Chapter 12

Superparamagnetic Nanoparticle Delivery of DNA Vaccine

Fatin Nawwab Al-Deen, Cordelia Selomulya, Charles Ma, and Ross L. Coppel

Abstract

The efficiency of delivery of DNA vaccines is often relatively low compared to protein vaccines. The use of superparamagnetic iron oxide nanoparticles (SPIONs) to deliver genes via magnetofection shows promise in improving the efficiency of gene delivery both in vitro and in vivo. In particular, the duration for gene transfection especially for in vitro application can be significantly reduced by magnetofection compared to the time required to achieve high gene transfection with standard protocols. SPIONs that have been rendered stable in physiological conditions can be used as both therapeutic and diagnostic agents due to their unique magnetic characteristics. Valuable features of iron oxide nanoparticles in bioapplications include a tight control over their size distribution, magnetic properties of these particles, and the ability to carry particular biomolecules to specific targets. The internalization and half-life of the particles within the body depend upon the method of synthesis. Numerous synthesis methods have been used to produce magnetic nanoparticles for bioapplications with different sizes and surface charges. The most common method for synthesizing nanometer-sized magnetite Fe_3O_4 particles in solution is by chemical coprecipitation of iron salts. The coprecipitation method is an effective technique for preparing a stable aqueous dispersions of iron oxide nanoparticles. We describe the production of Fe_3O_4-based SPIONs with high magnetization values (70 emu/g) under 15 kOe of the applied magnetic field at room temperature, with 0.01 emu/g remanence via a coprecipitation method in the presence of trisodium citrate as a stabilizer. Naked SPIONs often lack sufficient stability, hydrophilicity, and the capacity to be functionalized. In order to overcome these limitations, polycationic polymer was anchored on the surface of freshly prepared SPIONs by a direct electrostatic attraction between the negatively charged SPIONs (due to the presence of carboxylic groups) and the positively charged polymer. Polyethylenimine was chosen to modify the surface of SPIONs to assist the delivery of plasmid DNA into mammalian cells due to the polymer's extensive buffering capacity through the "proton sponge" effect.

Key words Superparamagnetic iron oxide nanoparticles, SPION, Polyethylenimine, PEI, DNA vaccine, Magnetofection

1 Introduction

Superparamagnetic iron oxide nanoparticles (SPIONs) have attracted significant attention in gene delivery applications because of their relatively low toxicity, low cost of production,

Monica Rinaldi et al. (eds.), *DNA Vaccines: Methods and Protocols*, Methods in Molecular Biology, vol. 1143,
DOI 10.1007/978-1-4939-0410-5_12,

ability to immobilize biological materials on their surfaces, and potential for direct targeting using external magnets. Magnetic particle-assisted gene delivery, also known as magnetic transfection or magnetofection, has been shown to improve both the efficiency of gene delivery and the rapidity of uptake in different tissues in vitro [1]. Magnetofection originated from the concept of magnetic drug delivery in the late 1970s, with the technique demonstrating applicability to gene delivery with viral and non-viral vectors [2]. Magnetic particles appear to be generally useable with any gene delivery vector, and the duration of the transfection process can be significantly reduced down to 10 min, compared to 4-h incubation usual with standard protocols [2]. Magnetofection is an appropriate tool for rapid and specific gene transfection needing only low doses in vitro and allowing site-specific in vivo applications [3, 4].

In biotechnology, the critical characteristics of magnetic nanoparticles are their nanoscale dimensions, magnetic properties, and ability to bind particular biomolecules and deliver them to specific targets. Studies performed over the last decade have used several types of iron oxides, among them maghemite, γ-Fe_2O_3, or magnetite, Fe_3O_4, which consist of a single domain of about 5–20 nm in diameter [5, 6]. Magnetite, Fe_3O_4, is the most common magnetic iron oxide candidate because its biocompatibility in biological systems has already been proved [7]. This form of iron oxide is stable in water or physiological saline under neutral pH conditions. It has a large surface area that can be modified to attach biological agents [8]. Nanoparticles of this composition with suitable surface coating materials can disperse widely in suitable solvents to produce a homogenous suspension called ferrofluid that permits further biochemical functionalization. Numerous synthesis methods have been used to produce magnetic nanoparticles for bioapplications including coprecipitation, microemulsions, polyols, sol–gel synthesis, sonochemical synthesis, hydrothermal, hydrolysis, thermolysis of organic precursors, flow injection, and electrospray [5, 9]. These methods have been used to prepare magnetic particles with homogeneous composition and narrow size distribution. However, the most common method for synthesizing magnetite particles in solution within the nanometer range is chemical coprecipitation of iron salts. The technique is probably the simplest and most efficient wet chemical route to obtain magnetic particles for biomedical applications [10].

The stabilization of iron oxide nanoparticles is an important feature in obtaining ferrofluid colloids that do not aggregate in both biological media and magnetic field. The hydrophobic surface of magnetic particles means that in the absence of coating

materials, these particles tend to interact with each other to form large clusters, resulting in the increase of aggregate size [10]. Coating layers not only provide stability to nanoparticles in solution but also help to bind various biological ligands to the particle surface for various biomedical applications. Various materials have been used as protective coatings for magnetic nanoparticles.

Polyethyleneimine (PEI) is one of the most efficient cationic compounds for delivery of plasmid DNA into mammalian cells due to its extensive buffering capacity through the "proton sponge" effects [11, 12]. PEI polymer is known to form cationic complexes with SPIONs that then interact nonspecifically with negatively charged DNA and enter the cell via endocytosis [13]. In contrast to other cationic polymers, PEI has high transfection efficiencies even in the absence of endosomolytic agents such as fusogenic peptides or chloroquine which facilitates cellular uptake [14]. Many types of linkages have been used to couple magnetic nanoparticles to nucleic acids, and the simplest one is a physical method based on electrostatic interaction between positively charged magnetic particles with a cationic polymer coating layer and negatively charged nucleic acids. Different factors have been examined for their effects on magnetic gene complex preparation such as molecular weight and different structures (branched and linear structure) of PEI as well as charge density and charge-to-mass ratio of the polymer and DNA molecules [13]. For instance, our previous work showed that SPIONs/branched PEI complexes at pH 4.0 showed a better binding capability for DNA than at a neutral pH, despite negligible differences in the size and surface charge of the complexes [15]. This finding might be a result of protonation and mutual charge repulsion between PEI amine groups in acidic conditions, expanding the polymeric network to increase the amount of entrapped genetic material and consequently increasing gene expression upon injection. In contrast, the stiff stable structure of the polymer's six-membered rings under neutral conditions would decrease the particle's ability to entrap more DNA molecules, subsequently decreasing DNA dosage (*see* Fig. 1) [15].

In this chapter, we describe a coprecipitation method to produce SPIONs. This method is exceptionally useful as it is able to produce magnetic particles of a specific size within the nanometer range and with good magnetic properties. The produced particles can then be coated with PEI polymers as an example of useful cationic polymers that can form complexes with DNA molecules for gene delivery.

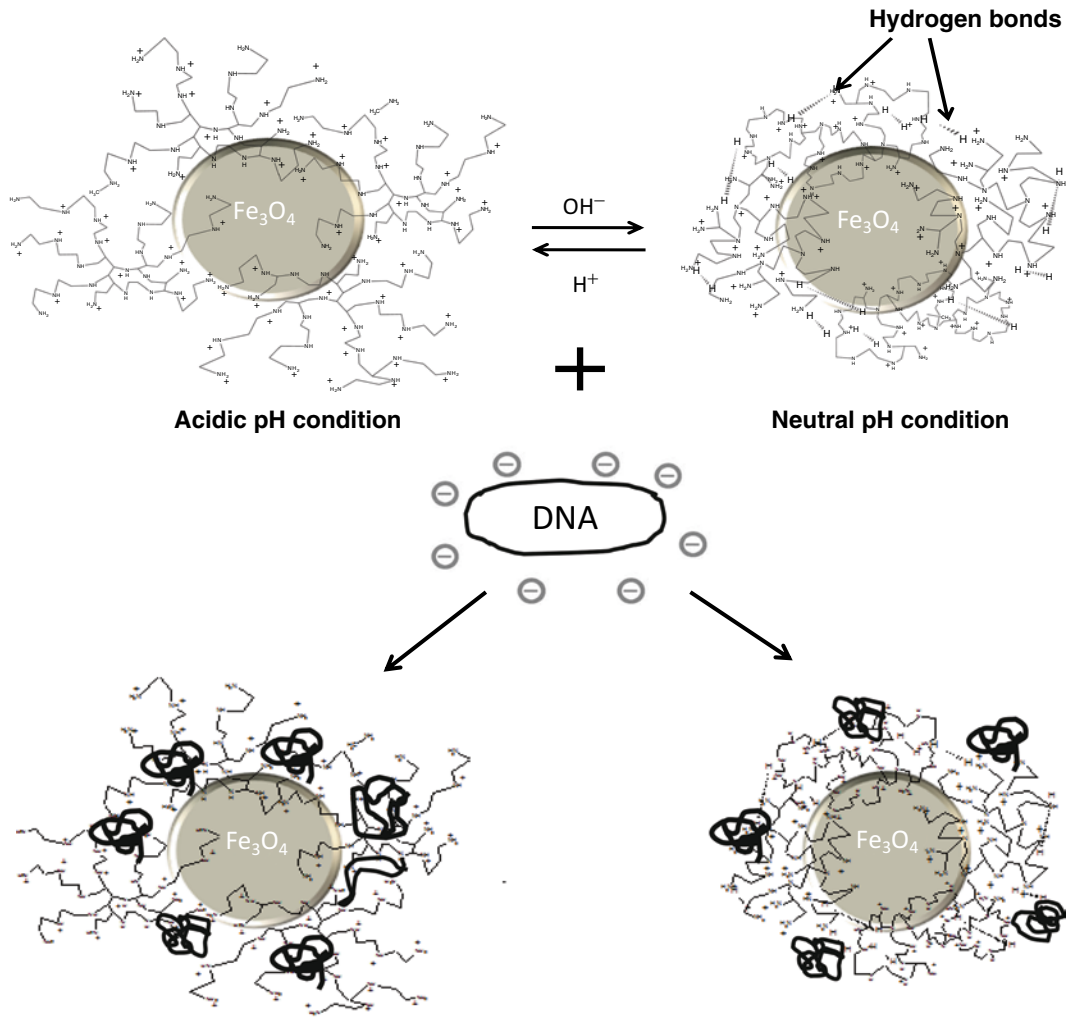

Fig. 1 A schematic demonstrating PEI structures under acidic and neutral pH conditions, showing a relatively branched structure due to mutual charge repulsion between amine groups under acidic condition and a stiff structure under neutral pH condition, with DNA likely to be entrapped within the respective structures. Adapted from Al-Deen et al. [15], with permission from American Chemical Society (ACS) publications of Langmuir

2 Materials

Prepare all solutions using ultrapure water (prepared by purifying deionized water dd H_2O to attain a sensitivity of 18 MΩ cm at 25 °C) and analytical grade reagents. Prepare and store all reagents at room temperature (unless indicated otherwise).

2.1 Iron Oxide Nanoparticle Preparation from Iron Salts

1. Fe (III) chloride ($FeCl_3.6H_2O$) and Fe (II) chloride ($FeCl_2.7H_2O$) (from Ajax Finechem and Ajax Chemicals, respectively).

2. Trisodium citrate dihydrate ($C_6H_5Na_3O_7$. $2H_2O$) (Sigma Aldrich).
3. Sodium hydroxide ACS reagent, ≥97.0 % in pellets (Sigma Aldrich).
4. Cooking oil for oil bath.
5. Zetasizer Nano ZS (Malvern Instruments Ltd., UK).
6. 1140 PW diffractometer with nickel-filtered Cu Kα radiation ($\lambda = 1.5405$ Å) (Philips).
7. Vibrating sample magnetometer (VSM) (Riken Denshi).
8. Transmission electron microscope (TEM) CM20 (Philips).

3 SPION/PEI Complexes

1. PEI solution: 10 % PEI in water (w/v), pH 7.9. Weigh 10 g of PEI (molecular weight of 25 kDa branched, Sigma Aldrich) and dissolve in 75 ml of H_2O. Adjust pH to 7.9 with concentrated HCL and add water to a volume of 100 ml. Filter the PEI solution through a 0.22 μm nitrocellulose filter. Store the solution at 4 °C (*see* **Note 1**).
2. 0.5 M HCl.
3. 0.5 M NaOH.
4. Zetasizer Nano ZS (Malvern Instruments Ltd., UK).
5. Dialysis tubing Spectra/Por® membranes (MWCO = 12,000–14,000) (Spectrum Medical Industries, Inc., Los Angeles, CA).

3.1 SPION/PEI/DNA Polyplexes

1. Endotoxin-free plasmid DNA: 10 μg/ml.
2. 1× PBS, pH 7.4.

3.2 Agarose Gel Electrophoresis

1. Agarose.
2. 6× Sample loading buffer: Weigh ~0.05 mg bromophenol blue and transfer to a 2 ml tube with 1 ml sterile H_2O and 1 ml glycerol. Add enough bromophenol blue to make the buffer deep blue. For long-term storage, keep the sample loading buffer frozen.
3. Ethidium bromide (EtBr) stock solution (10 mg/ml): 0.02 g in 1 ml sterile H_2O.
4. DNA ladder standard.
5. 50× tris–acetate–EDTA (TAE) buffer: 242 g tris base, 100 ml of 0.5 M EDTA solution, 57.1 ml glacial acetic acid, pH 8.5. Add 800 ml water to a 1 l graduated cylinder. Weigh 242 g tris base and transfer to the cylinder. Add 100 ml of 0.5 M EDTA and 57.1 ml glacial acetic acid, mix, and adjust the pH to 8.5

using KOH. Add up to 1 l with H_2O. Store the buffer at room temperature (*see* **Note 2**).

6. Electrophoresis chamber.
7. Power supply.
8. Gel casting tray and combs.

3.3 Reflux System

1. 100 ml three-necked round-bottom flask.
2. Dropping funnel.
3. Air evacuation vacuum pump.
4. Ultrasonic bath (Power Sonic 405, 40 kHz and 350 W).
5. Probe sonicator (Sonics vibra cell, 40 kHz and130 W).
6. N_2 gas cylinder.
7. Water-cooled condenser.
8. Temperature controller.
9. Heating magnetic stirrer.
10. Stir bar.

4 Methods

4.1 Synthesis and Characterization of SPIONs

This method involves coprecipitation of ferrous and ferric salts in an alkaline solution by the addition of a base such as concentrated ammonium hydroxide (NH_4OH) or sodium hydroxide (NaOH) in a non-oxidizing environment (N_2 gas atmosphere) with the following chemical reaction [16, 17]:

$$Fe^{2+} + 2Fe^{3+} + 8OH^- \rightarrow Fe_3O_4 + 4H_2O.$$

Control over size and shape of nanoparticles depends on the Fe^{2+} and Fe^{3+} ratio, the type of salts (e.g., sulphate, nitrate, chloride), and the pH of the reaction media [10].

1. Weigh 1.35 g (0.005 mol) of Fe (III) chloride ($FeCl_3.6H_20$) and 0.70 g (0.0025 mol) of Fe (II) chloride ($FeCl_2.7H_2O$) dihydrate (1:2 M ratios), and dissolve them in 20 ml of H_2O in the first beaker [1].
2. Weigh 1.2 g (1.5 mol) of NaOH and 1.47 g (0.005 mol) of trisodium citrate dihydrate, and dissolve them in 20 ml of Milli-Q H_2O in the second beaker [2] (*see* **Note 3**).
3. Sonicate these beakers in an ultrasonic bath with the homogenization shaking rate (3,600–9,000 rpm) for 10–15 min.
4. Transfer the solution in the first beaker [1] into 100 ml three-necked flask, and place a small magnetic bar inside the flask. Place the flask in the oil bath which has already been placed on the magnetic stirrer, and set the stirring rate to 1,000–1,500 rpm (*see* **Note 4**). Heat the oil bath to 80 °C (*see* Fig. 2).

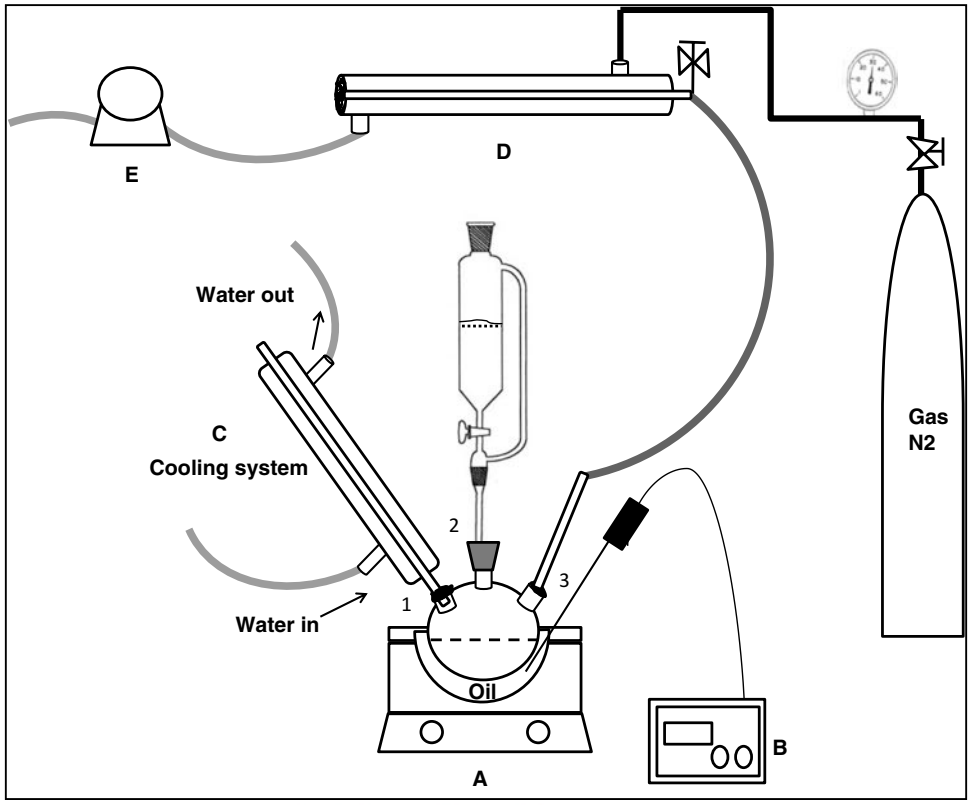

Fig. 2 A reflux system for synthesis of superparamagnetic iron oxide nanoparticles (SPIONs). (*A*) Heating magnetic stirrer, (*B*) temperature controller, (*C*) water-cooled condenser, (*D*) water-cooled condenser, (*E*) air evacuation vacuum pump

5. Transfer the solution in the second beaker [2] into a dropping funnel, and connect the funnel with the three-necked round-bottomed flask via neck [2]; make sure that its stopcock is closed (*see* **Note 5**) (*see* Fig. 2).
6. Introduce a separate hose of the nitrogen stream to the three-necked round-bottomed flask via neck 3 (*see* Fig. 2). Separate hose connected to the condenser which has been connected to N2 gas cylinder via a gas-trap arrangement connected to the top of the condenser.
7. Connect the water-cooled condenser to the three-necked round-bottomed flask via neck 1 (*see* Fig. 2) (*see* **Note 6**). Start water circulation through turning on a water tap. Be sure that cold water is flowing through the condenser in moderate water flow rates.
8. Wrap the connection of equipment in the reflux system with each other by a thin strip of paraffin film to avoid any outside air entering the system.
9. Remove the air out of the system by using a vacuum pump for 4–5 min. Open the cylinder tap cautiously to allow N_2 gas to enter the system for 4–5 min at a steady but controlled rate until 18.2 g to provide a nitrogen blanket to the reaction. Flowing N_2 gas through the reaction medium during the synthesis reaction can afford protection to the produced iron oxide particles from oxidation. The three-necked flask is kept under a positive nitrogen pressure by means of a gas-trap arrangement connected to the top of the condenser.
10. Once the system temperature reaches 80 °C, turn the funnel stopcock partially to start the aqueous coprecipitation of the iron salt solution with NaOH and trisodium citrate dihydrate solution.
11. After 1 h of reaction, collect the resulting black precipitates and remove them from solution by applying an external magnet. Then wash the precipitates four times, firstly with ddH_2O, then twice with ethanol, and finally with deionized (DI) water to remove excess ions and salts from the suspension. The concentration of the solution will be about 8 mg/ml.
12. Disperse the washed precipitate in 20 ml DI water. Zetasizer Nano ZS is used to determine the hydrodynamic diameter and zeta potential of these particles in suspension, while TEM CM20 is used to confirm the size and morphology of dry particles. X-ray powder diffraction (XRD), by means of diffractometer with nickel-filtered Cu Kα radiation ($\lambda = 1.5405$ Å), is used to determine the crystallinity and phase of iron oxide particles. Magnetic saturation is measured using a VSM under a magnetic field of up to 15 kOe at room temperature.

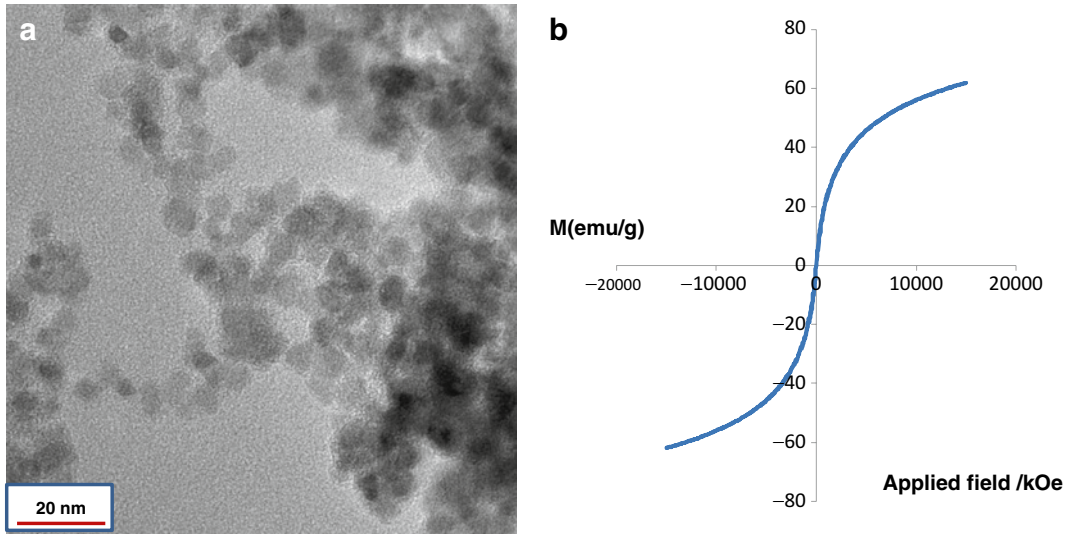

Fig. 3 (**a**) A TEM image of as-synthesized SPIONs, (**b**) VSM data for SPIONs, with *X*- and *Y*-axes in the graph indicating the applied field (kOe) and magnetization (emu/g), respectively

An example of size and morphology of prepared SPIONs under TEM and magnetic saturation using a VSM is shown in Fig. 3.

4.2 Coating SPIONs with PEI Polymer

In magnetofection, magnetic nanoparticles need appropriate surface coatings to form gene complexes, which also increase their stability in solution. The stability of magnetic nanoparticles in biological fluid can be improved by modifying their surface using materials including inorganic and polymeric materials to increase repulsive forces between particles, thus balancing magnetic and van der Waals attractive forces [18] (*see* **Note 7**).

1. Mix the prepared iron oxide suspension (0.1 mg/ml) with 10 % (w/v) PEI solution (25 kDa branched PEI), at PEI/Fe mass ratios of $(R) = 10$, while sonicating using a probe sonicator using a Sonics vibra cell 130 W apparatus at 40 kHz for 5 min.
2. Dialyze the produced SPION/PEI complexes using Spectra/Por membranes (MWCO = 12,000–14,000) against deionized water for 3 days to remove any unbound/excess PEI.
3. Acidify the mixture of SPION/PEI complex to pH 2.0 using 0.5 M HCl, and retain at this pH for 10 min to stabilize the complexes.
4. Divide each sample into two aliquots: increase the pH of the first part to 4.0 (referred to as SPION/PEI-A), while the other part is neutralized to pH 7.0 (referred to as SPION/PEI-N) using 0.5 M NaOH.

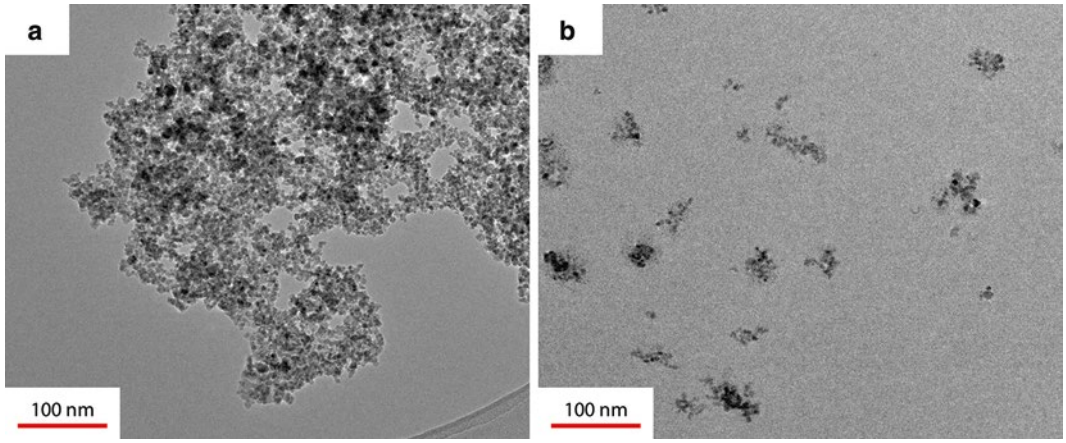

Fig. 4 TEM images of (**a**) as-synthesized SPIONs and (**b**) SPIONs/PEI (ratio = 10) at pH 4 displaying better dispersion. Adapted from Al-Deen et al. [15], with permission from American Chemical Society (ACS) publications of Langmuir

Zetasizer Nano ZS (Malvern Instruments Ltd., UK) is used to determine the hydrodynamic diameter and zeta potential of SPIONs/PEI in suspension, while TEM CM20 is used to confirm the size and morphology of dry particles. An example of the small aggregate size of prepared SPIONs/PEI at pH 4.0 compared with bare SPIONs under TEM is shown in Fig. 4.

4.3 Preparation of SPION/PEI/DNA Polyplexes

1. Mix plasmid DNA at a concentration of 10 μg/ml in PBS (pH 7.4) with SPION/PEI complexes at R of 10 at different N/P ratios (i.e., the molar ratio of PEI nitrogen to DNA phosphate) (*see* **Note 8** for details of calculating different N/P ratios).

4.4 DNA Retardation Assay

The DNA binding capabilities of SPION/PEI/DNA polyplexes are determined using 1 % agarose gel electrophoresis. SPION/PEI complexes with plasmid DNA were formed at N/P ratios of 0.5–30.

4.5 Agarose Gel Electrophoresis of SPION/PEI/DNA Polyplexes

1. Measure out 1 g of agarose.
2. Pour agarose powder into flask along with 100 ml of 1×TAE.
3. Boil with swirling the agarose solution on a heater (until all of the small translucent agarose particles are dissolved, the solution becomes clear, and there is a nice rolling boil) (approximately 10 min).
4. Allow the agarose solution cool to about 50–55 °C, swirling the flask occasionally to cool regularly.

5. Add EtBr to a final concentration of approximately 0.5 μg/ml (usually about 2–3 μl of stock solution per 100 ml gel) (*see* **Note 9**).
6. Seal the ends of the casting tray with two layers of tape.
7. Place the combs in the gel-casting tray.
8. Pour the melted agarose solution into the casting tray and let cool until it is solid.
9. Carefully pull out the combs, and remove the tape. Place the gel in the electrophoresis chamber.
10. Add enough TAE buffer so that there is about 2–3 mm of buffer over the gel (*see* **Note 10**).

4.6 Loading the Gel

1. For each N/P ratio, mix the appropriate amount of SPIONs/PEI with 0.5 μg plasmid DNA in the 25 μl PBS.
2. Incubate all SPION/PEI/DNA polyplex solutions at 37 °C for 30 min.
3. Add 5–6 μl of 6× sample loading buffer to each 25 μl SPION/PEI/DNA polyplexes.
4. Carefully pipette 20 μl of each sample/loading buffer mixture into separate wells in the gel.
5. Pipette 10 μl of the DNA ladder standard into at least one well of each row on the gel.

4.7 Running the Gel

Carry out the electrophoresis at 60 V for 90 min, and then visualize the DNA bands using a UV illuminator.

An example of agarose gel of gel electrophoresis of SPION/PEI/DNA polyplexes at different N/P ratios is shown in Fig. 5.

Fig. 5 Agarose gel electrophoresis of SPION/PEI/DNA polyplexes. Lane N: Plasmid DNA (naked). Lanes 0.5–30 correspond to SPION/PEI/DNA polyplexes at different N/P ratios

5 Notes

1. The PEI 10 % (w/v) solution is the working PEI reagent solution that should be stable for a long period of time at 4 °C. If a 6 % PEI solution is needed, add 6 ml of 10 % of PEI to 4 ml of H_2O.
2. For convenience, a concentrated stock of TAE buffer (either 10× or 50×) is often made ahead of time and diluted with water to 1× concentration prior to use.
3. The presence of trisodium citrate ($C_6H_5Na_3O_7.2H_2O$) on the magnetic nanoparticles works as an electrostatic stabilizer [15]. Trisodium citrate has three carboxyl groups which promote their adsorption onto the iron oxide particles, whilst ionization of carboxyl groups supplies the coated magnetic particles with a negative charge to give a stable dispersion in water due to a strong mutual electrostatic repulsion. Moreover, these negatively charged particles are able to adsorb cationic polyelectrolytes such as PEI.
4. High stirring rate (1,500 rpm) might play effective roles during particle nucleation in preventing the nanocrystals from growing further into large single crystals. Furthermore, the size of nanoparticles becomes smaller when the stirring rate increases due to the increasing amount of energy that is transferred to the suspension. SPIONs with this narrow size range could be used for the delivery of gene vector because the small size of nanoparticles has been shown to influence the rate of their uptake as well as their cytotoxicity [19].
5. Ensure that the dropping funnel stopcock is completely closed to prevent the coprecipitation reaction from starting while contents are being added to the three-necked flask at this stage.
6. A condenser is attached to the heated three-necked flask, and cooling water is circulated to condense the vapor, returning it back to the flask as a liquid.
7. A highly positively charged coating agent for magnetic nanoparticles such as PEI cationic polymer has advantages over other polycations in that it not only increases repulsive forces between the particles but also readily associate with negatively charged plasmid DNA accompanied by intrinsic endosomolytic activity [20].
8. The calculation of the N/P ratio for the SPION/PEI/DNA complexes is defined as the molar relation of primary amine groups in the PEI cationic molecule (secondary and tertiary amines are neglected in this calculation due to their lower pK_a values), which represent the positive charges, to phosphate groups in the DNA, which represent the negative charges. The

calculation of the N/P ratio was based on the assumption that one repeating unit of PEI containing one nitrogen (N) corresponds to 43.1 g/mol and one repeating unit of DNA containing one phosphate group (P) corresponds to 330 g/mol [21].

For example, if we need to prepare SPION/PEI/DNA polyplexes containing 10 μg DNA at N/P ratio of 2:

For DNA, 330 g/mol corresponds to one phosphate atom.

1 μg × 330 g/1 μg = 1 mol phosphate.

1 μg DNA = 1 mol phosphate × 10 − 6/330.

1 μg DNA = 3.03 × 10 − 9 mol phosphate.

10 μg DNA = 10 × 3.03 × 10 − 9 mol phosphate.

10 μg DNA = 30.3 × 10 − 9 mol phosphate.

For PEI, the number of N atom in 25 KDa of PEI = 25,000/43.1 = 580.0464 ≈ 580.05.

If we need to prepare SPION/PEI/DNA polyplexes with an N/P ratio of 2:

N/P = 2.

N = 2 × P.

N = 2 × 30.3 × 10 − 9 = 60.6 × 10 − 9.

(1 mol of PEI = 580.05 mol of N.)

How many moles of PEI are in 60.6 × 10 − 9 mol of N?

60.6 × 10 − 9/580.05 = 0.10 × 10 − 9 mol of PEI.

PEI mass = 0.10 × 10 − 9 mol × 25,000 g/mol = 2.61 × 10 − 6 g = 2.61 μg.

Alternatively, we can use the ratio to calculate the amounts of PEI and DNA required.

The g/mol ratio for one N (PEI) to one P (DNA) is N:P = 43.1:330. For N/P = 2, the g/mol ratio is 86.2:330.

Thus, mass of PEI:mass of DNA = 86.2:330.

For 10 μg DNA, the ratio becomes

Mass of PEI:10 μg = 86.2:330 or, in the form of division,

Mass of PEI/10 μg = 86.2/330.

Rearranging, mass of PEI = 86.2/330 × 10 μg = 2.61 μg.

Since we previously coated SPION with PEI at PEI/Fe mass ratios of (*R*) = 10 (*see* Subheading 3.2), the mass of SPION could be calculated depending on the mass of PEI, assuming that all SPIONs are coated completely with PEI polymer:

R (10) = PEI/SPIONs.

Mass of SPIONs = 2.61 μg/10 = 0.261 μg.

9. EtBr is an intercalating agent commonly used as a fluorescent tag (nucleic acid stain) that binds to the DNA and allows the visualization of DNA under ultraviolet (UV) light.
10. Gels can be made several days prior to use and sealed in a plastic wrap (without combs).

References

1. Krotz F, Sohn HY, Gloe T et al (2003) Magnetofection potentiates gene delivery to cultured endothelial cells. J Vasc Res 40: 425–434
2. Scherer F, Anton M, Schillinger U et al (2002) Magnetofection: enhancing and targeting gene delivery by magnetic force in vitro and in vivo. Gene Ther 9:102–109
3. Plank C, Scherer F, Schillinger U et al (2003) Magnetofection: enhancing and targeting gene delivery with superparamagnetic nanoparticles and magnetic fields. J Liposome Res 13:29–32
4. Dobso J (2006) Gene therapy progress and prospects: magnetic nanoparticle-based gene delivery. Gene Ther 13:283–287
5. Tartaj P, Morales MD, Veintemillas-Verdaguer S et al (2003) The preparation of magnetic nanoparticles for applications in biomedicine. J Phys D Appl Phys 36:R182–R197
6. Roca AG, Costo R, Rebolledo AF et al (2009) Progress in the preparation of magnetic nanoparticles for applications in biomedicine. J Phys D Appl Phys. doi:10.1088/0022-3727/42/22/224002
7. Schwertmann U, Cornell RM (1991) Iron oxides in the laboratory: preparation and characterization. Wiley-VCH, Weinheim, NY
8. Ankamwar B, Lai TC, Huang JH et al (2010) Biocompatibility of Fe_3O_4 nanoparticles evaluated by in vitro cytotoxicity assays using normal, glia and breast cancer cells. Nanotechnology. doi:10.1088/0957-4484/ 21/7/075102
9. Laurent S, Forge D, Port M et al (2008) Magnetic iron oxide nanoparticles: synthesis, stabilization, vectorization, physicochemical characterizations, and biological applications. Chem Rev 108:2064–2110
10. Gupta AK, Gupta M (2005) Synthesis and surface engineering of iron oxide nanoparticles for biomedical applications. Biomaterials 26: 3995–4021
11. Boussif O, Lezoualch F, Zanta MA et al (1995) A versatile vector for gene and oligonucleotide transfer into cells and in-vivo-polyethylenimine. Proc Natl Acad Sci U S A 92:7297–7301
12. Behr JP (1997) The proton sponge: a trick to enter cells the viruses did not exploit. Chimia 51:34–36
13. Godbey WT, Wu KK, Mikos AG (1999) Poly(ethylenimine) and its role in gene delivery. J Control Release 60:149–160
14. Kichler A, Leborgne C, Coeytaux E et al (2001) Polyethylenimine-mediated gene delivery: a mechanistic study. J Gene Med 3: 135–144
15. Al-Deen FN, Ho J, Selomulya C et al (2011) Superparamagnetic nanoparticles for effective delivery of malaria DNA vaccine. Langmuir 27:3703–3712
16. Massart R (1981) Preparation of aqueous magnetic liquids in alkaline and acidic media. IEEE Trans Magn 17:1247–1248
17. Massart R, Dubois E, Cabuil V et al (1995) Preparation and properties of monodisperse magnetic fluids. J Magn Magn Mater 149:1–5
18. Wu W, He QG, Jiang CZ (2008) Magnetic iron oxide nanoparticles: synthesis and surface functionalization strategies. Nanoscale Res Lett 3:397–415
19. Steitz B, Hofmann H, Kamau SW et al (2007) Characterization of PEI-coated superparamagnetic iron oxide nanoparticles for transfection: Size distribution, colloidal properties and DNA interaction. J Magn Magn Mater 311: 300–305
20. Lungwitz U, Breunig M, Blunk T et al (2005) Polyethylenimine-based non-viral gene delivery systems. Eur J Pharm Biopharm 60: 247–266
21. Geisse S, Jordan M, Wurm FM (2005) Large-scale transient expression of therapeutic proteins in mammalian cells. Methods Mol Biol 308:87–98