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EXPERT REVIEW

Functional Magnetic Nanoparticles for Non-Viral Gene Delivery and MR Imaging

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ABSTRACT Gene therapy is becoming a promising strategy to treat various kinds of genetic and acquired diseases. However, the development of safe, efficient, and targetable gene delivery systems remains a major challenge in gene therapy. The unique material characteristics of magnetic nanoparticles (MNPs), including high surface area, facile surface modification, controllable size, and excellent magnetic properties, make them promising candidates for gene delivery. The engineered MNPs with modifiable functional surfaces and bioactive cores can result in several advantageous diagnostic and therapeutic properties including enhanced magnetic resonance imaging (MRI) signal intensity, long permeation and retention in the circulatory system, specific delivery of therapeutic genes to target sites. In this review, the updated research on the preparation and surface modification of MNPs for gene delivery is summarized.

KEY WORDS gene delivery · gene therapy · magnetic nanoparticles (MNPs) · magnetic resonance imaging (MRI)

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INTRODUCTION

With the rapid development of recombinant DNA biotechnology, nucleic acid molecules are anticipated as potential drugs for various kinds of diseases (e.g. cancer, cystic fibrosis, macular degeneration, and Parkinson's disease) (1). Generally, gene therapy relies on the delivery of genetic materials into targeted cells for disease treatment (2). With efficient delivery, the therapeutic prospects range from tackling genetic diseases and slowing the progress of tumors, to fighting viral infections and suppressing neurodegenerative diseases. However, many challenges in gene therapy still remain due to host immune reactions, inefficient delivery systems, lack of sustained expression and so on (3).

In the past decades, the most commonly used gene carriers were viral vectors (e.g. adenoviruses and retroviruses) which showed high efficiency of gene delivery. However, the potential drawbacks of viral vectors are non-trivial, such as immunogenicity, insertional mutagenesis in the host genome, and limited DNA delivery capacity (4). Thus, more focus has been turned to non-viral vectors which might elicit much less immune responses or integration of DNA into the host genome. To date, many types of synthetic non-viral vectors have been developed for gene therapy, including: (i) cationic lipids; (ii) cationic polymers; (iii) dendrimers; (iv) gold nanoparticles; (v) magnetic nanoparticles; (vi) quantum dots; (vii) silica nanoparticles; (viii) fullerenes; (ix) carbon nanotubes; and (x) supramolecular systems (5).

Magnetic nanoparticles (MNPs) which can respond to magnetic field have garnered great attention in the past few decades and are being explored for gene delivery and gene therapy due to their unique features (6–8). First, with uniform and controllable size, ranging from 1 to 100 nm, MNPs are able to readily pass through cellular membranes for gene transfection. Second, with easily modifiable surface, various kinds of polymers and molecules can be physically or chemically combined with MNPs to decrease the side effects of gene

transfer, while maintaining the selectivity and efficiency of transfection. Third, superparamagnetism makes MNPs easily manipulated through magnetic field and the ferrimagnetic nanoparticles have been well investigated for magnetic field enhanced gene delivery, known as magnetofection (9,10). Fourth, signal of nearby protons can strengthen the contrast in magnetic resonance imaging (MRI) (11,12). The MNPs-based gene vectors can potentially be tracked by MRI to develop new gene therapeutic protocols and guidelines. This review briefly summarizes recent studies concerning MNPs-based gene vectors, focusing on preparation and surface modification of MNPs, as well as their potential role in gene therapy.

PREPARATION OF MAGNETIC NANOPARTICLES

The MNPs commonly used in MR imaging are iron oxides (Fe_3O_4, Fe_2O_3), manganese oxides ($MnFe_2O_4$, Mn_2O_3, MnO), gadolinium oxides (Gd_2O_3) and gadolinium-based agents (13). As the physicochemical properties of nanoparticles are strongly affected by the synthesis methods (12), numerous methodologies have been developed to synthesize shape-controlled, highly stable, and monodisperse magnetic nanoparticles. The most widely used synthesis methods include microemulsions, sol–gel syntheses, sonochemical reactions, hydrothermal reactions, hydrolysis and thermolysis of precursors, flow injection syntheses, and electrospray syntheses (14). In the subsequent sections we will systematically present the updated synthesis methods of the MNPs mentioned above.

Iron Oxide Nanoparticles

Among all the iron oxide nanoparticles, Fe_3O_4 NPs present the most interesting properties for biomedical applications due to their chemical/magnetic stability and low cytotoxicity (15). Co-precipitation method is one of the simplest, most classic, and oldest methods for the preparation of Fe_3O_4 NPs from aqueous Fe^{2+}/Fe^{3+} salt solutions(e.g. chlorides, sulfates and nitrates) by the addition of a base (e.g. ammonium or sodium hydroxide) under inert atmosphere at room temperature or at elevated (designated) temperature. Under oxidizing conditions, Fe_3O_4 NPs are easily oxidized to γ-Fe_2O_3. However, this method usually yields polydisperse nanoparticles and the products need to be modified with stabilizing agents, such as surfactants, inorganic molecules and polymers to obtain narrow particle size distribution.

Compared to the reaction in aqueous solution, the following rising route, the thermal decomposition method which involves the thermal decomposition of organometallic species (iron precursors) in high-boiling organic solvents in the presence of stabilizing agents, such as oleic acid and oleylamine, can yield more crystalline and monodisperse nanoparticles easily with different kinds of shapes. For example, Hyeon *et al*. synthesized approximately 13 nm γ-Fe_2O_3 nanoparticles from the oxidation of iron pentacarbonyl ($Fe(CO)_5$) in a solution of oleic acid, trimethylamine ($(CH_3)_3NO$) and octyl ether at 120°C (16). Another representative study which was reported by the Sun group was high temperature (about 300°C) decomposition of iron (III) acetylacetonate ($Fe(acac)_3$) in a mixture of phenyl ether/benzyl ether, alcohol, oleic acid and oleylamine (17,18). The resulting sizes of the Fe_3O_4 NPs could be tuned between 3 and 20 nm (Fig. 1). Other simple protocol developed by the Hyeon group revealed that the decomposition of iron-oleate complex in different solvents (e.g. 1-hexadecene, octyl ether, 1-octadecene, 1-eicosene, or trioctylamine) at 240–320°C also formed monodisperse Fe_3O_4 NPs with sizes ranging from 5 to 22 nm (19).

Although the thermal decomposition methods indeed can lead to highly uniform and monodisperse Fe_3O_4 NPs, the extreme hydrophobic surface prevent them from biomedical applications. In order to enhance the dispersibility of hydrophobic Fe_3O_4 NPs in water while maintaining their unique properties, one-pot synthesis and modification methods have been developed. One process reported by Lee *et al*. showed that the decomposition of $Fe(CO)_5$ in polyvinylpyrrolidone (PVP) surfactant and DMF solvent could yield water-soluble and uniform PVP-iron oxide nanoparticles (20). Also, decomposition of $Fe(acac)_3$ in a high boiling polar solvent like 2-pyrrolidone can lead to water-soluble magnetite nanocrystals of different sizes (21). In these processes, high boiling polar solvent acts as both a solvent and a hydrophilic coating material for the fabrication of water-soluble nanoparticles (22).

Manganese Oxide Nanoparticles

Manganese oxide nanoparticles (e.g. MnO, Mn_3O_4) have been regarded as one of the most promising T_1 MRI contrast agents since the Hyeon group first reported that MnO nanoparticles can be used as a long-awaited T_1 MRI contrast agent for various biological/physiological tissues and organs (23). Till now, many methods have been reported for the synthesis of manganese oxide nanoparticles (24–26). The most commonly used one is the thermal decomposition of oleate-Mn in certain high boiling point solvent (e.g. 1-octadecene, 1-hexadecene) (Fig. 2a) (19,27). The accurately controlled-MnO (7–35 nm) nanoparticles can be synthesized in large scale by using this method (Fig. 2b). Unlike the mechanism of normally used T_2 contrast agents, the r_1 relaxivity of manganese-based T_1 contrast agents is mainly determined by the direct interaction between the surface Mn^{2+} ion and the surrounding water protons. Thus, compared to solid manganese oxide nanoparticles, the small and hollow ones which have a larger surface to volume ratio may possess a larger r_1 relaxivity (28).

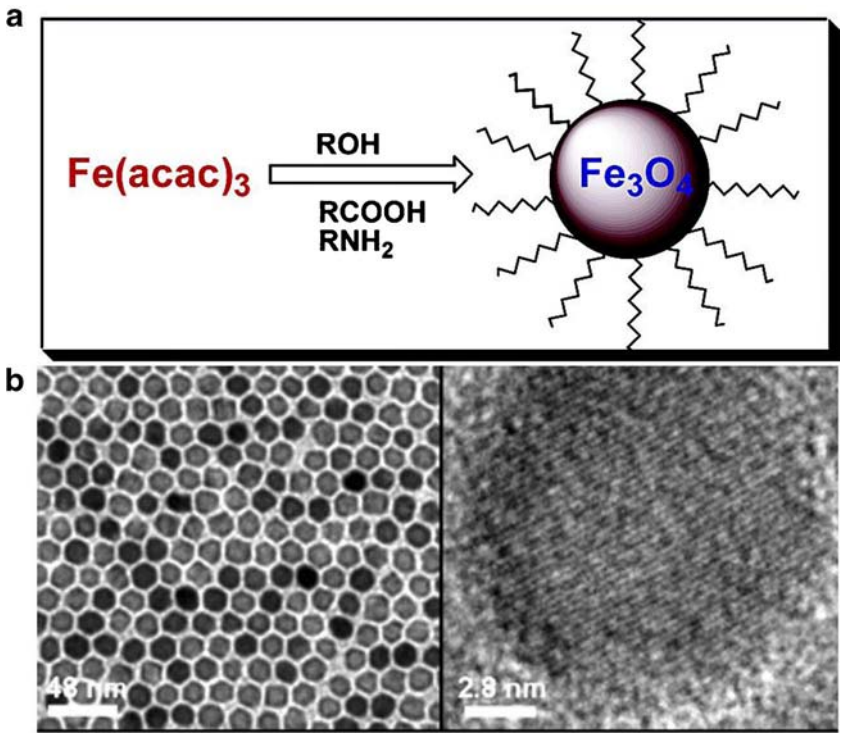

Fig. 1 (**a**) Schematic illustration of the synthesis of monodisperse Fe_3O_4 nanoparticles; (**b**) TEM bright field image of 16-nm Fe_3O_4 nanoparticles (*left*) and HRTEM image of a single Fe_3O_4 nanoparticle (*right*). Reproduced with permission (17). Copyright 2002 American Chemical Society.

Great efforts have been made to optimize synthesis approaches of smaller manganese oxide nanoparticles and hollow manganese oxide nanoparticles. Huang *et al*. utilized manganese stearate as the precursor to prepare 5 nm MnO/Mn_3O_4 nanoparticles (29). Later Baek *et al*. prepared monodispersed 2–3 nm MnO nanoparticles by a facile one-pot synthesis method in which the manganese chloride tetrahydrate is the related precursor (30). Moreover, the hollow structure of manganese oxide nanoparticles can also generate a higher r_1. As the Hyeon group reported that the MnO nanoparticles can be etched in technical grade trioctylphosphine oxide (TOPO) at 300°C to form hollow nanostructure (31). However, the drawback of the above method is the difficulty to remove the excess amount of TOPO for any biomedical applications. Many empirical studies have provided several routes to overcome the limitations. In the study by Shin *et al*. (32), the hollow particles were prepared by merely stirring the water-soluble solid manganese oxide nanoparticles in phthalate buffer solution at pH ~4.6. Very recently, Hao *et al*. obtained hollow manganese oxide (HMnO), hollow manganese phosphate (HMnP), and porous manganese phosphate (PMnP) NPs *via* directional ion transfer across different solid–liquid interfaces in a one-pot solvothermal process in which Mn(II) acetylacetonate ($Mn(acac)_2$), water and triethyl phosphate were used as reagents for the generation of ions (33) (Fig. 2c, d).

Compared to iron oxide nanoparticles, the magnetization of manganese oxide nanoparticles at physiologically relevant temperature is pretty low (5 emu/g) (34). However, Lee *et al*. recently reported that the manganese oxide nanoparticles can show a delayed increase in T_1 MRI signal intensity which is caused by cellular uptake *via* endocytosis into acidic compartments and the water-Mn^{2+} interaction (35). The delayed enhancement has obvious benefits for targeting MRI contrast to specific cells and receptors that are known to be recycled by endocytosis (32,35).

Gadolinium Oxide Nanoparticles and Complexes

Among all kinds of MRI contrast agents, the most frequently used clinical MRI contrast agents are gadolinium (Gd)-based contrast agents due to its highly paramagnetic properties of Gd^{3+} (36–38). Compared to other metal ions, Gd(III) possesses seven unpaired 4f electrons (8S7/2), generating a large electron magnetic moment that can efficiently induce the longitudinal relaxation of a water proton (39). The main commercialized Gd(III)-agents are Gd(III)-chelates (e.g. Gd-DTPA (Magnevist), Gd-DOTA (Dotarem), Gd-HP-DO3A (ProHance)), which have shown effective MRI signal intensity increase for disease diagnosis (40). Recently, more and more research demonstrated that gadolinium oxide (Gd_2O_3) nanoparticles which not only have higher r_1 relaxivity values than Gd^{3+}-chelates of certain sizes, but also possess functional surface for the targeted biomolecules combination, are much more popular in current research of developing T_1 MRI contrast agents. Various methods have been developed for the synthesis of Gd_2O_3 nanoparticles. Engstrom *et al.* demonstrated that the reaction of $GdCl_3$ and NaOH at elevated temperatures could yield gadolinium oxide nanoparticles with particle diameters in the range of 2–15 nm (41). The combustion of $Gd(NO_3)_3$ and amino acid glycine

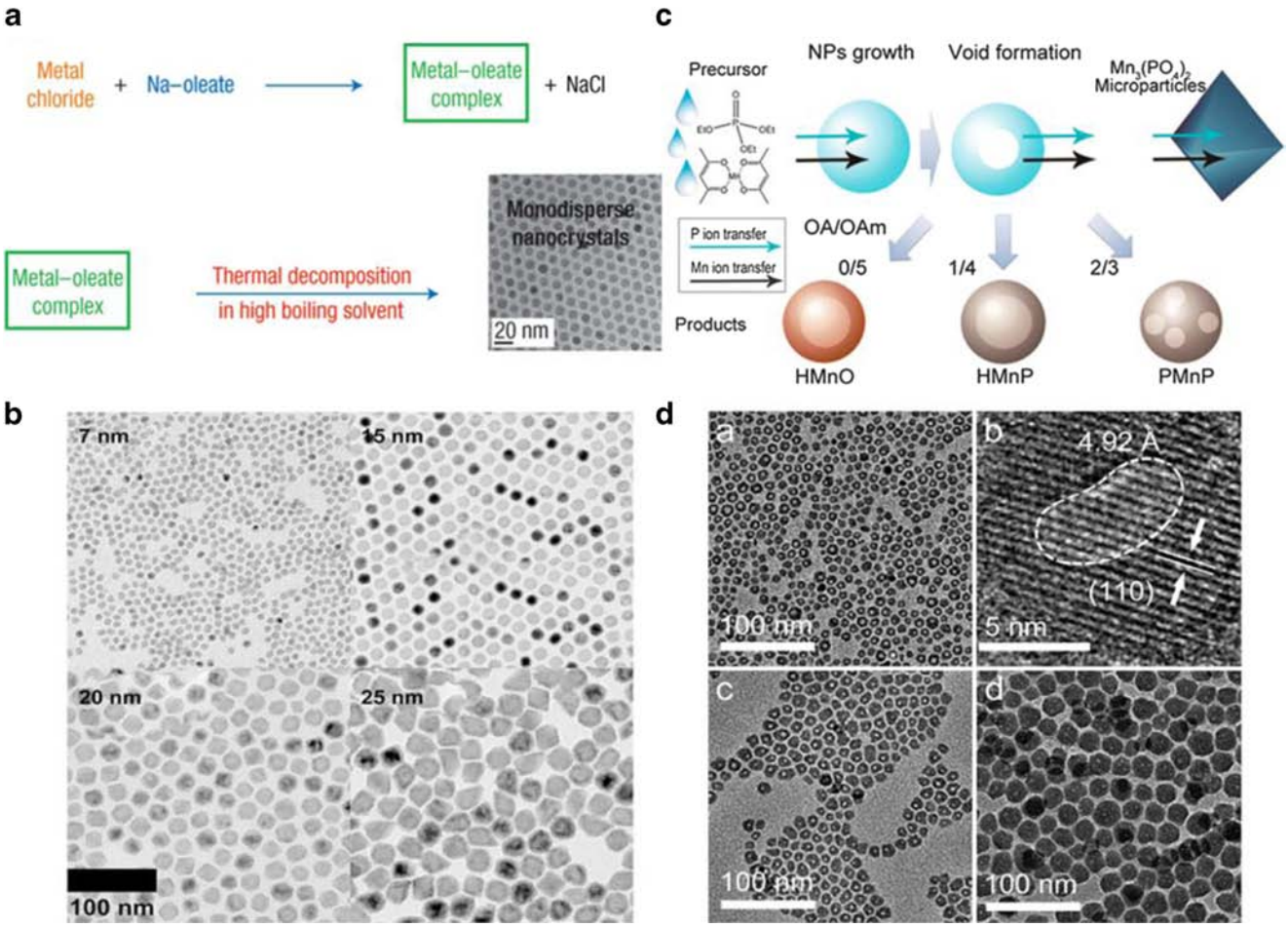

Fig. 2 (**a**) The scheme for the synthesis of monodisperse nanocrystals by using Metal-oleate complex; (**b**) TEM images of MnO nanoparticles with particle sizes of 7, 15, 20, and 25 nm. Reproduced with permission (19). Copyright 2004 Nature Publishing Group. (**c**) Schematic illustration of the synthesis of hollow and porous Manganese based nanoparticles by following Hou's method; (**d**) TEM images of the as-synthesized hollow nanoparticles: (**a**) 10 nm hollow manganese oxide; (**b**) single nanoparticle of 10 nm hollow manganese oxide; (**c**) 10 nm hollow manganese phosphate; (**d**) 25 nm porous manganese phosphate. Reproduced with permission (33). Copyright 2011 Royal Society of Chemistry.

successfully produced 10 nm uniformed Gd_2O_3 nanoparticles (42). Miyawaki *et al.* also reported that an average size of 2.3 nm nanoparticles could be synthesized by decomposing Gd(III) acetates encapsulated in single-walled carbon nanotubes (43). The ultra-small Gd_2O_3 nanoparticles with particle size of only 1 nm were prepared by using three different Gd(III) ion precursors and by refluxing each of them in tripropylene glycol under an O_2 flow (Fig. 3) (28).

Another strategy of Gd-based T_1 MRI contrast agents is to absorb Gd^{3+} into other nanocarriers, such as liposomes or polymer based nanoparticles, to enhance the T_1 MRI signal intensity. Trubetskoy *et al*. reported that Gd-diethylenetriaminepentaacetic-phosphatidylethanolamine (Gd-DTPA-PE) , an amphiphilic paramagnetic label modified liposomes, showed high T_1 enhancement of lymph node signal (44). By following the similar method, Wisner *et al*. prepared a prototypic gadolinium-chelated lipid *via* mixing lipidic N,N′-dimethylethylenediamine derivative containing a 10,12-diyne-diacyl domain, DTPA anhydride and $GdCl_3$ complexation (45). Another example of Gd^{3+}-based lipid was reported by Leclercq *et al*. which showed that the MCO-I-68 could be incorporated with DTPA-gadolinium to largely increase signal of MRI (46). Bhakta *et al*. synthesized Gd_2O_3-doped silica nanoparticles by using microemulsion method and $Gd(NO_3)_3$ was introduced as the precursor of Gd_2O_3 (47). The gold nanoparticles (AuNPs) coated with dithiolated diethylenetriamine pentaacetic acid (DTDTPA)-gadolinium (Gd(III)) chelate for MRI/computed tomography (CT) dual-imaging were also reported recently (48,49).

SURFACE MODIFICATION OF MAGNETIC NANOPARTICLES FOR GENE DELIVERY

As mentioned previously, the MNPs are usually coated with hydrophobic surfactants. To make these MNPs biocompatible for biological applications, the particles are usually modified with water-soluble molecules which can be attached to the

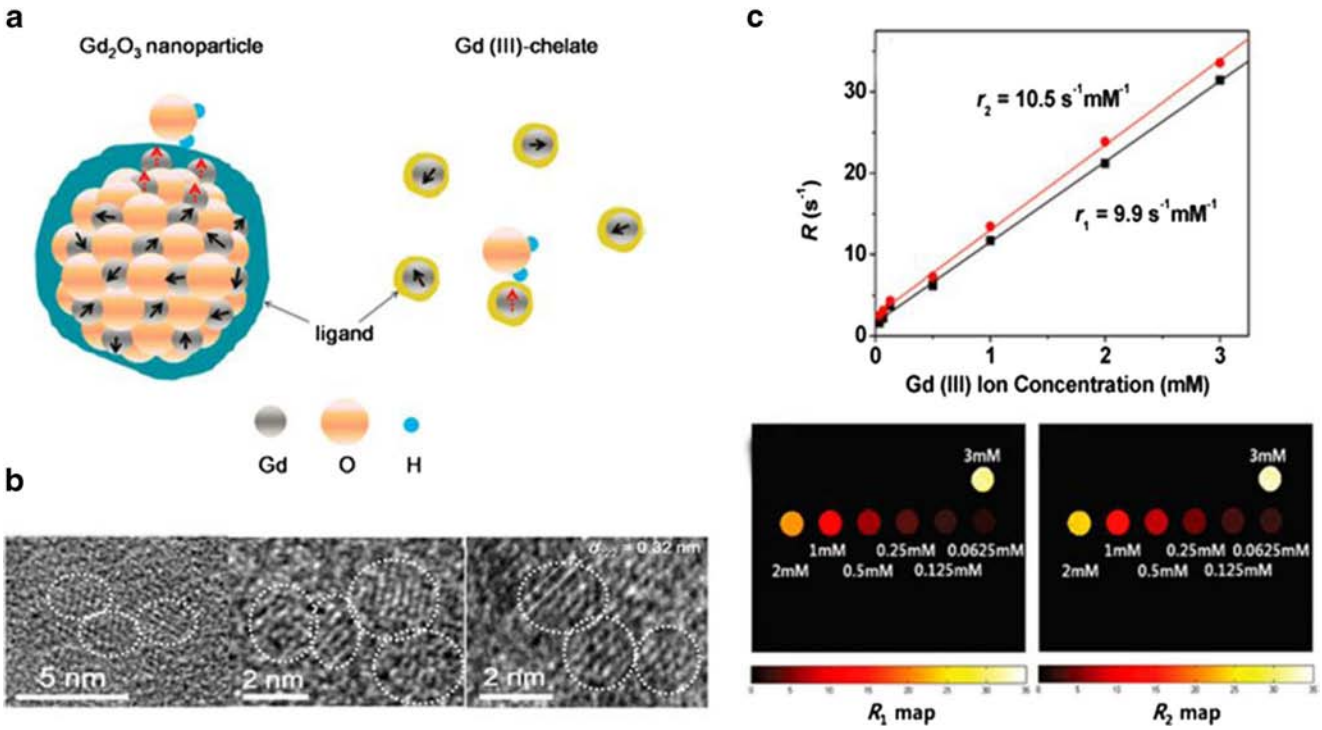

Fig. 3 (**a**) Schematic diagram showing that compared to individual Gd(III)-chelates, four surface Gd(III) ions cooperatively induce the longitudinal relaxation of the water proton in gadolinium oxide nanoparticles; (**b**) HRTEM images of ultrasmall gadolinium oxide nanoparticles synthesized from gadolinium chloride hydrate (*left*), gadolinium acetate hydrate (*middle*), and gadolinium acetylacetonate hydrate (*right*) by using Gd(III) ion as the precursors; (**c**) The r_1, r_2 relaxivity, R_1 and R_2 map images of the as-synthesized ultrasmall gadolinium oxide nanoparticles (28). Copyright 2002 American Chemical Society.

surface of the particles in a number of ways, for example, by employing cleavable linkers or by utilizing electrostatic interactions between the particle surface and the coating materials. For the application of MNPs in gene delivery, one choice of surface property is strongly positive charge to bind negatively charged nucleic acid molecules *via* electrostatic interactions and release them after being internalized into cells (50). To achieve this, cationic lipids, polymers, and dendrimers have been used frequently as MNPs coating materials for gene delivery.

Cationic Polymers

For gene therapy, cationic polymers which possess a net positive surface charge is an ideal type of vectors for therapeutic genes with negative charges since these two parts can generally form small complexes *via* electrostatic interactions (51). Currently several important cationic polymers are being used as vectors for gene delivery which include poly(ethylenimine) (PEI), poly(L-lysine) (PLL), and chitosan. Among these, PEI, a polymer of ethylenimine as polycation in aqueous solutions (pH <7.4) has emerged as a favorable candidate for *in vitro* and *in vivo* gene delivery due to its high efficiency and low cost (52–54). The high charge density of primary, secondary and tertiary amines of PEI allows DNA binding and protects them from nuclease degradation (55). It has been reported that the transfection efficiency is mainly related to the molecular weight (MW) of PEI, and higher MW PEI (e.g., MW >10 kDa) possesses much higher transfection efficiency due to high-density of positive charges. However, high MW PEI can induce higher cytotoxicity compared to low MW PEI (56).

To increase the transfection efficiency of low MW PEI, various protocols have been developed. One approach is to increase the positive charge density of low MW PEI. Choi *et al*. reported that cross-linked low MW PEI (1.8 kDa) *via* reversible disulfide bonds can have higher gene transfection efficiency compared with the high MW PEI (25 kDa) (57). Studies of Forrest and Thomas also revealed that cross-linked low MW PEI (0.4–2 kDa) could deliver DNA as efficiently as high MW PEI (25 kDa) to mammalian cells both *in vitro* and *in vivo* (58,59). Branched PEI (bPEI, 25 kDa) is the most commonly used form of PEI. A good number of studies have shown that PEI 25k can be modified onto Fe_3O_4 nanoparticles for *in vitro* gene delivery (9,60,61). However, according to the previous studies, PEI 25k has shown certain cytotoxic effects (e.g. cell death, apoptosis, inhibition of cell differentiation) (62). We demonstrated that low MW alkyl-polycation PEI 2k can be used for encapsulation of multiple hydrophobic superparamagnetic iron oxide (SPIO) nanoparticles. The resulting PEI 2k/SPIO nanocomposites possess higher siRNA transfection efficiency *in vitro* and *in vivo* and much lower cytotoxicity (63,64). The PEI modified Fe_3O_4

nanoparticles not only can absorb various kinds of genes, but also can be traced by T_2 MRI. However, SPIO based T_2 negative contrast agents are suboptimal for imaging when hemorrhages in tissues are present, such as in hemorrhagic stroke, tumors, or cell injection locations (65,66). Thus, the Park group reported PEI 25k coated hollow MONPs for efficient vascular endothelial growth factor (VEGF) siRNA delivery (67). Recently, we also synthesized low MW amphiphilic Alkyl-PEI2k/MONPs (Alkyl-PEI2k-MnOs) nanoclusters for efficient delivery of firefly luciferase (fluc) siRNA into 4T1-fluc cells.

Current research showed that the synchronization of gene expression and cell trafficking in transfected stem cells is crucial for augmentation of stem cell functions and real time *in vivo* monitoring. Yang *et al*. prepared a magnetic nanovector system which is PEI-coated $MnFe_2O_4$ nanoparticles. They demonstrated that the as synthesized nanocarriers which are highly stable and soluble in biological conditions can serve as an individual gene carrier, enabling easy cell trafficking, high gene loading capacity and high gene transfection efficiency in human mesenchymal stem cells (hMSCs) (68). Studies have also shown that MNPs based gene delivery carriers can dramatically improve the efficiency of gene delivery *in vivo* due to the utilizing of magnetic fields. Li *et al*. reported a different strategy which combined DNA with biotinylated high MW PEI, then conjugated the PEI/DNA complexes onto streptavidin coated magnetic nanobeads. After intravenous injection into mice, this type of MNPs carrier can be attracted to the left lung and the heart by an external magnet. The results showed that this formulation can effectively deliver genes (e.g. antiapoptotic gene Bcl-2, proangiogenic VEGF gene and antisense oligonucleotide aptamers) to the heart and tumor areas *in vivo* and showed high transfection efficiency (Fig. 4) (69).

Cationic Lipids

Lipid-based nanostructures are widely used in biological, pharmaceutical, and medical research, among those liposome is one of the most commonly used one. Liposome-mediated gene transfer, first reported by Felgner in 1987, is still one of the major strategies for delivery of genetic materials into host cells. It has been reported that various kinds of genes (exogenous globin mRNA, chromosomes, and DNA) (70–72) can be delivered into host cells due to the fusogenic ability of liposomes with cell membranes. The well-known mechanism of gene-lipid binding is the charge interaction, and the high positive surface charge of lipids allows them to combine with DNA *via* electrostatic interactions (73). Another effective strategy is to use the redox property of the thiol group to form gene-lipid complex (74). Compared to other transfection agents, lipid-based agents showed low toxicity, weak immunogenicity and relatively easy preparation.

Different strategies to increase the transfection efficiency of liposome have been studied which mainly focused on the modification of surface proteins or a combination of targeted ligands such as antibodies, transferrin, and lactose (75). Recently, lipid coated MNPs for enhanced gene delivery has been reported (76–81). One possible strategy is to utilize different cationic lipids coupled onto MNPs *via* charge interactions. For example, Hirao *et al*. developed magnetic cationic liposomes by incorporating magnetite particles into small cationic liposomes for pDNA delivery (82). This gene delivery system was able to achieve enhanced transfection efficiency in human osteosarcoma cells by magnetic induction. Yang *et al*. also reported that liposome bound Fe_3O_4 nanoparticles can transfect *Lac Z* gene and enhance green fluorescence protein gene (EGFP) more effectively than commercialized Lipofectamine™ 2000 and liposomes without MNPs (80). The Nantz group recently reported a click chemistry method which is a flexible oximation approach to attach lipid onto functionalized iron oxide nanoparticles. They demonstrated that by reacting an ammonium ion-based oxime ether lipid with iron oxide nanoparticles, they can generate a highly active, non-cytotoxic, high transfection vector *in vitro* and *in vivo* (83). All the results above showed that by incorporating MNPs, the liposome-MNPs gene vectors can enhance the transfection efficiency *in vitro* and *in vivo* and be tracked by MRI at the same time (84).

Generally, the preparation of classical lipid-coated magnetic nanocrystals is time-consuming, since liposomes need to be prepared separately and the liposome-MNPs always require tedious washing process to remove the uncoated MNPs. To overcome this problem, Namiki *et al.* reported a novel formulation of magnetic nanocrystals termed 'LipoMag'. The 'LipoMag', which was assembled as oleic acid-coated magnetite nanocrystal cores with cationic lipid shells, can be used as an efficient magnetic vector for delivering siRNA into tumor sites and tumor cells in mice (Fig. 5) (85). Compared with other formulas (e.g. PolyMag, which is commercially available polymer-coated magnetic nanocrystals), the LipoMag appears to possess better transfection efficiency *in vitro* and *in vivo* (85). Another interesting study by Holzbach *et al*. demonstrated that perfluoropropane-filled 'magnetobubbles' prepared by combination of MNPs, cationic lipid and DNA showed high transfection efficiency of VEGF to the ischemic skin flaps in rats *in vivo* (86). Oliver *et al*. even fabricated a complicated structure, MAGfect, a liposome formulation containing a lipidic gadolinium (Gd-DOTA-Chol 1) contrast agent for MRI (87). This kind of liposome-gadolinium which was demonstrated as an effective vehicle for transport of plasmid DNA into cells, showed potential for molecular imaging of gene therapy.

Dendrimer Coating

Dendrimers, which are multivalent macromolecules based on a well-defined cascade motif with spherical shapes, defect-free and perfectly monodisperse characteristics, have been developed and

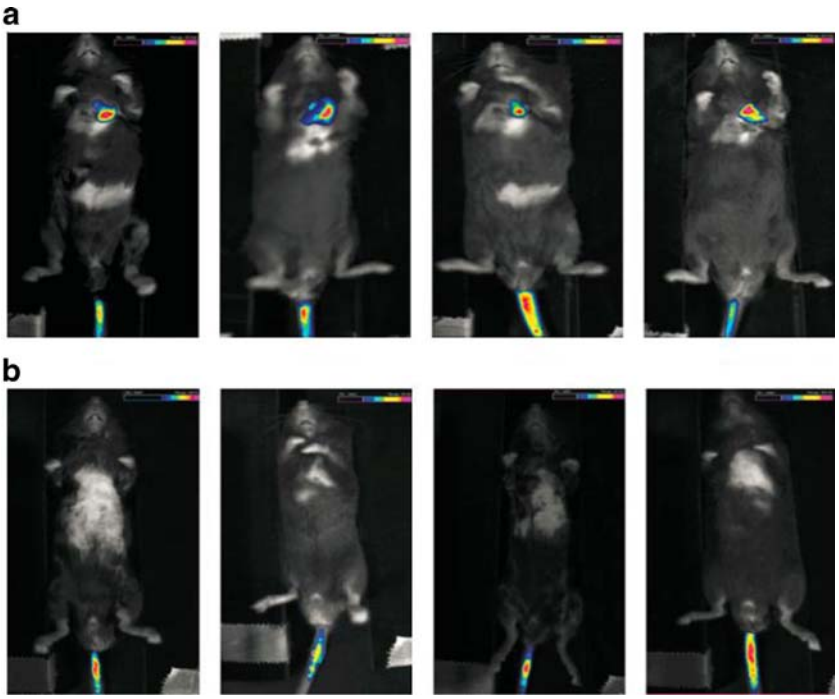

Fig. 4 Non-invasive *in vivo* trafficking of MNB/PEI/DNA complexes 2 h after systemic administration. (**a**) Mag^{+} group and (**b**) Mag^{-} group. Reproduced with permission (69). Copyright 2008 Wiley.

utilized in chemistry, pharmaceutics, and gene/drug delivery (88,89). For gene delivery, dendrimers-based vectors are attracting more and more attention due to its defined structures, inner cavities, and more controllable inner or outer surface (90,91). Among all the available dendrimers, the mostly studied one for gene delivery is polyamidoamine (PAMAM) dendrimer (92). PAMAM dendrimer is a class of highly branched macromolecules with well-defined structure and composition. The terminal primary amines of PAMAM dendrimer can be easily protonated, and the positive charges can interact with the negative phosphate groups of nucleic acid for high-efficiency gene delivery by electrostatic interactions (93,94).

However, the drawbacks of PAMAM dendrimers, such as low water solubility and certain cytotoxicity still need to be overcome for *in vivo* applications (95). Many enhanced transfection efficiency strategies have been reported (96). One common strategy is to link poly(ethylene glycol) (PEG) with PAMAM dendrimer since PEG is more biocompatible and non-immunogenic (97,98). Jevprasesphant *et al.* found that the surface of PAMAM modified with six lauroyl or four PEG chains could decrease the cytotoxicity remarkably due to the shielding effect (95). Another strategy is conjugation with suitable biomolecules, such as proteins and positively charged amino acids. Kim *et al*. reported that di-arginine-conjugated PAMAM (G3 or G4) dendrimer through amide bonds could improve complex stability, intra-nuclear localization and transfection efficiency (99). Histidine-conjugated PAMAM G4 dendrimer through amide bonds could enhance transfection efficiency in 10% serum and reduce cytotoxicity in Bel 7402 or Hela cells (100).

Jeong *et al*. reported that enhancement of the delivered gene activity in cells is also important for efficient gene delivery, and one useful strategy is to use rapid biodegradable polymers (101). Nam *et al*. reported e-PAM-R G2, 3 and 4 which are biodegradable PAMAM dendrimers as gene carriers for pDNA delivery showed high transfection efficiency due to rapid olidonucleotide release (102). As mentioned above, MNPs-based gene vectors are powerful since MRI is very useful in gene tracking and magnetic guiding is effective in increasing transfection efficiency. Dendrimer coating is also an alternative choice to modify MNPs as a gene carrier. Pan *et al*. prepared dendrimer-modified MNPs (dMNP) for *survivin* gene which showed quick accumulation into cells and high transfection efficiency (103). Liu *et al*. reported PAMAM dendrimer modified magnetic iron oxide nanoparticle/DNA/PEI 25k ternary complexes used for the magnetofection of mammalian cells. The results indicated that the transfection efficiency of COS 7 cells with ternary magnetoplexes was significantly increased when a magnetic field was applied, especially in the solution of 10% serum (104). Another interesting study conducted by Parker-Esquivel *et al*. showed that PAMAM coated MnO nanorods can effectively deliver macromolecular RNAs and minimize negative impacts on metabolic activity (105).

Multiple Polymer Strategy

Multiple polymer strategy which mainly focuses on the combination of different kinds of coating materials and nanoparticles has been applied to overcome the limitations of individual

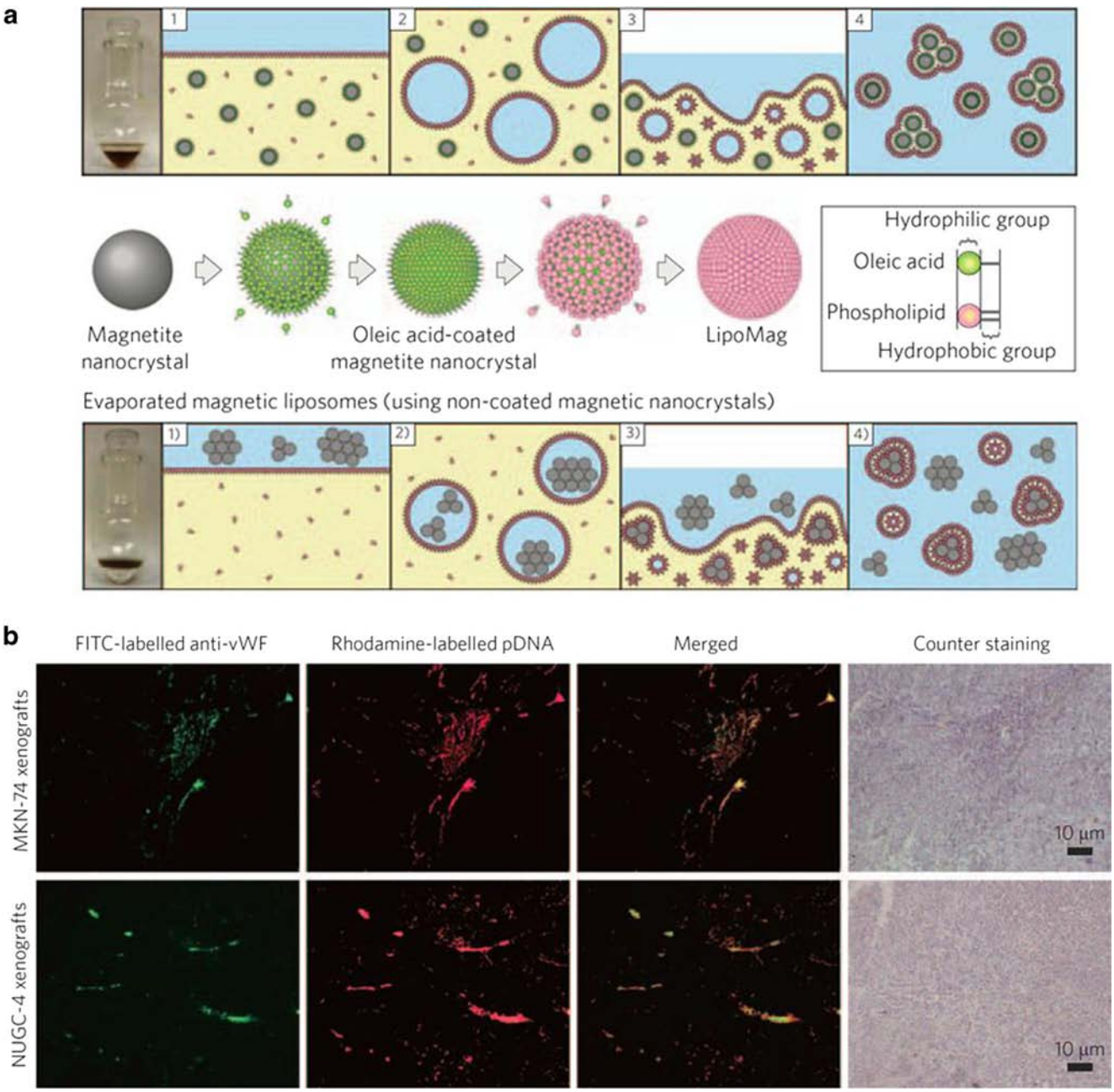

Fig. 5 (**a**) Schematic illustration of the preparation of LipoMag complex. (**b**) LipoMag-mediated delivery of nucleic acid to tumour lesions. pDNA distribution in xenografts after intravenous administration of fluorescently labeled LipoMag-mediated pDNA complex (85). Copyright 2009 Nature Publishing Group.

polymer molecules or polymer-coated nanoparticles based formulas. For example, grafting low MW PEI to proper polymers is one kind of method to manipulate low MW PEI as an efficient gene carrier. Wang *et al*. conjugated PEI to poly (5-methyl-5-allyloxycarbonyl-trimethylene carbonate) and produced a biodegradable polycation, which could enhance transfection efficiency. Namgung *et al*. grafted PEI 2.5k to multiarm PEG to form a complex, which showed significant improvement of transfection efficiency in human cervical adenocarcinoma epithelial cells (106).

In addition to the mentioned methods, another promising approach is to combine PEI with PAMAM. Zhao *et al.* reported that a starburst low MW PEI gene vector which contains a PAMAM core enclosed with a shell composed of PEI and PEG (PAMAM-PEI-PEG) could effectively condense DNA and improve the transfection efficiency (107). Patil *et al*. prepared a novel triblock nanocarrier, PAMAM-PEG-PLL, which combined individual features of PAMAM dendrimer, PEG, and PLL, were effectively taken up by cancer cells and induced the knock down of the target Bcl-2 gene (108).

Besides the water-soluble polycationic polymers, self-assembled cationic nanoparticles from amphiphilic cationic copolymers have also been applied for gene delivery (109,110). Sun *et al*. prepared an amphiphilic cationic triblock copolymer consisting of monomethoxy PEG, poly (3-caprolactone) (PCL) and poly (2-aminoethyl ethylene phosphate) denoted as mPEG45-b-PCL100-b-PPEEA12. They demonstrated that the synthesized nanoparticles can be used for efficient siRNA delivery (111). The multiple polymers can be coated onto the surface of MNPs for gene delivery. Kievit *et al.* prepared NP-CP-PEI nanoparticle system which is comprised of short-chain PEI and PEG grafted to the natural polysaccharide, chitosan (CP) and SPIO nanoparticles. They demonstrated that this kind of system showed effective gene transfection both *in vitro* and *in vivo* compared to SPIO-PEI, SPIO-CP and commercially available transfection agents (112). Very recently, the authors improved the SPIO-CP-PEI nano system to target deliver genes to C6 glioma cells in a xenograft model by using chlorotoxin (CTX) modification for the SPIO-CP-PEI nanoparticles (113,114). The results showed that the targeted delivery system can promote specific uptake of nanovectors into glioma cells, exposing a higher transfection of genes into target cells.

For Gd-based gene vectors, packaging of Gd^{3+} contrast agents into the multiple polymers is a newly developed strategy for preparing the MNPs-based gene vectors for MRI tracking. Kamaly *et al*. showed the synthesis of a novel gadolinium lipid, Gd.DOTA.DSA, designed for liposomal cell labeling and tumor imaging. Liposome formulations consisting of this lipid were optimized in order to achieve maximum cellular entry, and to label HeLa cells *in vitro* (115). Recent studies have shown that the mesoporous silica nanoparticles (MSNs) can be easily taken up by mammalian cells and showed non-toxicity and good biocompatibility (116,117). Li *et al*. successfully packaged siRNA into the mesopores of magnetic mesoporous silica nanoparticles (M-MSNs), and mixed with PEI to form a polymer layer (M-MSN_siRNA@PEI). The obtained delivery vehicles can lead to both exogenous EGFP gene knockdown and endogenous B-cell lymphoma 2 (Bcl-2) gene knockdown *in vitro* (118).

To date, "all-in-one" nanoparticle probes for simultaneous delivery and multimodal imaging have become a promising strategy for gene and drug delivery. The MNPs-based multifunctional nanoprobe for gene delivery usually consists of a magnetic core for MRI, highly positive charge polymers for gene binding, targeted molecules, and other molecules for optical imaging or PET imaging. Lee *et al.* reported a multifunctional "all-in-one" magnetic nanoprobe comprising a magnetic core(manganese-doped magnetism-engineered iron oxide (MnMEIO)), a cell-specific targeting moiety(RGD), a fluorescent dye(Cy5), and therapeutic siRNA(siGFP) in one system. The nanosystem can be delivered into target cells *via* endocytosis and show excellent siGFP delivery efficiency (119) (Fig. 6). In another representative study, Kumar *et al*. described a multifunctional gene nanovector, including MN-EPPT-siBIRC5 that consists of SPIO (for MRI), the dye Cy5.5 (for near-infrared optical imaging), peptides (EPPT) that specifically targets uMUC-1, and a synthetic siRNA that targets the tumor specific anti-apoptotic gene BIRC5. This formula permits the simultaneous tumor specific delivery of

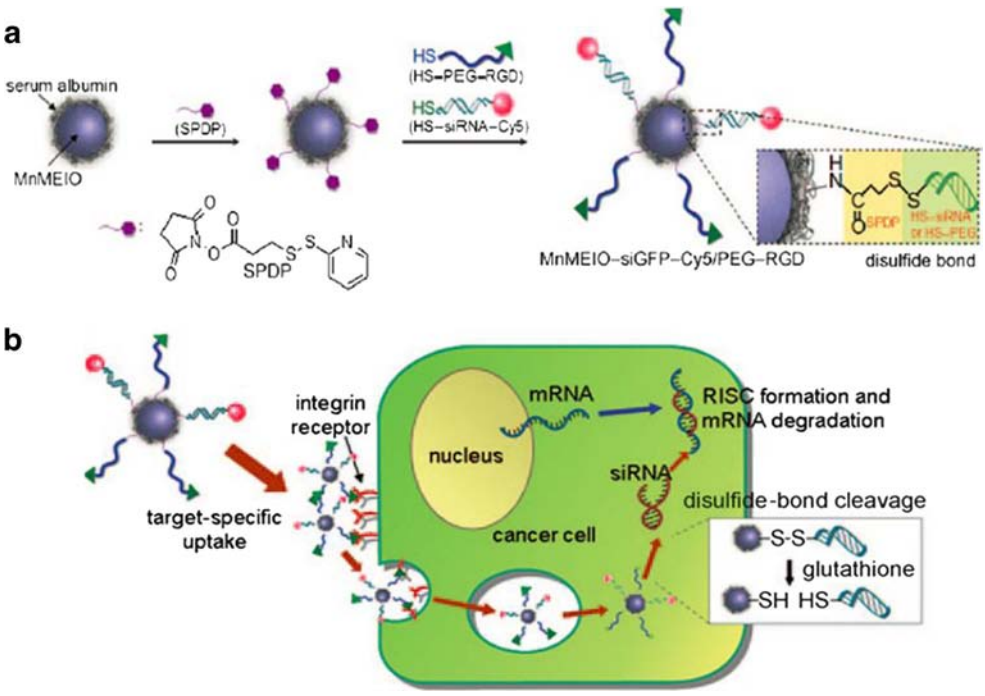

Fig. 6 Fabrication of "all-in-one" nanoprobe. (**a**) Synthetic scheme for MnMEIO-siGFP-Cy5/PEG-RGD multifunctional nanoprobe. (**b**) Schematic illustration of intracellular processes for gene therapy (119). Copyright 2009 Wiley.

siRNA to tumors and the imaging of the delivery process (120), showing the functional MNP to be promising theranostics for future gene therapy applications.

CONCLUSIONS AND PERSPECTIVES

There has been tremendous progress in the design and synthesis of MNPs-based non-viral transfection agents for gene delivery in the past few decades. Various kinds of polymers, lipids, and dendrimers have been developed to prepare MNPs with accurate size, shape, composition, magnetization, relaxivity and surface charge control. These capabilities have greatly expanded the applications of MNPs as imaging and transfection agents. Since many MNPs have been used in clinical settings for many years, there is a high potential that these functional targeted MNPs will be applicable in clinical gene therapy in the future.

Typically, for effective *in vivo* gene therapy, the MNPs-based vectors usually possess long circulation time, efficient delivery of target genes into cells of interest and further transport into the cell nucleus. MNPs-based gene vectors are particularly useful for molecular imaging of cell/gene delivery due to their unique physicochemical properties. That means the functional nanovectors not only can load and deliver genes effectively, but also can be tracked by MRI. With advances in nanotechnology, MNPs-based gene delivery systems deserve more research efforts as they can be integrated for noninvasive and quantitative imaging and targeted gene therapy within one entity.

The most important issue that needs to be addressed is *in vivo* application of MNPs for targeted gene delivery. Methods to increase the loading capacity of therapeutic gene in the MNPs and control their release at target cells also remain quite challenging: 1) functional surface modification during conjugation may change MNPs chemical properties, 2) quick release of therapeutic gene from MNPs in the blood before entering into target sites, 3) various physiological barriers preventing the nanovectors from reaching the targeted cells. It is necessary to maximize the interaction of the MNPs formulation with the target tissues/cells and to eliminate or minimize the uptake by other organs.

In summary, the ultimate goal is that functional MNPs allow for efficient, specific *in vivo* delivery of genes without systemic toxicity, and the gene delivered as well as the therapeutic efficacy can be accurately measured noninvasively and spatiotemporally. Thus, future work will be focused on the development of multifunctional MNPs-vectors in order to obtain an efficient and nontoxic transfection method for gene delivery *in vitro* and *in vivo* which also can be used for tracking and disease diagnosis. To reach this, the materials which need to be used for the synthesis of MNPs-based gene vectors should be safe, biocompatible and degradable in the organism. Furthermore, targeted MNPs-vectors and their mechanism should be characterized in detail in order to bring them to the clinical translation.

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