Rev.Adv.Mater.Sci. 5 (2003) 375-380

# A NOVEL METHOD FOR EFFICIENT GENE DELIVERY USING AMINO-MODIFIED SILICA COATED MAGNETIC NANOPARTICLES

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*Received: July 27, 2003*

**Abstract.** We have developed a novel method for efficient gene delivery using amino-modified silica coated magnetic nanoparticles. The positively charged amino-modified silica coated magnetic nanoparticles have been directly prepared using the synchronous hydrolysis of tetraethoxysilane and N-(β-amimoethyl)-γ-aminopropyltriethoxysilane in water-in-oil microemulsion after the synthesis of aqueous magnetic ferrofluid with the precipitation of the chloride mixture with the base. Plasmid DNA can be easily combined onto the positively charged nanoparticle surface to form Nanoparticles-plasmid DNA complexes. The combined plasmid DNA was effectively protected from enzymatic digestion of DNaseI. And the nanoparticles-plasmid DNA complexes can successfully cross various systemic barriers to COS-7 cells as well as mediate high expression of Green Fluorescence Protein (GFP) gene in cells by use of this new amino-modified silica coated magnetic nanoparticles as gene delivery. It would provide a new technology and measure for gene expression, gene filtration, gene function research and gene therapy.

## 1. INTRODUCTION

Efficient DNA delivery is critical for molecular biology research and gene therapy. With the implement of human genome project, a number of 'ex vivo' and 'in vivo' strategies for gene delivery have been proposed continuously [1-3], including viral vectors [4], liposome [2,4], synthetic gene delivery system [5,6] and so on. Especially following the fast development of nanotechnology, a novel non-viral gene carriers has been appeared under the combination of nanotechnology and biotechnology, which greatly increases the efficiency of DNA transfection, and brings new opportunities to the development and application of gene engineering technology. At present, a variety of synthetic nanoparticles materials have been studied for enrichment and transport of negatively charged DNA, such as cationic polymers nanoparticles, dendrimers, modified inorganic nanoparticles and so on [7-13]. The inorganic nanoparticles have a number of advantages over organic ones in vivo applications with low polydispersity and high biocompatibility [12-14]. We have used amino-modified silica nanoparticles successfully for gene transfection [14,15].

Herein, we report a novel protective DNA nano-enricher for gene delivery based on amino-modified silica coated magnetic nanoparticles. The approach takes advantage of the amino-modified silica coated magnetic nanoparticles with a net positive charge at neutral pH. Nanoparticles-plasmid DNA complexes can be easily formed through electrostatically binding between the positive charges of the amino-modified silica coated magnetic nanoparticles and the negative charges of the plasmid DNA. The enriched plasmid DNA was effectively protected from

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enzymatic digestion of DNaseI. And the nanoparticles-plasmid DNA complexes can successfully cross various systemic barriers to COS-7 cells as well as mediate high expression of Green Fluorescence Protein (GFP) gene in cells by use of this novel DNA nano-enricher. In comparison with the liposome gene carrier, it shows high transfection efficiency. It would provide a new technology and measure for gene expression, function research and gene therapy. Further, the amino-modified silica nanoparticles are super- paramagnetic. This method maybe used in the filtration of the successfully transfected cells under the magnet.

## 2. EXPERIMENTAL SECTION

### 2.1.Reagents and biological materials

N-(β-amimoethyl)-γ-aminopropyl-triethoxysilane was obtained from Sigma company. DNaseI and was obtained from Beijing Sino-American Biotechnology Corporation. Green fluorescent protein (GFP) expression carrier (pIRGFP, 4.3kb) was presented by Dr. Yu Xinglong of Farrier Institute of Quartermaster University of PLA. COS-7 cell lines were provided in our lab. All other chemicals were purchased from the Reagent & Glass Apparatus Corporation and used without further purification.

Nanopure water was used for the preparation of all solutions. The reagents or solutions were all sterilized.

### 2.2. Instruments

The amino-modified silica coated magnetic nanoparticles have been characterized by transmission electron microscopy (TEM) and alternating gradient magnetometer (AGM). Zeta potential was measured using Malvern zetasizer 3000HS (England, Malvern). UVPCDS8000PC gel imaging analytical system (USA, UVP) was used to capture the images of the agarosegel electrophoresiser of plasmid DNA. Nanoparticles were prepared with 19HVV magnetic stirrer (Zhejiang). The images of the transfected cells were taken by Nikon TE300 fluorescent microscope (Japan, Nikon).

### 2.3. Preparation of amino-modified silica coated magnetic nanoparticles

Amino-modified silica coated magnetic nanoparticles were prepared using the synchronous hydrolysis of tetraethoxysilane and N-(β-amimoethyl)-γ-aminopropyltyiethoxysilane in water-in-oil microemulsion. Briefly, the procedure was first down by synthesis of aqueous magnetic ferrofluid with the precipitation of the chloride mixture with the base [16]. Then the amino-modified silica coating was down by using water-in-oil microemulsion technique [14]. Well-distributed mixture of tetraethoxysilane and N-(β-amimoethyl)-γ-aminopropyltriethoxysilane (with the ratio of volume: 1/1) was added to the microemulsion. With the initiation of ammonia, tetraethoxysilane and N-(β-amimoethyl)-γ-aminopropyltriethoxysilane synchronously hydrolyzed in microemulsion to produce amino-modified silica coated magnetic nanoparticles. Pure silica coated magnetic nanoparticles were prepared simultaneously by controlled hydrolysis of tetraethoxysilane in microemulsion with the initiation of ammonia.

### 2.4. Characterization of nanoparticles

**a. Zeta potential of nanoparticles.** The zeta potentials of aqueous magnetic ferrofluid, amino-modified silica coated magnetic nanoparticles and pure silica coated magnetic nanoparticles in different pH were respectively determined by Malvern zetasizer 3000HS pH auto titration system as follows: these nanoparticles were suspended in nanopure water, and were taken pH titration with 0.1 mol/l NaCl and 0.25 mol/l NaOH solution. The zeta potentials in different pH were obtained.

**b. The size and figure of nanoparticles.** Based on the results of nanoparticles' zeta potential, we selected the proper dispersant (when zeta potential >30 mv or < -30 mv, nanoparticles suspension is stable and disperse) to make nanoparticles suspension. The suspension was then added dropwise onto the carbon-coated copper membrane, and dried at room temperature. The size was measured by Hitachi-800 transmission electron microscope. And the aqueous magnetic ferrofluid, amino-modified silica coated magnetic nanoparticles are super-paramagnetic.

### 2.5. Combination analysis of amino-modified silica coated magnetic nanoparticles and plasmid DNA

**a. Combination of amino-modified silica coated magnetic nanoparticles with plasmid DNA.** The nanoparticles and plasmid DNA were mixed well by certain mass ratio, and reacted for 10 min at room temperature. In every experimental group, the weigh of nanoparticles was 0.25 mg and the concentration of plasmid DNA was 0.36 ug/ul, the amount of

**Table 1.** Combination of amino-modified silica coated magnetic nanoparticles with plasmid DNA (4.3 kb)/ 0.25 mg nanoparticles.

| Experimental Group | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Plasmid DNA (μL) 0.36 μg/μL | 0 | 2.5 | 5.0 | 7.5 | 10.0 | 12.5 | 15.0 | 20 | 30 | 60 |

DNA changed with the volume. Experiments were designed as described in Table 1. The combination results were measured by Malvern zetasizer 3000HS hand-sampling system, and agarosegel electrophoresiser (1% w/v agar, vatage: 60 v, time: 2 h).

**b. Protection of DNA by DNA-nanoparticle complexes.** We added equal DNaseI respectively to plasmid DNA and plasmid DNA-nanoparticle complexes, let them digest in water bath (37 °C) for 1h. Agarsegel electrophoresis was used to gain results.

## 2.6. Application of the amino-modified silica nanoparticles for gene delivery

In this work, green fluorescent protein (GFP) expression carrier (pIRGFP, 4.3kb) served as report gene. If GFP plasmid DNA can be efficiently delivered into the cells and exert its function, green fluorescence protein will be synthesized in cells through the expression of GFP gene and can then be imaged in real-time with fluorescence microscopy. COS-7 cells were selected as the receptor cells. The COS-7 cells were inoculated in six-hole cell-dish, 5% $CO_2$ chamber. The cells were cultured until the cover rate reached 50%-70%. The nanoparticles were used to deliver pIRGFP plasmid DNA as follows: the nanoparticles and pIRGFP plasmid DNA were mixed well as definite ratio of mass, and placed at room temperature for 10 min. The cells were rinsed twice with 2ml non-serum substrate and resuspended with 1.5 ml non-serum substrate. The cells were incubated with nanoparticle-plasmid DNA complexes for 6 h at 37 °C, and replaced with absolute substrate including 15% calf serum.

# 3. RESULTS AND DISCUSSION

## 3.1. Characterization of nanoparticles

**3.1.1. The size of nanoparticles.** TEM images of both magnetic core and amino-modified silica coated magnetic nanoparticles are shown in Figs.1a and 1b, The starting aqueous ferrofluid contains regularly shaped magnetite nanoparticles with a number-average TEM diameter of about 8 nm. They are not aggregated even after several weeks as our experience. When the aqueous ferrofluid was dilled

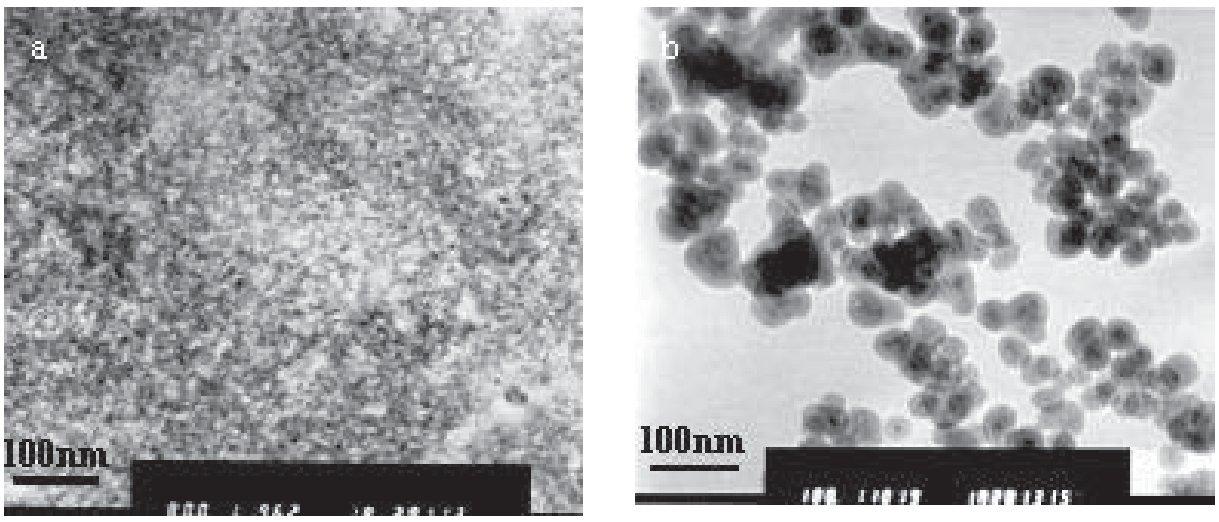


**Fig. 1.** TEM image of magnetic core and amino-modified silica coated magnetic nanoparticles. (a) : Magnetic core, (b): amino-modified silica coated magnetic nanoparticles.

with W/O microemulsion with the presence of tetraethoxysilane (TEOS) and N-(β-amimoethyl)-γ-aminopropyltriethoxysilane, the silica was coated around the magnetite core to grow to the size of about 40±5 nm.

**3.1.2. Zeta potential determination of nanoparticles.** The amino-modified silica coated magnetic nanoparticles were found sufficient to produce a net positive charge at neutral pH. At lower pH, the zeta potential of the amino-modified silica nanoparticles increased due to the protonation of amino group on the surface. At pH 10 and above, the amino groups were not protonated, the nanoparticles possessed negative zeta potential. The zeta potential of aqueous magnetic ferrofluid is near zero at neutral pH. However, pure silica nanoparticles had positive zeta potential only at pH 5.3 or below. It implied that only amino-modified silica coated magnetic nanoparticles could electrostatically bind negative DNA to form DNA-nanoparticles complexes at neutral pH, which provide theoretic foundation for the application of amino-modified silica coated magnetic nanoparticles as DNA enricher for gene delivery.

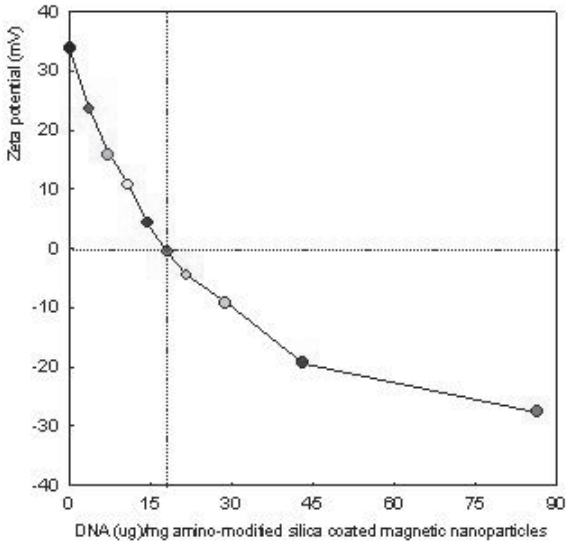


**Fig. 2.** Zeta potential of amino-modified silica coated magnetic nanoparticles-DNA complexes (Plasmid DNA, 0.36 μg/μL, pH 7.20).

## 3.2. Analysis of the combination of amino-modified silica coated magnetic nanoparticles and plasmid DNA (pIRGFP)

**3.2.1. Combination of amino-modified silica coated magnetic nanoparticles and plasmid DNA.** The binding ability of amino-modified silica coated magnetic nanoparticles with plasmid DNA has been quantitatively investigated by using Malvern zetasizer 3000HS hand-sampling system and agarosegel electrophoresis. Agarosegel electrophoresis was performed in a 1% (w/v) gel, ethidium bromide included for visualization, for 2 h at 60 V. The zeta potential of nanoparticles-plasmid DNA complexes were decreased with the increasing amount of plasmid DNA due to the binding plasmid DNA on the surface of amino-modified silica coated magnetic nanoparticles (see Fig. 2). The zeta potential reached near to zero when 4.5 ug DNA was added to 0.25 mg nanoparticles. When the mass ratio of nanoparticles to plasmid DNA was 10/0.18, the binding ability of the nanoparticles to plasmid DNA has reached saturation, and further increase of plasmid DNA resulted in negative zeta potential in the nanoparticles-DNA complexes suspensions. Agarosegel electrophoresis also demonstrated that amino-modified silica coated magnetic nanoparticles could bind the negative plasmid DNA. And the nanoparticles-DNA complexes retained at the baseline. The reasons that the nanoparticles-DAN complexes did not move towards the positive electrode were the charge and the size of the plasmid DNA-NP complexes. But as addition of amount of DNA increases, More DNA is observed progressing toward the positive electrode (Fig. 3), which further support the combination ability of amino-modified silica coated magnetic nanoparticles with plasmid DNA.

**3.2.2. Protection of bound DNA by DNA-nanoparticle complexes.** To investigate whether surface binding is sufficient to provide protection against enzymatic degradation, 2U DNaseI was added respectively to the uncomplexed plasmid DNA and plasmid DNA-nanoparticles complexes containing 3.6 ug of DNA, and incubated for 1h at 37 °C. Free plasmid DNA was degraded completely into small fragments and could not be visualized in the agargel. However, DNA-nanoparticles complexes digested by DNaseI were still visualized around sample pore. And the characteristics of the released DNA from the DNA-nanoparticle complexes that have been incubated with DNaseI have not changed. These results showed that DNA-nanoparticle complexes formation was efficiently to provide protection against enzymatic degradation.

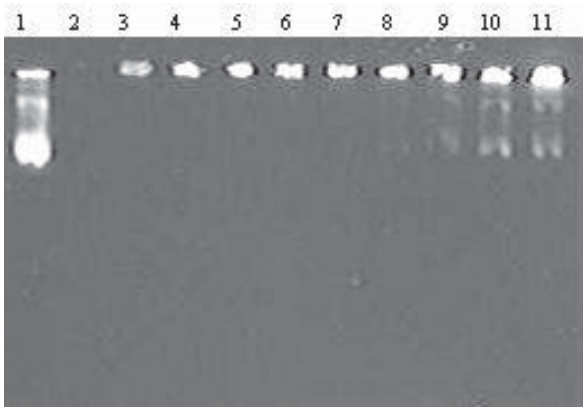


**Fig. 3.** Agarosegel electrophoresis of the amino-modified silica coated magnetic nanoparticle-DNA complexes. Exterior lane is uncomplexed DNA. The second line is the pure silica coated magnetic nanoparticles incubated with DNA. Lane3-11 contain increasing amount of plasmid DNA (0.9 μg, 1.8 μg, 2.7 μg, 3.6 μg, 4.5 μg, 5.4 μg, 7.2 μg, 10.8 μg, 21.6 μg) with 0.25 μg nanoparticles. The results demonstrated that amino-modified silica coated magnetic nanoparticles could bind the negative plasmid DNA. And the nanoparticles-DNA complexes retained at the baseline. But as addition of amount of DNA increases, More DNA is observed progressing toward the positive electrode.

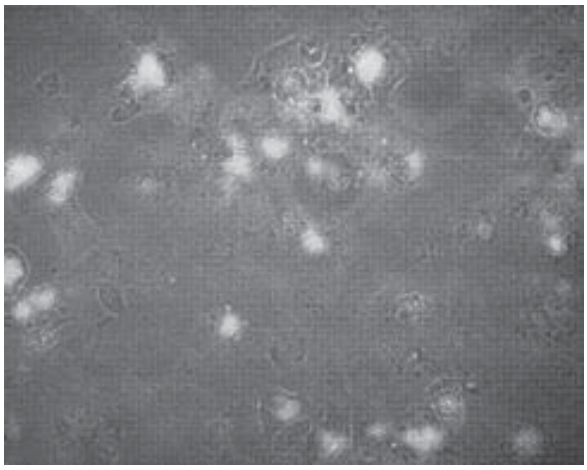

**Fig. 4.** Fluorescent image of transfected COS-7 cells based on the DNA nano-enricher.

### 3.3. Application of the novel gene carrier based on amino-modified silica coated magnetic nanoparticles in gene transfection

Based on the aforementioned analysis of interaction between the plasmid DNA and the amino-modified silica coated magnetic nanoparticles, the combination of the amino-modified silica coated magnetic nanoparticles with the plasmid DNA appeared to be quite effective. After being bound, the DNA was protected from digestion of DNaseI. All of advantages of the amino-modified silica coated magnetic nanoparticles provided theoretic foundation and practical guide to elucidate the potential of amino-modified silica coated magnetic nanoparticles as gene delivery. In this work, COS-7 cells were seeded in six-hole cell-dish in 5% $CO_2$ with an initial concentration of $1 \cdot 10^5$ cells/dish. The cells were cultured until the cover rate reached 50% ~ 70%. The nanoparticles were used to deliver plasmid DNA. The process was followed. The nanoparticles and plasmid DNA were mixed well with the mass ratio of 20:1 containing 1 μg of plasmid DNA, and incubated together at room temperature for 10 min. The cells were cultured with 1.5ml medium without serum after rinsing twice with 2 ml medium without serum. The nanoparticles-DNA complexes were added to the medium slowly and mix well. After the cells were incubated at 37 °C for 6 hours, the medium without serum was replaced with complete medium including 15% fetal bovine serum. The complexes possessed positive potential in neutral conditions and were fused with cell membrane easily through electrostatically interaction. And the plasmid DNA was delivered into cells. The transfected cells were observed by use of fluorescent microscope. The results showed that the nanoparticles DNA enricher could efficiently deliver DNA into COS-7 cells as well as mediate high expression of pIRGFP (Fig. 4).

## 4. CONCLUSIONS

In conclusion, this work is important because it provides a unique platform for delivery of DNA because of ease of fabrication, especially in contrast to the complex synthesis of modified nanoparticles. Indeed, The inorganic nanoparticles, such as hychoxyapatite and inert matrix material, e.g. silica, used as a non-viral gene carrier for DNA delivery has also been reported. However, the nanoparticles should be functionally modified with polycations or aminosilanes, which makes procedure more complex. In this regard, the preparation and modifica-

tion of the functionalized nanoparticles can be finished in one step. And the enriched plasmid DNA was protected effectively from enzymatic digestion of DNaseI. Further, this method maybe used in the filtration of the successfully transfected cells under the magnet.

## ACKNOWLEDGEMENTS

This work was supported by the Pre-Key Project of Basic Research of the Sci. and Tec. Ministry of China '2001-51', Key Project of Natural Science Foundation of P.R.China '20135010', National Key Basic Research Program (2002CB513100) and Oversea Youth Scholar Co-research Foundation of P.R.China (20028506).

## REFERENCES.

[1] O. Boussif, F. Lezoualch and M. A.Zanta // *Proc Natl. Acad. Sci. USA* **92** (1995) 7297.
[2] R. Reszka, J. H. Zhu and F. Weber // *J. Liposome Res.* **5** (1995) 149.
[3] M. Singh, M. Briones and G. Ott // *PNAS J* **97** (2000) 811.
[4] P. Blezinger, P. Blezinger, J. Wang, M. Gondo, A. Quezada, D. Mehrens, M. French, A. Singhal, S. Sullivan, A. Rolland, R. Ralston and W. Min // *Nature* Biotechnol. **17** (1999) 343.
[5] Y. Wang and D. Becker // *Nature Med.* **3** (1997) 887.
[6] M. C. Morris, L. Chaloin and F. Hetitz // *Curr. Opin. Biotechnol.* **11** (2000) 461.
[7] S. S. Davis // *Trends In Biotech.* **15** (1997) 217.
[8] A. R. Bender, H. V. Briesen and J. Kreuter // *Antimicrobial Agents & Chemotherapy* **40** (1996) 1467.
[9] M. Junghans, J. Kreuter and A. Zimmer // *Nucleic Acid Res.* **28** (2000) e45.
[10] D. Luo and W. M. Saltzman // *Nat. Biotech.* **18** (2000) 893.
[11] E. Dauty, J. S. Remy and T. B. Blessing // *J. Am. Chem. Soc.* **38** (2001) 9227.
[12] C. Kneuer, M. Sameti, E. G. Haltner, T. Schiestel, H. Schirra, H. Schmidt and C. M. Lehr // *Int. J. Pharm*. **196** (2000) 257.
[13] K. K. Sandhu, C. M. McIntosh, J. M. Simard and S. W. Smith // *Bioconjug. Chem.* **13** (2002) 3.
[14] X. X. He, K. M. Wang, W. H. Tan, B. Liu, X. Lin, S. S. Huang, D. Li, C. M. He and J. Li // *Chinese Sci. Bull.* **48** (2003) 223.
[15] X. X. He, K. M. Wang, W. H. Tan, B. Liu, X. lin, C. M. He, D. Li, S. S. Huang and J. Li // *J. Am. Chem. Soc.* **125** (2003) 7168.
[16] X. X. He, K. M. Wang, W. H. Tan, D. Xiao, X. H. Yang and J. Li, *SPIE, International Conference on Sensors Technology* **4414** (2001) 394.