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DNA/PEI nano-particles for gene delivery of rat bone marrow stem cells

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Abstract

The objective of this study was to deliver the DNA/PEI nano-particles into rat bone marrow stem cells (rBMSCs). The formation of nano-particles from DNA and PEI was performed by the adding of the PEI solution to the DNA solution. Gel retardation assay showed the optimal N/P charge ratio where PEI completely binds DNA. The particle size of DNA/PEI complexes measured by dynamic light scattering (DLS) showed diameters of about 200–400 nm. These results indicated that DNA was sufficiently condensed by PEI to give DNA/PEI nano-particles and the optimal packing was achieved at N/P charge ratio of 4. From AFM image, PEI completely condensed DNA into round-shaped. The diameters of the DNA/PEI nano-particles observed by AFM were in good agreement with the results of the DLS. rBMSCs viability in the presence of DNA/PEI nano-particles was evaluated by means of the MTT assay. The DNA/PEI nano-particles were transfected into rBMSCs. After gene transfection, enhanced green fluorescence protein (EGFP) was monitored by flow cytometry. Naked DNA showed no EGFP expression, whereas delivery of DNA/PEI nano-particles to rBMSCs showed EGFP expression and resulted in 2–10% transfection.
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Keywords: Gene delivery; Bone marrow stem cell; Nano-particle; Transfection

1. Introduction

Stem cells possess the inherent capability of transforming and differentiating into many cell types and specific cell type [1]. Thus, the use of stem cells is currently under intensive investigation such as cell-based therapies in regenerative medicine for neurological disease or injury, diabetes, and myocardial infarction [2–5]. The *in vitro* differentiated derivatives of stem cells through genetic manipulation are thought to be able to repair or replace damaged cells, tissues or organs. Gene delivery could be a powerful strategy for specific differentiation of stem cells through genetic manipulation since several transcription factors have been demonstrated to regulate stem cell differentiation to specific cells for various organs such as heart, pancreas, liver, and neurons [6,7]. Especially stem cells can be safely introduced into *in vivo* without immune-suppressants such as cyclosporine A [8]. Among several stem cells, bone marrow stem cells (BMSCs) are broadly studied on the aspect of multi-lineage differentiation [9] and rule out the ethical controversy.

Considering the existing approaches for gene delivery, the system using viral vectors such as retroviruses, adenoviruses, and adeno-associated viruses have been extensively investigated and shown superior transfection efficiency [10,11]. However, viral systems suffer from their potential life-threatening effects of immunogenicity and carcinogenicity. Thus, conventional viral ways of gene transfer to such progenitor cells suffer from a number of disadvantages such as safety issues. Recently, gene delivery using non-vial vectors have been received many attention because non-vial vectors possess several advantages such as easy preparation, stability, and safety, even though it has significant limitation in terms of efficacy [12–15]. Non-viral gene delivery systems can be classified into lipoplex and polyplex forming carriers. Among the cationic polymers

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to form polyplex, poly(ethyleneimine) (PEI), poly-L-lysine (PLL), and chitosan were reported. PEI was described as an effective transfection reagent due to easy available, easy functionalization, and low cost [16–18]. Previous study was reported that PEI has about two to fourfold effective than PLL [19]. The PEI forms complexes through condensation of negatively charged DNA via electrostatic interaction. The DNA/PEI complexes were entered into nucleus through several steps such as uptake into the cell, endosomal release, and decondensation of DNA inside or outside nucleus.

Several groups have successfully developed DNA nano-particles using PEI. Ideally, such gene delivery systems using nano-particles could protect DNA without the removal from systemic circulation by phagocytic cells [20–22]. To our knowledge, there are a few studies reported about the gene delivery into stem cells using nano-particles prepared from DNA and PEI [23]. Thus, the aim of this study is to evaluate gene delivery and transfection efficiency in rat bone marrow stem cells (rBMSCs) using DNA/PEI nano-particles.

2. Experiment

2.1. Materials

Branched PEI (M_n: 10 kDa) was purchased from Aldrich and lipofectamine^{TM} as a reference was purchased from Invitrogene. All other reagents were commercially available special grade reagents.

2.2. Plasmid DNA preparation

The plasmid used in this study was plasmid enhanced green fluorescence protein (pEGFP, Clontech). The plasmid was expanded in *Escherichia coli* (*E. coli* strain DH5α) and purified with QIAfilter Maxi Kits (Qiagen) following the manufacturer's protocol. The quantity and quality of the purified plasmid DNA were assessed by optical density at 260 and 280 nm and by electrophoresis in 0.8% agarose gel. The purified plasmid DNA was resuspended in PBS buffer and kept in aliquots at a concentration of 0.8 mg/ml.

2.3. Formation of DNA/PEI nano-particles

Complexes of PEI and plasmid DNA were formed by first diluting plasmid and the addition of appropriate amount of optimum medium. The PEI solution was then added to the plasmid solution to achieve the desired N/P charge ratio and vortexed immediately. The complexes were allowed to interact for 15 min before use. The PEI concentrations were calculated to make the desired N/P charge ratio for 1 μg of plasmid, assuming that 43.1 g/mol corresponded to each repeating unit of PEI containing one nitrogen atom and 330 g/mol corresponded to each repeating unit of DNA containing one phosphorus atom.

2.4. Agarose gel retardation assay

DNA/PEI complexes were prepared at N/P ratio of 0–32. Loading dye (1 μl 10× Blue Juice, Sigma) was added to the samples. Aliquots of the solutions (10 μl) were loaded onto 0.8% agarose gel stained with 25 μl of 12.5 μg ethidium bromide solution and run on an electrophoresis cell. A UV image station (BioDok-ItTM Imaging System) was used to record gel images.

2.5. Particle size analysis

The particle size of DNA/PEI suspension (1 ml) was determined by dynamic light scattering (DLS, ELS-8000, Photal, Japan) at room temperature. Atomic force microscopy (AFM) measurements were carried out in the tapping mode with a Nanoscope IV instrument (Digital Instruments Inc.).

2.6. Rat bone marrow stem cell isolation

The rBMSCs were harvested from the femurs and tibias of 5-week-old female Fischer rats. Briefly, bones were aseptically excised from the hind limbs of rats following ether euthanasia. The soft tissue was removed, and the femur and tibia were placed in 50 ml DMEM. The proximal end of the femur and distal end of the tibia were removed using sterile scissor. A hole was then created in the knee joint end of each bone marrow was flushed from the shaft with phosphate-buffered saline (PBS) (pH 7.4) with a 26-gauge needle. The marrow solution was then resuspended in PBS and the combined cell suspensions from all bone marrow were placed in a centrifuge tube and spun down at 300 × *g* for 5 min. The supernatant was aspirated, and the pellet was resuspended in fresh primary media (DMEM supplemented with 10% fetal bovine serum (Gibco BRL), 100 U/ml penicillin, and 100 g/ml streptomycin) and seeded into tissue culture flasks at 1×10^5 cells/cm^2. The flasks were rinsed three times with PBS on the second day of expansion to remove the non-adherent cells. The medium was then exchanged every 3–4 days throughout the studies. Adherent cells were rinsed thoroughly with PBS and trypsinized for use in the gene delivery.

2.7. MTT assay

Cell viability was determined by using water-soluble enzyme substrate MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide) which was converted to blue water-insoluble product formazan accumulated in the cytoplasm of viable cells. Cell viability of 3-well plates performed individually and then calculated as average value. In brief, 100 μl of PBS solution of the MTT tetrazolium substrate (5 mg/ml) was added at 24 h after post transfection. After incubation for 4 h at 37 °C, the resulting violet formazan precipitate was solubilized by the addition of 1 ml of DMSO and shaken for 30 min. The solutions were then read using a plate reader of an ELISA (E-max, Molecular Device, USA). The optical density of each well determined at 590 nm.

2.8. Transfection experiments

rBMSCs were seeded in 12-well plates at a density of 3×10^4 cells/well for 24 h before transfection. One microgram of plasmid DNA loaded at each well. Complexes of DNA/PEI

were prepared using N/P charge ratio of 8 and 16, whereas complexes composed of 3 μl lipofectamine and 1 μg plasmid were added to each well as reference. Cells were incubated for 4 h with each complex. The cells were then washed and replated with culture medium. At 24 and 48 h after the transfection, transfection efficiency was determined by flow cytometry. Transfection experiments were performed in triplicate.

2.9. Flow cytometry

Cells were analyzed on a flow cytometer (Beckman-Coulter, USA) equipped with laser. Fluorescence was detected using 520 and 570 nm bandpass filters for EGFP. Approximately 10,000 events were acquired per sample. Data analysis was carried out using the program WinMDI.

3. Results and discussion

3.1. Nano-particle formation from DNA and PEI

The condensation of polyanionic DNA into nano-particles is an important prerequisite for gene delivery employing poly cations [24–26]. Thus, the formation of DNA/PEI polyplex nano-particles was performed by the adding of the PEI solution to the DNA solution. Gel electrophoresis showed the optimal charge ratio where DNA completely binds with PEI as shown in Fig. 1. DNA with PEI completely condensed to the nano-particles at N/P charge ratio above 4. Moreover, particle size showed diameters of about 200–400 nm as summarized in Table 1. This indicated that amine cationic group in PEI condensed with anionic group of DNA through electrostatic interaction to give DNA/PEI nano-particles. AFM was also used to characterize structure and morphology of nano-particles. DNA/PEI complex formed at N/P charge ratio of 8 was imaged as shown in Fig. 2 and particle diameters measured in tapping mode. The diameters of all DNA/PEI nano-particles observed by AFM were in good agreement with the results of the DLS. The nano-particles were spherical in shape. These results indicated that DNA was sufficiently condensed by PEI.

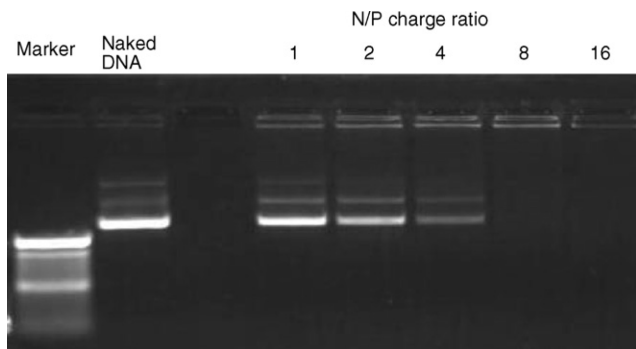

Fig. 1. The effect of increasing N/P charge ratio on the electrophoretic migration of plasmid DNA through 0.8% agarose gel (DNA was visualized by ethidium bromide staining). Increasing amounts of PEI were added to a constant amount of 1 μg DNA. The complex composition was calculated on basis of PEI nitrogen per DNA phosphate and expressed as DNA/PEI equivalents.

Table 1
Particle size of nano-particles formed with plasmid DNA and PEI at different N/P ratios

N/P charge ratio	Particle size (nm)[a]
0.5	231
1	428
2	320
4	527
8	283
16	306
32	420

[a]Measured by the DLS.

3.2. Cytotoxicity

Cytotoxicity might be induced by electrostatic interactions between polycations and cell membranes [27,28]. Thus, it depended on the concentration of excess cationic charge of DNA/PEI nano-particles. The cytotoxicity of DNA/PEI nano-particles at different N/P charge ratios was analyzed (Fig. 3). N/P charge increasing exhibited low cell viability, indicating that increasing amount of PEI affected the cytotoxicity due to

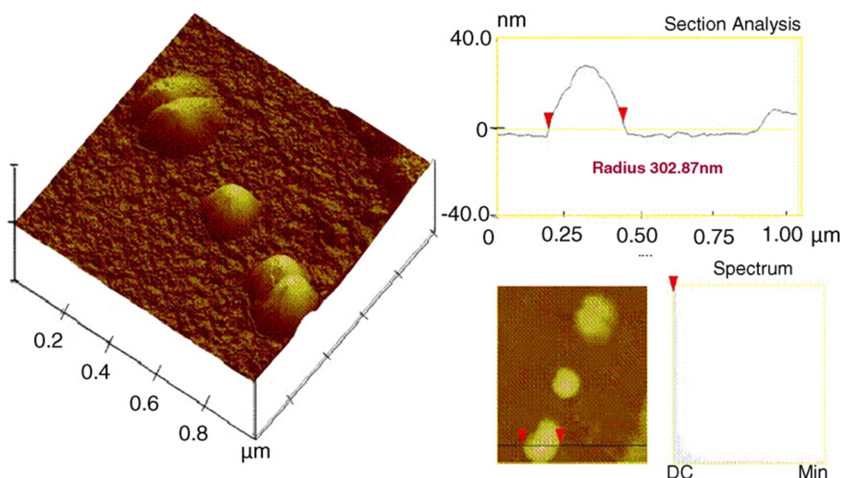

Fig. 2. AFM image of DNA/PEI complexes at N/P charge ratio of 8.

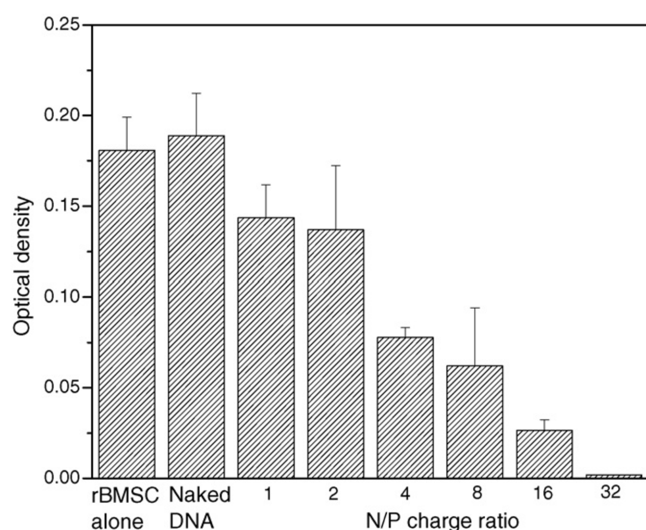

Fig. 3. rBMSCs viability in the presence of plasmid DNA and DNA/PEI complexes with different N/P charge ratios.

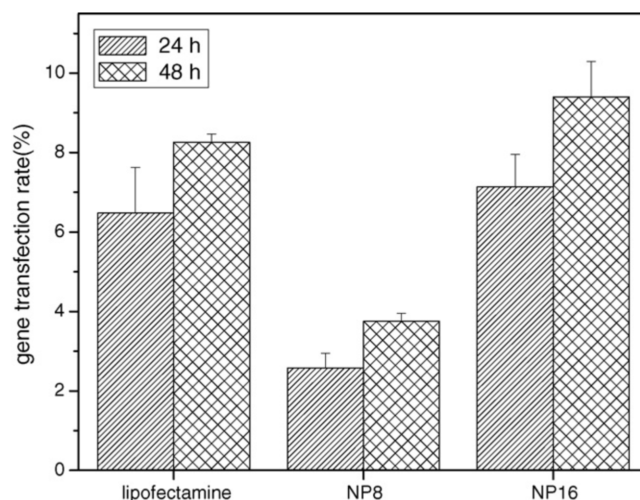

Fig. 5. Transfection results of rBMSCs from FACS, 24 and 48 h post-transfection using lipofectamine^{TM}, PEI (N/P charge ratio of 8) and PEI (N/P charge ratio of 16).

increasing cationic charge. Collectively, these results implied that DNA/PEI complexes of N/P charge below 16 might be used to safely deliver gene as the optimal N/P charge ratio. Thus, this experiment was limited up to N/P charge of 16 as most efficient ratio.

3.3. Transfection efficiency

DNA/PEI nano-particles prepared at different N/P charge ratios of 8 and 16 by constantly keeping the amounts of plasmid DNA. Hence, equal volumes of nano-particles suspension were added to the cells, each containing 1 μg of plasmid DNA per well. DNA/PEI nano-particles were incubated with rBMSCs. Transfection efficiencies were compared to the following: naked DNA, lipofectamine^{TM}, and DNA/PEI nano-particles at N/P charge ratio of 8, 16 and 32. After 24 and 48 h, rBMSCs were analyzed by fluorescence microscopy (Fig. 4). No EGFP expression was observed at naked DNA, while DNA/PEI nano-particles and lipofectamine^{TM} produced green fluorescent cells after gene transfection. DNA/PEI complexes with N/P charge of 16 exhibited large number of green fluorescent cell than that of 8. A higher N/P charge ratio of 32 resulted in severe cytotoxicity, which could be observed microscopically, as well as strongly reduced green cells (data not shown). By increasing culture time, fluorescence microscopy showed a more green fluorescence cells. We attempted to determine the transfection efficiency. Cells were harvested and analyzed by flow cytometry. Obviously, DNA/PEI nano-particles and lipofectamine^{TM} displayed effective ability to achieve transfection ratio of 2–10%, much greater than those observed for naked DNA (Fig. 5). DNA/PEI nano-particles of N/P charge ratio of 16 exhibited a similar transfection efficiency with lipofectamine^{TM} and approximate twofold higher transfection efficiency than that of 8. Transfection efficacy of the nano-particles increased with increasing N/P

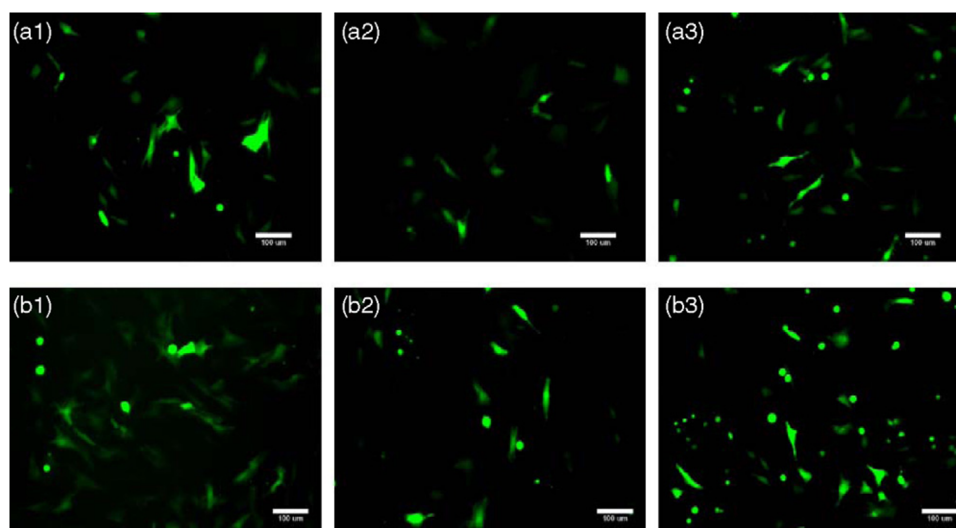

Fig. 4. Green fluorescent images of rBMSCs (a) 24 h and (b) 48 h post-transfection using: (1) lipofectamine^{TM}; (2) PEI (N/P charge ratio of 8); (3) PEI (N/P charge ratio of 16).

charge ratio. An increase in incubation time also leads to an increase in transfection efficiency. This is obviously due to the cell proliferation. A further increase in incubation time after 48 h was not examined, due to an increase in PEI cytotoxicity after longer incubation periods.

4. Conclusion

In this study, we tried to the first time to evaluate the most effective nano-particles with PEI and DNA for rBMSCs. The nano-particles were formed by the addition of PEI to DNA solution and the formation of nano-particles was confirmed by DLS and AFM. The particle size of DNA/PEI complexes showed diameters of about 200–400 nm. From AFM image, PEI completely condensed DNA into round-shaped DNA complexes. These results indicated that DNA was sufficiently condensed by PEI to give DNA/PEI nano-particles. The increasing cationic charges of DNA/PEI nano-particles have been shown to cause cytotoxicity. The DNA/PEI complexes with N/P charge ratios of 8 and 16 were transfected into rBMSCs. Naked DNA showed no EGFP, whereas delivery of DNA/PEI nano-particles to rBMSCs resulted in around 2–10% transfection. In conclusion, we have shown that DNA/PEI nano-particles allow for gene delivery into rBMSCs.

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References

[1] Z. Lin, C. Willers, J. Xu, M.H. Zheng, Tissue Eng. 12 (2006) 971.
[2] O. Lindvall, Z. Kokaia, Nature 29 (2006) 1094.
[3] S. Lim, W. Chang, S. Lee, B.W. Song, H.J. Kim, M.J. Cha, S.Y. Kwon, S.M. Han, Y.S. Jang, N.S. Chung, K.C. Hwang, Tissue Eng. Reg. Med. 3 (2006) 301.
[4] D.I. Kim, J.E. Lim, A.K. Kim, H.S. Eo, H.J. Jeon, J.S. Kim, I.S. Jang, S.W. Cho, B.S. Kim, Tissue Eng. Reg. Med. 3 (2006) 125.
[5] W. Chang, J.Y. Kim, S. Lim, S. Lee, B.W. Song, H.J. Kim, M.J. Cha, S.Y. Kwon, S.M. Han, B.H. Min, Y. Jang, N. Chung, K.C. Hwang, Tissue Eng. Reg. Med. 3 (2006) 327.
[6] P.S. Becker, Acta Haematol. 114 (2005) 188.
[7] A. Srivastava, Hum. Gene Ther. 16 (2005) 792.
[8] S. Aggarwal, M.F. Pittenger, Blood 105 (2005) 1815.
[9] P.H. Krebsbach, S.A. Kuznetsov, P. Bianco, P.G. Robey, Crit. Rev. Oral Biol. Med. 10 (1999) 165.
[10] A. Breen, P. Strappe, A. Kumar, T. O'Brien, A. Pandit, J. Biomed. Mater. Res. A 78 (2006) 702.
[11] C.E. Lilley, R.H. Branston, R.S. Coffin, Curr. Gene Ther. 1 (2001) 339.
[12] S. Nimesh, R. Kumar, R. Chandra, Int. J. Pharm. 320 (2006) 143.
[13] M.S. Kim, S.L. Diamond, Bioorg. Med. Chem. Lett. 16 (2006) 4007.
[14] Y.C. Liu, H.L. Chen, C.J. Su, H.K. Lin, W.L. Liu, U.S. Jeng, Macromolecules 38 (2005) 9434.
[15] J.H. Chung, K.C. Hwang, Y.S. Jang, Tissue Eng. Reg. Med. 3 (2006) 268.
[16] P. Banerjee, R. Weissleder, A. Bogdanov, Bioconjug. Chem. 17 (2006) 125.
[17] E. Kleemann, M. Neu, N. Jekel, L. Fink, T. Schmehl, T. Gessler, W. Seeger, T. Kissel, J. Control. Release 109 (2005) 299.
[18] W.T. Godbey, K.K. Wu, A.G. Mikos, J. Control. Release 5 (1999) 149.
[19] L.L. Farrell, J. Pepin, C. Kucharski, X. Lin, Z. Xu, H. Uludag, Eur. J. Pharm. Biopharm. 65 (2007) 388.
[20] B. Brissault, C. Leborgne, C. Guis, O. Danos, H. Cheradame, A. Kichler, Bioconjug. Chem. 17 (2006) 759.
[21] M.S. Kim, S.L. Diamond, Bioorg. Med. Chem. Lett. 16 (2006) 5572.
[22] G. Özgel, J. Akbuğa, Int. J. Pharm. 315 (2006) 44.
[23] A. Eguchi, H. Furusawa, A. Yamamoto, T. Akuta, M. Hasegawa, Y. Okahata, M. Nakanishi, J. Control. Release 104 (2005) 507.
[24] T. Hwang, H.D. Han, D.H. Seo, H. Seong, J.H. Kim, B.C. Shin, Tissue Eng. Reg. Med. 3 (2006) 114.
[25] S.W. Kim, T. Ogawa, Y. Tabata, I. Nishimura, J. Biomed. Mater. Res. 71 (2004) 308.
[26] P. Chen, Colloids. Surf. A: Physicochem. Eng. Asp. 261 (2005) 3.
[27] N. Oku, Y. Yamazaki, M. Matsuura, M. Sugiyama, M. Hasegawa, M. Nango, Adv. Drug Deliv. Rev. 52 (2001) 209.
[28] J. Kloeckner, S. Bruzzano, M. Ogris, E. Wagner, Bioconjug. Chem. 17 (2006) 1339.