The Feasibility of Using Magnetic Nanoparticles Modified as Gene Vector

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ABSTRACT

Objective: *To evaluate the feasibility of using magnetic nanoparticles (MNPs) as gene vector and the effect of magnetic field on efficiency of transfection.*

Methods: *Magnetic nanoparticles were prepared by controlling some chemical reaction parameters through a partially reduction precipitation method with ferric chloride aqueous solution as precursor material. The surface of particles was modified by polyethyleneimine (PEI) agents. The appearance, the size distribution, structure and phase constitute of MNPs were characterized by Transmission electron microscope (TEM), X-ray diffraction (XRD); the potential of absorbing DNA of MNPs was analysed by electrophoresis. Transfection was determined by delivering reporter gene, PGL2-control encoding luciferase, to different cell lines using MNPs-PLL as vector. The effect of magnetic field on the efficiency of transfection was determined using Nd-Fe-B permanent magnet.*

Results: *Foreign gene could be delivered to various cell lines by MNPs-PLL and expressed with high efficiency but the transfection efficiency and time course varied in the different cell lines studied. Magnetic field could enhance the efficiency of transfection by 5–10 fold.*

Conclusion: *MNPs- PLL can be used as a novel non-viral gene vector in vitro, which offers a basis for gene delivery in vivo.*

Keywords: Magnetic field, magnetic nanoparticles (MNPs), transfection

Viabilidad del uso de Nanopartículas Magnéticas Modificadas como Vectores Genéticos

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RESUMEN

Objetivo: *Evaluar la viabilidad del uso de nanopartículas magnéticas (MNPs) como vectores genéticos y el efecto de campo magnético en la eficiencia de la transfección.*

Métodos: *Se prepararon nanopartículas magnéticas mediante el control de algunos parámetros de la reacción química a través de un método de precipitación de reducción parcial con soluciones acuosas de cloruro férrico como el material precursor. La superficie de las partículas fue modificada mediante agentes de polietileneimina (PEI). La apariencia, el tamaño, distribución, estructura y constitución de fase de las MNPs, se caracterizaron mediante el microscopio electrónico de transmisión (MET), difracción de rayos X (DRX); el potencial de adsorber ADN de las MNPs se analizó mediante electroforesis; la transfección se determinó mediante el suministro del gene reportador de la luciferasa control PGL2, a diferentes líneas celulares usando MNPs – PLL como vectores. El efecto de campo magnético sobre la eficacia de la transfección se determinó usando el imán permanente NdFeB.*

Resultados: *El gene foráneo pudo suministrarse a varias líneas celulares mediante MNPs – PLL y expresarse con alta eficiencia pero la eficiencia de la transfección y el curso de tiempo variaron en las diferentes líneas celulares estudiadas. El campo magnético pudo mejorar la eficiencia de la transfección en 5–10 veces.*

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Conclusión: *Las MNPs – PLL pueden usarse como un nuevo vector genético no viral* in vito, *lo cual ofrece una base para el suministro del gene* in vivo.

Palabras claves: campo magnético, nanopartículas magnéticas (MNPs), transfección

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INTRODUCTION

Gene therapy refers to introduction of exogenous gene into cells, by recovery or supplement of gene expression; it can correct disorder of gene structure or function of the human body, prevent pathogenic progress, kill diseased cells or inhibit replication of genetic substance of exogenous pathogen to treat diseases. The key to gene therapy is to introduce DNA into cells and then into the nucleus. So success of gene therapy not only depends on safe and effective target gene but highly efficient and safe gene vector is also a key factor (1).

In clinical experiments on gene therapy to tumours, two-thirds adopt viral vectors which have various defects (2) such as possessing immunity and cell toxicity, lack of histological specificity, size limit of loading exogenous gene, potential carcinogenesis and active viral particles generated in recombination processes. For this reason, people gradually turn to non-viral vectors. Traditional non-viral vectors are mostly cationic polymers and derivatives of liposomes; their major defect is inefficiency of gene transfer, especially *in vivo.* They are easily digested by intracellular and extracellular hydrolytic enzymes. In addition, they lack histological specificity but have certain cell toxicity. To overcome such defects of non-viral vectors, some researchers proposed using inorganic nanometer particles as gene vectors. Compared with the present non-viral vectors, inorganic nanometer particles have nano-effect and have little cell toxicity. They can resist digestion by digestive enzymes.

The present study first used an aqueous solution of iron trichloride as raw material; a partial reduction precipitation method was used to prepare magnetic nanoparticles and PEI was used for surface modification. Then luciferase, as report gene system, was used to test transfection characteristics of MNPs-PEI which transfected exogenous genes to different cell lines and test the influence of active magnetic field on the efficiency of gene vector.

MATERIALS AND METHODS

COS-7 cells of African green monkey renal cell line, NIH/3T3 cells of mice fibroblast, Hela cells of human cancer of the uterine cervix and MA 782/5S-8102 cells of mice cell line were frozen and revived by our laboratory, cultured in RPMI-1640 culture medium containing 10% calf serum and then put in a culture box with 5% CO2. PGL2-control plasmid and luciferase reagent kit were from Promega, USA.

Such chemical reagents as dextran, ferrous chloride, ferric chloride and ammonia water were analytical reagents from a chemical reagent company. Cell culture reagent RPMI-1640 and calf sera were from Gibico, polyvinylimine was from Sigma and BCA protein quantitative reagent kit was from Sigma.

A 1mol/L $FeCl_3$ solution was added into a 500 ml three-necked flask and it was diluted to 100 ml with distilled water. Certain 0.5 mol/L Na_2SO_3 solution was added into a 150 ml beaker and it was diluted to 100 ml with distilled water; then it was put into a dropping funnel and dropped into a flask. Ammonia water was dropped into the flask quickly while stirring vigorously. Black precipitation could be seen and the pH value was adjusted to 8. After the product was heated for 30 minutes in 60–80°C water bath, the magnetic separation was carried out and the product was washed and dried in *vacuo*. MNPs were dispersed in a dispersion system with certain PEI, and stirred with thermostatic magnetic agitator for several hours for full reaction and forming of stable MNPs with core-shell structure. MNPs were separated from the solution with a magnetic separation method and washed repeatedly with distilled water and methanol. After vacuum drying, modified MNPs were stored in a dry vessel for later use.

X-ray diffraction was used to analyse MNPs before and after modification; HITACHI 800 TEM was used to analyse the appearance and particle size of MNPs before and after modification.

Magnetic nanoparticles -PEI were mixed with PGL2-control plasmid DNA in a 30:1 ratio at different acid-base values. At pH = 7, MNPs-PEI were mixed with plasmid DNA at different proportions (0:1, 10:1, 30:1, 50:1, 100:1, 150:1) while DNA concentration was constant at 0.01 μg/μl. After the mixture was left standing for 1 hour the products were taken for agarose gel electrophoresis 80 v for 2 hour, and the results were observed with gel imaging system and pictures were taken.

One hundred thousand cells were planted into 6-well plates. After 24 hours, 1640 culture medium without serum was used to wash them twice. When 1640 culture medium was free from serum, 2 μg PGL2-control expression plasmid was transfected into each well. MNPs-PEI with plasmid DNA at ratio 50:1 were mixed and oscillated for 10s, and then it was loaded on single layer culture cells; five hours after transfection, 1640 culture medium with 15% calf serum was added, transfected PGL2-control cells were harvested at different times of transfection, washed twice with PBS, cells were cracked with cracking solution and the cells were scraped with cell scraper and oscillated for 20s and centrifuged at 12 000 r/min for 1 minute; the supernatant fluid was collected, 20μl supernatant was added to the luciferase assay

substrate (Promega) and the luciferase activity was tested with a single photon detector. The protein content was measured with BCA protein quantitative reagent kit and the relative luciferase activity (RLU) was calculated in each milligram of protein. For applied magnetic field group, strong magnetic block was put under 6-well plate with MNPs-PEI/DNA compound (mass ratio 30:1) and transfected for half an hour. Other steps were the same as above.

RESULTS

Figure 1 shows XRD of unmodified MNPs obtained from the experiment. XRD analysis showed that the XRD main peak

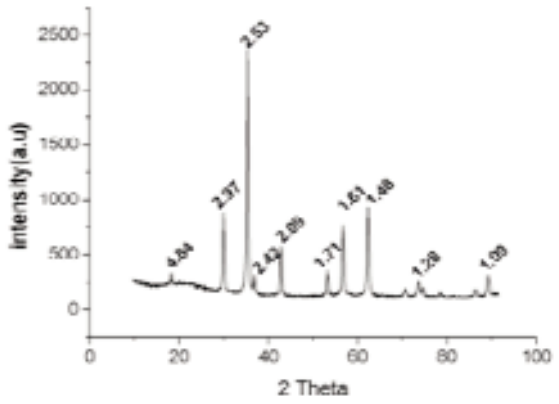


Fig. 1: X-ray diffraction of MNPs.

of the powder was very close to Fe_3O_4 (19–629). So the main phase of MNPs was Fe_3O_4. Fig. 2 is a TEM picture of

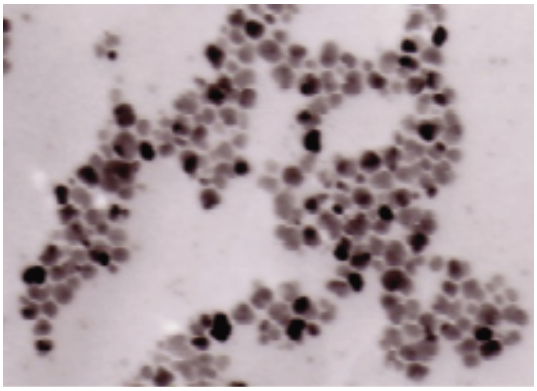


Fig. 2: Appearance of MNPs under electronic microscope.

unmodified MNPs under 50 000 amplified factors. We could see that MNPs were uniformly distributed with certain dispersion. A columnar drawing of particle size distribution was obtained by imaging analysis software (Fig. 3). It could be seen that particle size was usually in normal distribution, concentrated at the 5–25 nm zone (97%) of which particles of 10–20 nm accounted for 76%.

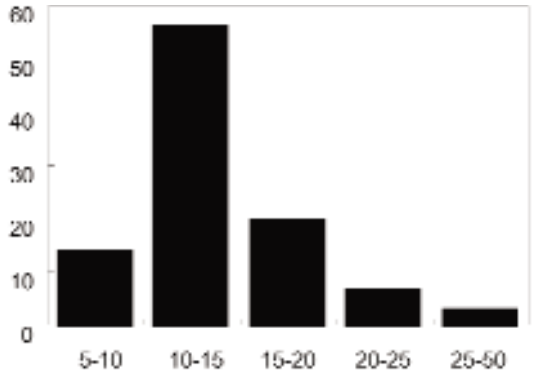


Fig. 3: Particle size distribution of MNPs.

Agarose gel electrophoresis showed that under acid and neutral conditions, MNPs-PEI has the ability to combine with DNA. Electrophoresis showed no movement of DNA or decrease of the moving speed of DNA. But under basicity, DNA combination ability was poor (Fig. 4A). Fig. 4A shows

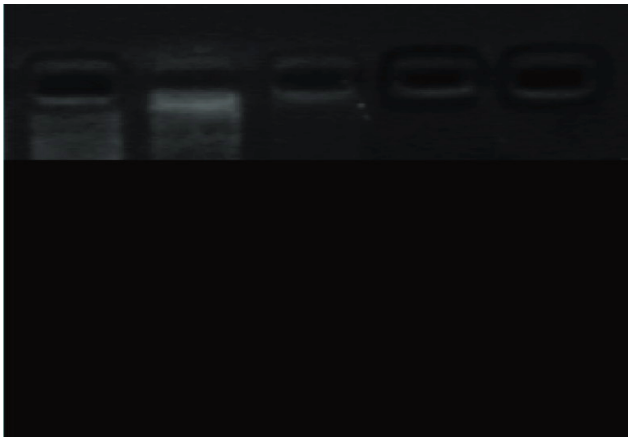
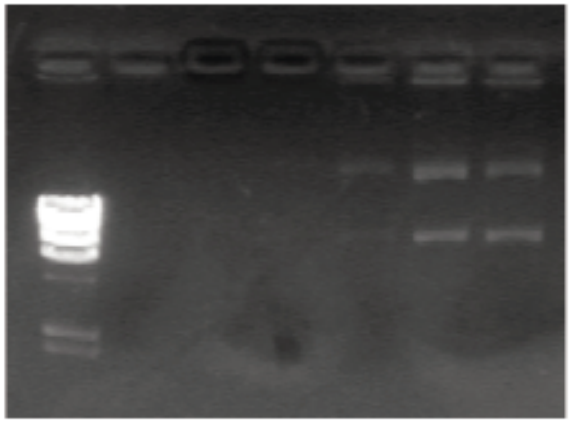


Fig. 4A: Experiment of combination between PEI modified Mn-Zn ferrite magnetic nano-particle and DNA at various acid-base conditions.

combination of MNPs-PEI and DNA when their mass ratio was 30:1 at various acid-base conditions. Control plasmid DNA at pH = 10, pH = 7 and pH = 3 were in 1–4 electrophoretic channels respectively. When pH = 3, their combination ability was strong, the speed of DNA movement was low, DNA combined with MNPs-PEI was in the well and no free DNA was seen. When pH = 10, their combination ability was weak, the obvious DNA band could be seen in the corresponding channel. Their combination ability was sensitive to the pH value which meant that combination depended on electrostatic attraction. When the surface of ferric oxide nanoparticle was modified by the cationic polymer polyvinyl imine, it could combine with negative DNA at acid, neutral and basic conditions. Fig 4B shows the combination of PEI modified Mn-Zn ferrite magnetic nanoparticle with DNA at different mass ratio. From agarose gel electrophorogram, it could be seen that from 1:30 on, PEI

modified Mn-Zn ferrite magnetic nanoparticle could combine with DNA effectively (Fig. 4B). There was no obvious DNA band on the gel. When plasmid was added as per 1:0 and 1:10, an obvious DNA band could be seen on the electrophoretic channel.



4B

Fig. 4B Combination between PEI modified magnetic nano-particle and DNA at different mass ratio.

Magnetic nanoparticles polyethyleneimine transfer reports gene expression vector PGL2-control in different cell lines, including COS-7 cell, NIH/3T3 cell, HeLa cell and mice MA-782 cell. Single photon monitor was used to detect activity of expression product luciferase after PGL2-control was transfected to cells. Results showed that MNPs-PEI could transfect exogenous plasmid PGL2-control to cell lines; transfection efficiency and expression ageing were different (Fig. 5).

Magnetic nanoparticles polyethyleneimine had a crystal core of magnetic Fe_3O_4 with diameter of 10 nm, which had superparamagnetism. Permanent magnet was put under the transfection cell culture plate and when the magnetic field was applied to various cell lines, transfection efficiency increased by 4–5 times (Fig. 6).

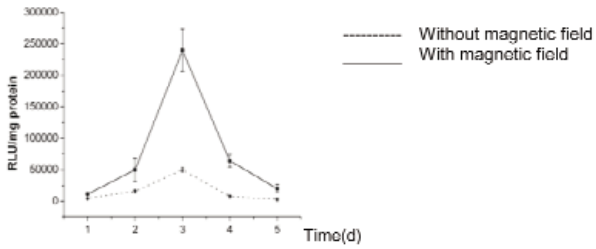
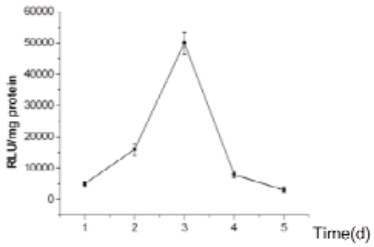


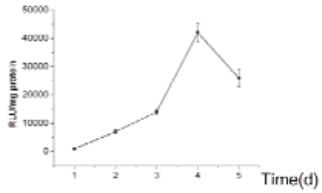
Fig. 6: Influence of active magnetic field on efficiency of MNP5-PEI transfection of COS-7 cell line.

DISCUSSION

At present, commonly used gene vectors are classified into viral and non-viral types. Though viral vector is more efficient, it may induce immune reaction of the host and has a potential for carcinogenesis, limited loading capacity and is expensive. Non-viral vectors have such advantages as safety, low toxicity, large loading capacity and easy operation, but transfection efficiency is low (3).



5a



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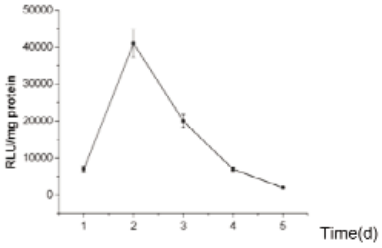

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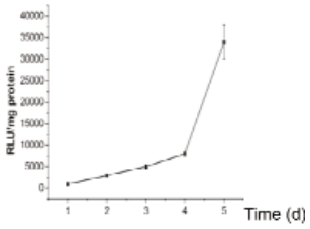

5d

Fig. 5: Time-efficiency curve of MNPs-PEI in vitro transfer of PGL2-control.

5a Time-efficiency relationship of MNPs-PEI transfection of PGL2-control to COS7 cell line.

5b Time-efficiency relationship of MNPs-PEI transfection of PGL2-control to NIH/3T3 cell line.

5c Time-efficiency relationship of MNPs-PEI transfection of PGL2-control to HeLa cell line.

5d Time-efficiency relationship of MNPs-PEI transfection of PGL2-control to MA-782 cell line.

Commonly used non-viral vectors include liposome, phosphate of lime and cationic polymer. Nano-gene vector belongs to the non-viral vectors. When the particle size of the gene vector is as small as a nanometer, such substance can be called nano-gene introduction vector. Nanoparticle as a gene vector has the following advantages (4–6). It can wrap, concentrate and protect nucleotide and prevent its degradation by nuclease. Nanoparticle has a large specific surface area and biological affinity. It is easy to couple specific target molecules on its surface to achieve specificity of gene therapy. Controllable degradation of nanoparticles result in controllable release of gene vector which can prolong the acting time effectively and reduce adverse effect. It can avoid the potential risk of conventional viral vectors which may result in sequential change of normal nucleotide of the host. Combination of cationic polymer nanoparticles with DNA mainly depends on charge action. When DNA combines with a suitable molecule of cationic polymer, the DNA molecule with negative charge is concentrated into an ordered nanoparticle with diameter of 50~200 nm, which is the basis of the supermolecular chemical action of the cationic polymer vector. Studies have shown that the compound of cationic polymer nano-gene vector and DNA is one of the most promising non-viral gene introduction vectors (7).

Polyethyleneimine is a cationic polymer which is an artificial synthetic organic macromolecule with a positive charge of extremely high density. Research demonstrated that PEI has high transduction efficiency *in vitro* and *in vivo* (8) but it lacks target. Magnetic ferric oxide nanoparticle is the latest result of domestic and foreign research to guide medication and medical nano materials. In addition to the general advantages of nanoparticles, it has superparamagnetism. In an active magnetic field it can guide a loaded substance to move and concentrate directionally for target gene therapy (9). There were studies that used nanoparticles of dextran wrapping over ferric oxide as a gene vector but the transduction efficiency was low (10).

The present study used PEI that can combine with DNA and protect it and magnetic nano material that has magnetic orientation, and ferric oxide magnetic nanoparticle wrapped with PEI as a gene vector for *in vitro* gene transfection experiment. We tried to find a new type of gene vector which can integrate combination and protection of DNA with orientation, safety and high efficiency. Electronic microscopy reviewed that the particle size of MNPs-PEI is about 10 nm and the particles are uniform. Experimental combination of MNPs-PEI and DNA demonstrated their efficency.

Experiment of *in vitro* gene transfection found that MNPs-PEI can introduce PGL2-control plasmid DNA into tumour cells and express luciferase. When a magnetic field was applied, transfection efficiency could be increased by several times. So magnetic transfection could be one of the effective methods that improve transfection efficiency of gene introduction vector. We hold that this might be the main reason for the action of the magnetic field, which enabled the suspending magnetic gene vector to reach the cell surface easily, and allow more gene vectors to enter the cells. So MNPs-PEI might become a good vector for *in vitro* gene transfection. Domestic and foreign scholars hold that magnetic transfection was a good method to overcome slow aggregation of vectors and low concentration in target tissues (11–12).

Introduction of PEI into a vector and combination with magnetic nanoparticles can improve transfection efficiency, increase expression level of gene but also reduce the volume of vector used, decrease toxicity and cost and shorten required time of effective transduction. In addition, ferric oxide magnetic nanoparticles were easily prepared, particle size may reach 10 nm or smaller, cell toxicity is low and particles can be engulfed by cells. It has superparamagnetism. In particular, the vessel endothelium gap of tumour tissue is enlarged and ferric oxide magnetic nanoparticle can easily pass through vessel endothelium and be swallowed by tumour cells. At present, ferric oxide magnetic nanoparticle is widely used in the contrast agent of nanomagnetic resonance (NMR), especially in NMR research of tumours (13). We believe that the properties of ferric oxide magnetic nanoparticle also conform to the requirements of gene vector; and can be used for gene therapy of tumours. Therefore, introduction of the new gene PEI and magnetic nanoparticle into a vector has wide application.

Since great importance is now attached to functional gene combination and gene therapy, gene transfer technology has become a hot spot of research. The mechanism of MNPs-PEI entering cells is not clear, but conjecture is that structural and functional change of the cell membrane resulted in cell phagocytosis. The present study confirmed the feasibility of MNPs-PEI as a gene vector *in vitro;* active magnetic field can improve transfection efficiency effectively. MNPs-PEI was easy to prepare, cheap and might become a substitute for existing gene transfection vector for gene therapy *in vivo*.

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