y.T.; Youn, Y.S.; Lee, E.S. The reversal of drug-resistance in tumors using a drug-carrying nanoparticular system. *Int. J. Mol. Sci.* **2009**, *10*, 3776–3792.
12. Jordan, A.; Scholz, R.; Wust, P.; Fahling, H.; Roland, F. Magnetic fluid hyperthermia (MFH): Cancer treatment with AC magnetic field induced excitation of biocompatible superparamagnetic nanoparticles. *J. Magn. Magn. Mater.* **1999**, *201*, 413–419.

13. Mornet, S.; Vasseur, S.; Grasset, F.; Veverka, P.; Goglio, G.; Demourgues, A.; Portier, J.; Pollert, E.; Duguet, E. Magnetic nanoparticle design for medical applications. *Prog. Solid State Chem.* **2006**, *34*, 237–247.
14. Kim, D.H.; Kim, K.N.; Kim, K.M.; Lee, Y.K. Targeting to carcinoma cells with chitosan- and starch-coated magnetic nanoparticles for magnetic hyperthermia. *J. Biomed. Mater. Res., Part A* **2009**, *88*, 1–11.
15. Ito, A.; Shinkai, M.; Honda, H.; Kobayashi, T. Heat-inducible TNF-alpha gene therapy combined with hyperthermia using magnetic nanoparticles as a novel tumor-targeted therapy. *Cancer Gene Ther.* **2001**, *8*, 649–654.
16. Tang, Q.S.; Zhang, D.S.; Cong, X.M.; Wan, M.L.; Jin, L.Q. Using thermal energy produced by irradiation of Mn-Zn ferrite magnetic nanoparticles (MZF-NPs) for heat-inducible gene expression. *Biomaterials* **2008**, *29*, 2673–2679.
17. Salloum, M.; Ma, R.H.; Weeks, D.; Zhu, L. Controlling nanoparticle delivery in magnetic nanoparticle hyperthermia for cancer treatment: Experimental study in agarose gel. *Int. J. Hyperthermia* **2008**, *24*, 337–345.
18. Wust, P.; Gneveckow, U.; Johannsen, M.; Bohmer, D.; Henkel, T.; Kahmann, F.; Sehouli, J.; Felix, R.; Ricke, J.; Jordan, A. Magnetic nanoparticles for interstitial thermotherapy—feasibility, tolerance and achieved temperatures. *Int. J. Hyperth.* **2006**, *22*, 673–685.
19. Ito, A.; Honda, H.; Kobayashi, T. Cancer immunotherapy based on intracellular hyperthermia using magnetite nanoparticles: A novel concept of "heat-controlled necrosis" with heat shock protein expression. *Cancer Immunol. Immunother.* **2006**, *55*, 320–328.
20. Tanaka, K.; Ito, A.; Kobayashi, T.; Kawamura, T.; Shimada, S.; Matsumoto, K.; Saida, T.; Honda, H. Intratumoral injection of immature dendritic cells enhances antitumor effect of hyperthermia using magnetic nanoparticles. *Int. J. Cancer* **2005**, *116*, 624–633.
21. Ito, A.; Tanaka, K.; Honda, H.; Abe, S.; Yamaguchi, H.; Kobayashi, T. Complete regression of mouse mammary carcinoma with a size greater than 15 mm by frequent repeated hyperthermia using magnetite nanoparticles. *J. Biosci. Bioeng.* **2003**, *96*, 364–369.
22. Muggia, F.M. Doxorubicin-polymer conjugates: Further demonstration of the concept of enhanced permeability and retention. *Clin. Cancer Res.* **1999**, *5*, 7–8.
23. Gabizon, A.; Chemla, M.; Tzemach, D.; Horowitz, A.T.; Goren, D. Liposome longevity and stability in circulation: Effects on the *in vivo* delivery to tumors and therapeutic efficacy of encapsulated anthracyclines. *J. Drug Target.* **1996**, *3*, 391–398.
24. Sakakibara, T.; Chen, F.A.; Kida, H.; Kunieda, K.; Cuenca, R.E.; Martin, F.J.; Bankert, R.B. Doxorubicin encapsulated in sterically stabilized liposomes is superior to free drug or drug-containing conventional liposomes at suppressing growth and metastases of human lung tumor xenografts. *Cancer Res.* **1996**, *56*, 3743–3746.
25. Harrington, K.J.; Mohammadtaghi, S.; Uster, P.S.; Glass, D.; Peters, A.M.; Vile, R.G.; Stewart, J.S. Effective targeting of solid tumors in patients with locally advanced cancers by radiolabeled pegylated liposomes. *Clin. Cancer Res.* **2001**, *7*, 243–254.
26. Noe, L.L.; Becker, R.V., III; Gradishar, W.J.; Gore, M.; Trotter, J.P. The cost effectiveness of tamoxifen in the prevention of breast cancer. *Am. J. Manag. Care* **1999**, *5* (Suppl. 6), S389–S406.

27. Ibrahim, N.K.; Desai, N.; Legha, S.; Soon-Shiong, P.; Theriault, R.L.; Rivera, E.; Esmaeli, B.; Ring, S.E.; Bedikian, A.; Hortobagyi, G.N.; *et al.* Phase I and pharmacokinetic study of ABI-007, a Cremophor-free, protein-stabilized, nanoparticle formulation of paclitaxel. *Clin. Cancer Res.* **2002**, *8*, 1038–1044.
28. Ibrahim, N.K.; Samuels, B.; Page, R.; Doval, D.; Patel, K.M.; Rao, S.C.; Nair, M.K.; Bhar, P.; Desai, N.; Hortobagyi, G.N. Multicenter phase II trial of ABI-007, an albumin-bound paclitaxel, in women with metastatic breast cancer. *J. Clin. Oncol.* **2005**, *23*, 6019–6026.
29. Pinder, M.C.; Ibrahim, N.K. Nanoparticle albumin-bound paclitaxel for treatment of metastatic breast cancer. *Drugs Today* **2006**, *42*, 599–604.
30. Hamaguchi, T.; Kato, K.; Yasui, H.; Morizane, C.; Ikeda, M.; Ueno, H.; Muro, K.; Yamada, Y.; Okusaka, T.; Shirao, K.; *et al.* A phase I and pharmacokinetic study of NK105, a paclitaxel-incorporating micellar nanoparticle formulation. *Br. J. Cancer* **2007**, *97*, 170–176.
31. Hamaguchi, T.; Matsumura, Y.; Suzuki, M.; Shimizu, K.; Goda, R.; Nakamura, I.; Nakatomi, I.; Yokoyama, M.; Kataoka, K.; Kakizoe, T. NK105, a paclitaxel-incorporating micellar nanoparticle formulation, can extend *in vivo* antitumour activity and reduce the neurotoxicity of paclitaxel. *Br. J. Cancer* **2005**, *92*, 1240–1246.
32. Muggia, F.M.; Hainsworth, J.D.; Jeffers, S.; Miller, P.; Groshen, S.; Tan, M.; Roman, L.; Uziely, B.; Muderspach, L.; Garcia, A.; *et al.* Phase II study of liposomal doxorubicin in refractory ovarian cancer: Antitumor activity and toxicity modification by liposomal encapsulation. *J. Clin. Oncol.* **1997**, *15*, 987–993.
33. Kikumori, T.; Kobayashi, T.; Sawaki, M.; Imai, T. Anti-cancer effect of hyperthermia on breast cancer by magnetite nanoparticle-loaded anti-HER2 immunoliposomes. *Breast Cancer Res. Treat.* **2009**, *113*, 435–441.
34. Johannsen, M.; Thiesen, B.; Wust, P.; Jordan, A. Magnetic nanoparticle hyperthermia for prostate cancer. *Int. J. Hyperth.* **2010**, *26*, 790–795.
35. Rao, W.; Deng, Z.S.; Liu, J. A review of hyperthermia combined with radiotherapy/chemotherapy on malignant tumors. *Crit. Rev. Biomed. Eng.* **2010**, *38*, 101–116.
36. Yallapu, M.M.; Othman, S.F.; Curtis, E.T.; Gupta, B.K.; Jaggi, M.; Chauhan, S.C. Multi-functional magnetic nanoparticles for magnetic resonance imaging and cancer therapy. *Biomaterials* **2011**, *32*, 1890–1905.
37. Chen, B.; Wu, W.; Wang, X. Magnetic iron oxide nanoparticles for tumor-targeted therapy. *Curr. Cancer Drug Targets* **2011**, *11*, 184–189.
38. Zhang, H.; Lee, M.Y.; Hogg, M.G.; Dordick, J.S.; Sharfstein, S.T. Gene delivery in three-dimensional cell cultures by superparamagnetic nanoparticles. *ACS Nano* **2010**, *4*, 4733–4743.
39. Pickard, M.R.; Barraud, P.; Chari, D.M. The transfection of multipotent neural precursor/stem cell transplant populations with magnetic nanoparticles. *Biomaterials* **2011**, *32*, 2274–2284.
40. Lee, J.H.; Lee, K.; Moon, S.H.; Lee, Y.; Park, T.G.; Cheon, J. All-in-one target-cell-specific magnetic nanoparticles for simultaneous molecular imaging and siRNA delivery. *Angew. Chem., Int. Ed. Engl.* **2009**, *48*, 4174–4179.

41. Kievit, F.M.; Veiseh, O.; Bhattarai, N.; Fang, C.; Gunn, J.W.; Lee, D.; Ellenbogen, R.G.; Olson, J.M.; Zhang, M. PEI-PEG-Chitosan Copolymer Coated Iron Oxide Nanoparticles for Safe Gene Delivery: Synthesis, complexation, and transfection. *Adv. Funct. Mater.* **2009**, *19*, 2244–2251.
42. Mykhaylyk, O.; Antequera, Y.S.; Vlaskou, D.; Plank, C. Generation of magnetic nonviral gene transfer agents and magnetofection *in vitro*. *Nat. Protoc.* **2007**, *2*, 2391–2411.
43. Song, H.P.; Yang, J.Y.; Lo, S.L.; Wang, Y.; Fan, W.M.; Tang, X.S.; Xue, J.M.; Wang, S. Gene transfer using self-assembled ternary complexes of cationic magnetic nanoparticles, plasmid DNA and cell-penetrating Tat peptide. *Biomaterials* **2010**, *31*, 769–778.
44. Scherer, F.; Anton, M.; Schillinger, U.; Henke, J.; Bergemann, C.; Kruger, A.; Gansbacher, B.; Plank, C. Magnetofection: Enhancing and targeting gene delivery by magnetic force *in vitro* and *in vivo*. *Gene Ther.* **2002**, *9*, 102–109.
45. Shi, Y.; Zhou, L.; Wang, R.; Pang, Y.; Xiao, W.; Li, H.; Su, Y.; Wang, X.; Zhu, B.; Zhu, X.; Yan, D.; Gu, H. *In situ* preparation of magnetic nonviral gene vectors and magnetofection *in vitro*. *Nanotechnology* **2010**, *21*, 115103.
46. Ang, D.; Nguyen, Q.V.; Kayal, S.; Preiser, P.R.; Rawat, R.S.; Ramanujan, R.V. Insights into the mechanism of magnetic particle assisted gene delivery. *Acta Biomater.* **2011**, *7*, 1319–1326.
47. Morishita, N.; Nakagami, H.; Morishita, R.; Takeda, S.; Mishima, F.; Terazono, B.; Nishijima, S.; Kaneda, Y.; Tanaka, N. Magnetic nanoparticles with surface modification enhanced gene delivery of HVJ-E vector. *Biochem. Biophys. Res. Commun.* **2005**, *334*, 1121–1126.
48. Namgung, R.; Singha, K.; Yu, M.K.; Jon, S.; Kim, Y.S.; Ahn, Y.; Park, I.K.; Kim, W.J. Hybrid superparamagnetic iron oxide nanoparticle-branched polyethylenimine magnetoplexes for gene transfection of vascular endothelial cells. *Biomaterials* **2010**, *31*, 4204–4213.
49. Yiu, H.H.; McBain, S.C.; Lethbridge, Z.A.; Lees, M.R.; Dobson, J. Preparation and characterization of polyethylenimine-coated Fe_3O_4-MCM-48 nanocomposite particles as a novel agent for magnet-assisted transfection. *J. Biomed. Mater. Res., Part A* **2010**, *92*, 386–392.
50. Namiki, Y.; Namiki, T.; Yoshida, H.; Ishii, Y.; Tsubota, A.; Koido, S.; Nariai, K.; Mitsunaga, M.; Yanagisawa, S.; Kashiwagi, H.; *et al.* A novel magnetic crystal-lipid nanostructure for magnetically guided *in vivo* gene delivery. *Nat. Nanotechnol.* **2009**, *4*, 598–606.
51. Arsianti, M.; Lim, M.; Marquis, C.P.; Amal, R. Polyethylenimine based magnetic iron-oxide vector: The effect of vector component assembly on cellular entry mechanism, intracellular localization, and cellular viability. *Biomacromolecules* **2010**, *11*, 2521–3251.
52. Kim, T.S.; Lee, S.H.; Gang, G.T.; Lee, Y.S.; Kim, S.U.; Koo, D.B.; Shin, M.Y.; Park, C.K.; Lee, D.S. Exogenous DNA uptake of boar spermatozoa by a magnetic nanoparticle vector system. *Reprod. Domest. Anim.* **2009**, *45*, e201–e206.
53. Yang, S.Y.; Sun, J.S.; Liu, C.H.; Tsuang, Y.H.; Chen, L.T.; Hong, C.Y.; Yang, H.C.; Horng, H.E. *Ex vivo* magnetofection with magnetic nanoparticles: A novel platform for nonviral tissue engineering. *Artif. Organs* **2008**, *32*, 195–204.
54. Tresilwised, N.; Pithayanukul, P.; Mykhaylyk, O.; Holm, P.S.; Holzmuller, R.; Anton, M.; Thalhammer, S.; Adiguzel, D.; Doblinger, M.; Plank, C. Boosting oncolytic adenovirus potency with magnetic nanoparticles and magnetic force. *Mol. Pharmaceutics* **2010**, *7*, 1069–1089.

55. Hashimoto, M.; Hisano, Y. Directional gene-transfer into the brain by an adenoviral vector tagged with magnetic nanoparticles. *J. Neurosci. Methods* **2011**, *194*, 316–320.
56. Mah, C.; Fraites, T.J., Jr.; Zolotukhin, I.; Song, S.; Flotte, T.R.; Dobson, J.; Batich, C.; Byrne, B.J. Improved method of recombinant AAV2 delivery for systemic targeted gene therapy. *Mol. Ther.* **2002**, *6*, 106–112.
57. Basti, H.; Ben Tahar, L.; Smiri, L.S.; Herbst, F.; Vaulay, M.J.; Chau, F.; Ammar, S.; Benderbous, S. Catechol derivatives-coated Fe_3O_4 and gamma-Fe_2O_3 nanoparticles as potential MRI contrast agents. *J. Colloid Interface Sci.* **2010**, *341*, 248–254.
58. Gamarra, L.F.; Amaro, E., Jr.; Alves, S.; Soga, D.; Pontuschka, W.M.; Mamani, J.B.; Carneiro, S.M.; Brito, G.E.; Figueiredo Neto, A.M. Characterization of the biocompatible magnetic colloid on the basis of Fe_3O_4 nanoparticles coated with dextran, used as contrast agent in magnetic resonance imaging. *J. Nanosci. Nanotechnol.* **2010**, *10*, 4145–4153.
59. Jung, C.W.; Jacobs, P. Physical and chemical properties of superparamagnetic iron oxide MR contrast agents: Ferumoxides, ferumoxtran, ferumoxsil. *Magn. Reson. Imaging* **1995**, *13*, 661–674.
60. Martina, M.S.; Fortin, J.P.; Menager, C.; Clement, O.; Barratt, G.; Grabielle-Madelmont, C.; Gazeau, F.; Cabuil, V.; Lesieur, S. Generation of superparamagnetic liposomes revealed as highly efficient MRI contrast agents for *in vivo* imaging. *J. Am. Chem. Soc.* **2005**, *127*, 10676–10685.
61. Widder, D.J.; Greif, W.L.; Widder, K.J.; Edelman, R.R.; Brady, T.J. Magnetite albumin microspheres: A new MR contrast material. *Am. J. Roentgenol.* **1987**, *148*, 399–404.
62. Sun, X.; Gutierrez, A.; Yacaman, M.J.; Dong, X.; Jin, S. Investigations on magnetic properties and structure for carbon encapsulated nanoparticles of Fe, Co, Ni. *Mater. Sci. Eng. A* **2000**, *286*, 157–160.
63. Tomitaka, A.; Kobayashi, H.; Yamada, T.; Jeun, M.; Bae, S.; Takemura, Y. Magnetization and self-heating temperature of $NiFe_2O_4$ nanoparticles measured by applying ac magnetic field. *J. Phys.: Conf. Ser.* **2010**, *200*, 122010.
64. Cho, W.S.; Duffin, R.; Poland, C.A.; Duschl, A.; Oostingh, G.J.; Macnee, W.; Bradley, M.; Megson, I.L.; Donaldson, K. Differential pro-inflammatory effects of metal oxide nanoparticles and their soluble ions *in vitro* and *in vivo*; zinc and copper nanoparticles, but not their ions, recruit eosinophils to the lungs. *Nanotoxicology* **2011**, in press.
65. George, S.; Xia, T.; Rallo, R.; Zhao, Y.; Ji, Z.; Lin, S.; Wang, X.; Zhang, H.; France, B.; Schoenfeld, D.; *et al.* Use of a high-throughput screening approach coupled with *in vivo* zebrafish embryo screening to develop hazard ranking for engineered nanomaterials. *ACS Nano* **2011**, *5*, 1805–1817.
66. Giri, J.; Pradhan, P.; Somani, V.; Chelawat, H.; Chhatre, S.; Banerjee, R.; Bahadur, D. Synthesis and characterizations of water-based ferrofluids of substituted ferrites [Fe1-xBxFe2O4, B = Mn, Co (x = 0−1)] for biomedical applications. *J. Magn. Magn. Mater.* **2008**, *320*, 724–730.
67. Karlsson, H.L.; Cronholm, P.; Gustafsson, J.; Moller, L. Copper oxide nanoparticles are highly toxic: A comparison between metal oxide nanoparticles and carbon nanotubes. *Chem. Res. Toxicol.* **2008**, *21*, 1726–1732.

68. McBain, S.C.; Yiu, H.H.; Dobson, J. Magnetic nanoparticles for gene and drug delivery. *Int. J. Nanomed.* **2008**, *3*, 169–180.
69. Buyukhatipoglu, K.; Clyne, A.M. Superparamagnetic iron oxide nanoparticles change endothelial cell morphology and mechanics via reactive oxygen species formation. *J. Biomed. Mater. Res., Part A* **2011**, *96*, 186–195.
70. Schroder, U.; Segren, S.; Gemmefors, C.; Hedlund, G.; Jansson, B.; Sjogren, H.O.; Borrebaeck, C.A. Magnetic carbohydrate nanoparticles for affinity cell separation. *J. Immunol. Methods* **1986**, *93*, 45–53.
71. Berry, C.C.; Wells, S.; Charles, S.; Curtis, A.S. Dextran and albumin derivatised iron oxide nanoparticles: Influence on fibroblasts *in vitro*. *Biomaterials* **2003**, *24*, 4551–7455.
72. Nitin, N.; LaConte, L.E.; Zurkiya, O.; Hu, X.; Bao, G. Functionalization and peptide-based delivery of magnetic nanoparticles as an intracellular MRI contrast agent. *J. Biol. Inorg. Chem.* **2004**, *9*, 706–712.
73. Ito, A.; Ino, K.; Kobayashi, T.; Honda, H. The effect of RGD peptide-conjugated magnetite cationic liposomes on cell growth and cell sheet harvesting. *Biomaterials* **2005**, *26*, 6185–6193.
74. de la Fuente, J.M.; Penades, S. Glyconanoparticles: Types, synthesis and applications in glycoscience, biomedicine and material science. *Biochim. Biophys. Acta* **2006**, *1760*, 636–651.
75. McDonald, M.A.; Watkin, K.L. Investigations into the physicochemical properties of dextran small particulate gadolinium oxide nanoparticles. *Acad. Radiol.* **2006**, *13*, 421–427.
76. Mertz, C.J.; Kaminski, M.D.; Xie, Y.; Finck, M.R.; Guy, S.; Rosengart, A.J. *In vitro* studies of functionalized magnetic nanospheres for selective removal of a simulant biotoxin. *J. Magn. Magn. Mater.* **2005**, *293*, 572–577.
77. Mikhaylova, M.; Jo, Y.; Kim, D.; Bobrysheva, N.; Andersson, Y.; Eriksson, T.; Osmolowsky, M.; Semenov, V.; Muhammed, M. The Effect of Biocompatible Coating Layers on Magnetic Properties of Superparamagnetic Iron Oxide Nanoparticles. *Hyperfine Interact.* **2004**, *156–157*, 257–263.
78. Qiu, X.P.; Winnik, F. Preparation and characterization of PVA coated magnetic nanoparticles. *Chin. J. Polym. Sci.* **2000**, *18*, 535–539.
79. Yiu, H.H.P.; Wright, P.A.; Botting, N.P. Enzyme immobilisation using SBA-15 mesoporous molecular sieves with functionalised surfaces. *J. Mol. Catal. B: Enzym.* **2001**, *15*, 81–92.
80. Ameur, S.; Martelet, C.; Jaffrezic-Renault, N.; Chovelon, J.-M. Sensitive immunodetection through impedance measurements onto gold functionalized electrodes. *Appl. Biochem. Biotechnol.* **2000**, *89*, 161–170.
81. Arsianti, M.; Lim, M.; Lou, S.N.; Goon, I.Y.; Marquis, C.P.; Amal, R. Bi-functional gold-coated magnetite composites with improved biocompatibility. *J. Colloid Interface Sci.* **2011**, *354*, 536–545.
82. Williams, D.; Gold, K.; Holoman, T.; Ehrman, S.; Wilson, O. Surface modification of magnetic nanoparticles using gum arabic. *J. Nanopart. Res.* **2006**, *8*, 749–753.
83. Klabunde, K.J.; Stark, J.; Koper, O.; Mohs, C.; Park, D.G.; Decker, S.; Jiang, Y.; Lagadic, I.; Zhang, D. Nanocrystals as stoichiometric reagents with unique surface chemistry. *J. Phys. Chem.* **1996**, *100*, 12142–12153.

84. Zhang, H.; Xia, T.; Meng, H.; Xue, M.; George, S.; Ji, Z.; Wang, X.; Liu, R.; Wang, M.; France, B.; *et al.* Differential expression of syndecan-1 mediates cationic nanoparticle toxicity in undifferentiated versus differentiated normal human bronchial epithelial cells. *ACS Nano* **2011**, *5*, 2756–2769.
85. Sunoqrot, S.; Bae, J.W.; Jin, S.E.; Ryan, M.P.; Liu, Y.; Hong, S. Kinetically controlled cellular interactions of polymer-polymer and polymer-liposome nanohybrid systems. *Bioconjugate Chem.* **2011**, *22*, 466–474.
86. Schweiger, C.; Pietzonka, C.; Heverhagen, J.; Kissel, T. Novel magnetic iron oxide nanoparticles coated with poly(ethylene imine)-g-poly(ethylene glycol) for potential biomedical application: Synthesis, stability, cytotoxicity and MR imaging. *Int. J. Pharm.* **2011**, *408*, 130–137.
87. Boussif, O.; Lezoualc'h, F.; Zanta, M.A.; Mergny, M.D.; Scherman, D.; Demeneix, B.; Behr, J.P. A versatile vector for gene and oligonucleotide transfer into cells in culture and *in vivo*: Polyethylenimine. *Proc. Natl. Acad. Sci. USA* **1995**, *92*, 7297–7301.
88. Thomas, M.; Lu, J.J.; Ge, Q.; Zhang, C.; Chen, J.; Klibanov, A.M. Full deacylation of polyethylenimine dramatically boosts its gene delivery efficiency and specificity to mouse lung. *Proc. Natl. Acad. Sci. USA* **2005**, *102*, 5679–5684.
89. Abdallah, B.; Hassan, A.; Benoist, C.; Goula, D.; Behr, J.P.; Demeneix, B.A. A powerful nonviral vector for *in vivo* gene transfer into the adult mammalian brain: Polyethylenimine. *Hum. Gene Ther.* **1996**, *7*, 1947–1954.
90. Zuo, K.H.; Jiang, D.L.; Zhang, J.X.; Lin, Q.L. Forming nanometer TiO_2 sheets by nonaqueous tape casting. *Ceram. Int.* **2007**, *33*, 477–481.
91. Ieda, M.; Fu, J.D.; Delgado-Olguin, P.; Vedantham, V.; Hayashi, Y.; Bruneau, B.G.; Srivastava, D. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Cell* **2010**, *142*, 375–386.
92. Takeuchi, J.K.; Bruneau, B.G. Directed transdifferentiation of mouse mesoderm to heart tissue by defined factors. *Nature* **2009**, *459*, 708–711.
93. Vierbuchen, T.; Ostermeier, A.; Pang, Z.P.; Kokubu, Y.; Sudhof, T.C.; Wernig, M. Direct conversion of fibroblasts to functional neurons by defined factors. *Nature* **2010**, *463*, 1035–1041.
94. Zhou, Q.; Brown, J.; Kanarek, A.; Rajagopal, J.; Melton, D.A. *In vivo* reprogramming of adult pancreatic exocrine cells to beta-cells. *Nature* **2008**, *455*, 627–632.
95. Takahashi, K.; Yamanaka, S. Induction of pluripotent stem cells from mouse embryonic and adult fibroblast cultures by defined factors. *Cell* **2006**, *126*, 663–676.
96. Laurent, N.; Sapet, C.D.; Le Gourrierec, L.; Bertosio, E.; Zelphati, O. Nucleic acid delivery using magnetic nanoparticles: The Magnetofection™ technology. *Ther. Deliv.* **2011**, *2*, 471–482.
97. Sonawane, N.D.; Szoka, F.C., Jr.; Verkman, A.S. Chloride accumulation and swelling in endosomes enhances DNA transfer by polyamine-DNA polyplexes. *J. Biol. Chem.* **2003**, *278*, 44826–44831.
98. Brunner, S.; Sauer, T.; Carotta, S.; Cotten, M.; Saltik, M.; Wagner, E. Cell cycle dependence of gene transfer by lipoplex, polyplex and recombinant adenovirus. *Gene Ther.* **2000**, *7*, 401–407.
99. Nishiyama, N.; Kataoka, K. Current state, achievements, and future prospects of polymeric micelles as nanocarriers for drug and gene delivery. *Pharmacol. Ther.* **2006**, *112*, 630–648.