Role of weakly polarized nanoparticles in electroporation†

Jeong Ah Kim[a] and Won Gu Lee*[b]

Received 28th June 2010, Accepted 3rd September 2010
DOI: 10.1039/c0nr00448k

In this study, we present a role for weakly polarized nanoparticles as engineered gene transporters that can enhance electromediated gene delivery. To validate this proof-of-concept, fluorescent poly(ethylene glycol) (PEG)-coated silica nanoparticles (SiNPs) with opposite polarities, SiNPs(RITC)-PEG/PTMA(+) and SiNPs(RITC)-PEG/PMP(−), are used. To investigate the electroporative uptake direction of the nanoparticles–gene complex, we employ microscale electroporation to generate more symmetric and uniform electric fields. The effect of the polarity of the nanoparticles on EGFP gene transfection efficiency in HeLa cells is measured by flow cytometry analysis. The results show that, compared to cationic nanoparticles, anionic nanoparticles have potential as electromediated gene transporters at a low gene concentration. Furthermore, we believe that this finding can be useful for developing a platform that enables electroporation-based gene/drug delivery associated with functional nanoparticles.

Introduction

Today's pharmaceutical and biotechnological industries are striving to overcome the obstacles to safe and efficient gene delivery.[1] Gene delivery can be used to develop gene therapy or vaccination methods that enable the treatment of severe diseases such as heart diseases,[2] hepatic carcinoma,[3] and chronic inflammatory diseases.[4] One of the challenges of gene delivery is to develop a nonviral synthetic delivery method that can enhance the overall efficiency from uptake to transfection.[5] Since the transfection efficiency (TE) depends on the efficiency of gene uptake, delivery, and expression, a number of uptake quantities of target genes are a prerequisite for enhanced gene therapy.

Polymer-based gene delivery is emerging as a useful method for gene uptake and delivery applications.[6] Polymeric nanoparticles have been used to combine the salient features of viral and nonviral vectors as drug or gene carriers.[7–11] Since silica is a biocompatible material, silica nanoparticles (SiNPs) have been used for a number of pharmaceutical applications.[12–19] In addition, the SiNPs can be used as surface modifiers by controlling several properties such as zeta-potential and surface reactivity.[12] Poly(ethylene glycol) (PEG), a biocompatible material for fabrication, patterning and coating nano/microstructures for biological applications, can be used to modify the SiNPs.[20–23] The modified SiNPs could also be used to enhance gene uptake and expression by condensing gene-vector complexes in the vicinity of cells *via* gravity.[24]

Electroporation is one of the most efficient nonviral gene delivery methods. For clinical applications, however, it still has limitations such as low cell viability due to large electrodes and difficulty of transferring the electroporated cells into fresh media in a rapid manner. To address these issues, microscale electroporation methods have been developed and reviewed for a wide variety of applications, such as single cell electroporation and analysis in microfluidic environments.[25–27] In our previous study, we suggested the concept of microchannel electroporation within poly(dimethylsiloxane) (PDMS) channels and examined the effects of the channel geometries on the efficiency of cell electroporation.[28,29] In addition, we observed an impulsive, reversal flow against electroosmosis within the channel during electroporation and derived a flow model associated with acoustic gas evolutions.[30] More recently, we developed a microcapillary-type electroporation device that can increase both cell viability and transfection levels during electroporation.[31] This approach can be used to decrease a number of adverse effects such as large pH variations and electrode contamination due to large electrodes, enabling safer transfer of electroporated cells to fresh media through a microcapillary in a rapid and quantitative manner.

Here, we present a role for PEG-modified SiNPs as engineered gene transporters during electroporation. The hypothesis is that anionic SiNPs have the potential to promote electromediated uptake of negatively charged pEGFPs into living cells because the SiNPs can move in the same uptake direction as pEGFPs. We previously demonstrated that negatively charged DNAs can be used as gene transporters and improve the transfection efficiency of target genes in electro-mediated gene delivery.[32] Based on our previous work, we characterized the effect of weakly polarized SiNPs as engineered gene transporters on transfection efficiency in electroporation by flow cytometry analysis. The results were in good agreement with our hypothesis for a lower gene concentration level. Here we aimed to use weakly polarized nanoparticles to avoid unwanted electrical aggregates or strong bonding between gene-SiNPs complexes during electroporation.

Material and methods

Analytical procedures

To investigate the effect of the SiNPs on electromediated gene delivery, we analyzed the electroporation under different

[a]*School of Chemical & Biological Engineering, Seoul National University, 599 Gwanangno, Gwanak-gu, Seoul, 151-742, Republic of Korea*
[b]*Department of Mechanical Engineering, College of Engineering, Kyung Hee University, Seochon-dong, Giheung-gu, Yongin-si, Gyeonggi-do, 446-701, Republic of Korea. E-mail: termylee@khu.ac.kr; Fax: +82 31 202 8106; Tel: +82 31 201 3321*

† Electronic supplementary information (ESI) available: Supplementary Figures S1 and S2. See DOI: 10.1039/c0nr00448k

electrolyte conditions such as in SiNPs-suspended solutions. The overall experimental scheme is illustrated in Supplementary Figure S1.† Briefly, the effect of oppositely charged SiNPs on the TE can be examined with the following sequential processes: (i) preparation of oppositely charged, fluorescent SiNPs, (ii) preparation of biological samples, (iii) sample mixing and loading for electroporation, (iv) electroporation, (v) post-incubation (~24 h), and finally (vi) flow cytometry analysis.

Preparation of SiNPs with different polarities

Nanoparticles can be coated with a shell of stable and biocompatible silica to avoid potential toxic effects on cells.[33] The polyvinylpyrrolidone method was applied to particles having ionic surface charges to generate a sol–gel silica coating, the thickness of which could be altered by varying the amount of tetraethoxysilane loaded.[13] In our experiments, these SiNPs were characterized for polarity and then purchased from Biterials, Inc., South Korea. Briefly, the zeta potential of the nanoparticles was generated to give either a positive zeta potential (+4.06 mV) or a negative one (−5.51 mV) at pH 7.4. To focus more on the effects of the polarity of the SiNPs on gene transfection, the size was predetermined at 50 nm in average diameter (Supplementary Figure S2†). Note the size of these nanoparticles can be controlled by the concentration of the sol–gel solution. RITC, a luminescent organic dye, was incorporated into the silica shells by covalent coupling with the sol–gel precursor. The final concentration of the synthesized SiNPs was 4.5 mg ml^{-1} in deionized water. For cell electroporation, these SiNPs were centrifuged at 13 000 rpm for 5 min. Note that the concentration of the SiNPs can be changed, corresponding to specific samples, *e.g.*, RNA and DNA. Most of the supernatant was drawn off and replaced with Dulbecco's phosphate-buffered-saline (DPBS) without Mg^{2+} and Ca^{2+}.

TEM imaging

The transmission electron micrograph (TEM) image was taken on the JEM3010. The voltage was adjusted to 300 kV. The magnification was 10^5 and the exposure time was 1.0 s.

Measurement of zeta potential

The distribution of electrokinetic mobility and the zeta-potential for the oppositely charged SiNPs(RITC)-PEG were measured by using the Smoluchowski equation (Supplementary Figure S2†). In the case of SiNPs(RITC)-PEG/PTMA(+), the mobility and the zeta potential were 2.892×10^{-5} cm^2 V^{-1} and +4.06 mV, respectively; and for SiNPs(RITC)-PEG/PMP(−), -3.893×10^{-5} cm^2 V^{-1} and −5.51 mV. The migration was driven by the average electric field of −28.27 V cm^{-1}. The solvent was deionized water at 20.2 °C with a viscosity of 0.9954 cP and a dielectric constant of 80.4133.

Preparation of cell and gene samples

HeLa cells were cultured as a monolayer in Dulbecco's modified Eagle's medium (DMEM, HyClone, USA), containing 10% fetal bovine serum (FBS, Sigma), penicillin (100 units mL^{-1}), streptomycin (100 μg mL^{-1}) and L-glutamine (4 mM) at 37 °C in a humidified 5% CO_2 incubator. Cells were detached from the tissue culture flask using Trypsin–EDTA. The final cell concentration was adjusted from 3×10^6 to 5×10^6 cells mL^{-1}. The pEGFP-N1 gene was used as a marker of successful gene transfection. We used a Plasmid Isolation Kit (Qiagen, Germany) to extract and purify the pEGFP-N1 plasmids carrying the GFP from transformed *Escherichia coli* (DH5-α). The plasmid pEGFP-N1 was added to the final solution at a normal concentration of 0.05 μg μL^{-1}. One hundred microlitres (4.5 mg mL^{-1}) of a sonicated suspension of these SiNPs was added to 100 μL of HeLa cells with different pEGFP concentration levels.

Microscale electroporation

The electromediated delivery of pEGFP into HeLa cells in two different SiNPs-suspended media was performed at two different concentrations (pEGFP: 0.05 and 0.005 μg μL^{-1}). Electroporation was performed with the Microporator™ (Digital Bio Technology, Inc., South Korea). The biggest difference between this device and a conventional electroporator is related to its reaction chamber.[26] The protocol for HeLa cells was the following: electric field (335 V cm^{-1}), pulse width (35 ms), the number of pulses (twice), and the period of pulse (1 s). After electroporation, all samples were immediately transferred into 6-well plates filled with DMEM (1 mL $well^{-1}$).

Flow cytometry analysis

To examine the GFP expression efficiency, electro-transfected HeLa cells were detached from the well plate with Trypsin–EDTA, washed with PBS, and suspended in DMEM. The prepared sample was added into sample tubes for the flow cytometry analysis, which was quantitatively performed on the FACS Calibur (Becton Dickinson, USA) and analyzed with Win MDI software. For each sample, 10 000 events were collected. The transfection efficiency of pEGFP into HeLa cells at a normal gene concentration of 0.05 μg μL^{-1} and 10-times dilution of 0.005 μg μL^{-1} was measured, respectively (Fig. 3 and 4).

Fluorescence imaging

Fluorescent images of GFP transfection of HeLa cells were taken using the GFP (excitation at 488 nm and emission at 522 nm) and the RITC (excitation at 540 nm and emission at 560 nm) filters. All fluorescent images were collected on an inverted biological microscope IX71 (Olympus, Japan).

Statistical analysis

Transfection experiments were performed in triplicate and the efficiency value was represented as the mean ± S.D. Comparison between groups was analyzed using the unpaired *t*-test. The value of $p < 0.05$ was considered statistically significant.

Results and discussion

Electroporation is induced by transmembrane voltage *via* an electric field. Numerous pores are created on the cell membrane by the electric shock. In the hypothesis relating to the effect of relative uptake polarity, negatively charged DNAs and anionic

SiNPs can be delivered together in the same direction under electrodynamic force or electropulsation-driven flow (Fig. 1).[30] Our results show that anionic SiNPs could assist with greater pEGFP uptake into the cells with less aggregation (Fig. 2). On the other hand, the results also indicate that nonspecific binding by the aggregated pEGFP-SiNPs complexes might occur at the outer cell membrane.

Aggregation can potentially increase the size of those complexes. The large or electrically neutral particles are unlikely to obey the external electric field. The aggregated pEGFP-SiNPs might be slowly delivered into the cells even in the presence of the electric field. It is also anticipated that the delivery efficiency is inversely proportional to the square of the size of the pEGFP.[34,35] A bigger pEGFP-SiNPs complex may decrease the efficiency of gene uptake and delivery.

The electrically neutral complexes could increase the brightness of rhodamine B-isothiocyanate (RITC) by gathering close together. These aggregations could then promote endocytotic uptake in the vicinity of the cell membranes after electroporation. In our experiments, however, endocytotic uptake was rarely observed. To block endocytotic uptake, the samples could be pre-incubated before mixing to prevent such nonspecific adsorption by endocytosis.[36]

To examine the effects of the polarity of the SiNPs on the transfection, flow cytometry analysis was conducted (Fig. 3 and 4). The experiments were performed three times under the same conditions. The results show that the TE of pEGFP in two different SiNPs-suspended media was lower than that of pEGFP electroporation alone for a normal gene concentration of 0.05 μg μL^{-1} (Fig. 3 and 5A). The SiNPs-PEG/PTMA (5-propylsulfonyl-oxyimino-5*H*-thiophen-2-ylidene-(2-methylphenyl)acetonitrile) had much lower TE than SiNPs-PEG/PMP (poly(4-methyl-2-pentyne)) (Fig. 3). In addition, the final concentration of target

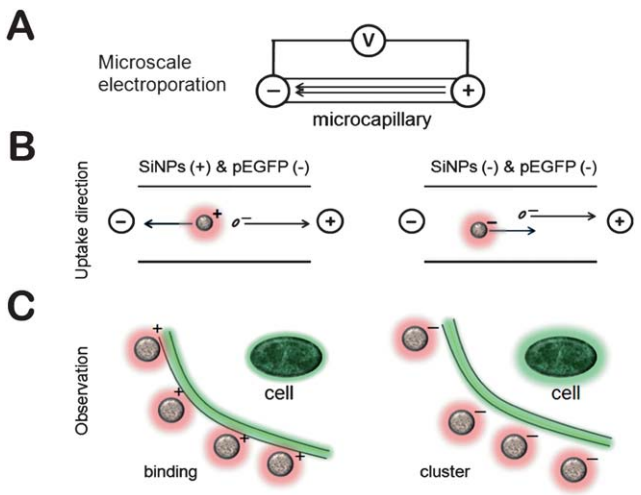

Fig. 1 Schematic of the concept of polarizing nanoparticles for electroporation: an approach to enhance uptake of SiNPs-gene complex. (A) Schematic for microscale electroporation that can produce more uniform or symmetric electric field in a microstructure. (B) Schematic for uptake direction of polarized particles or molecules under an electric field. (C) A cartoon for observed images of cells after electroporation: cationic SiNPs showed preference for nonspecific binding to cell membranes, while anionic SiNPs formed clusters in the vicinity of cells.

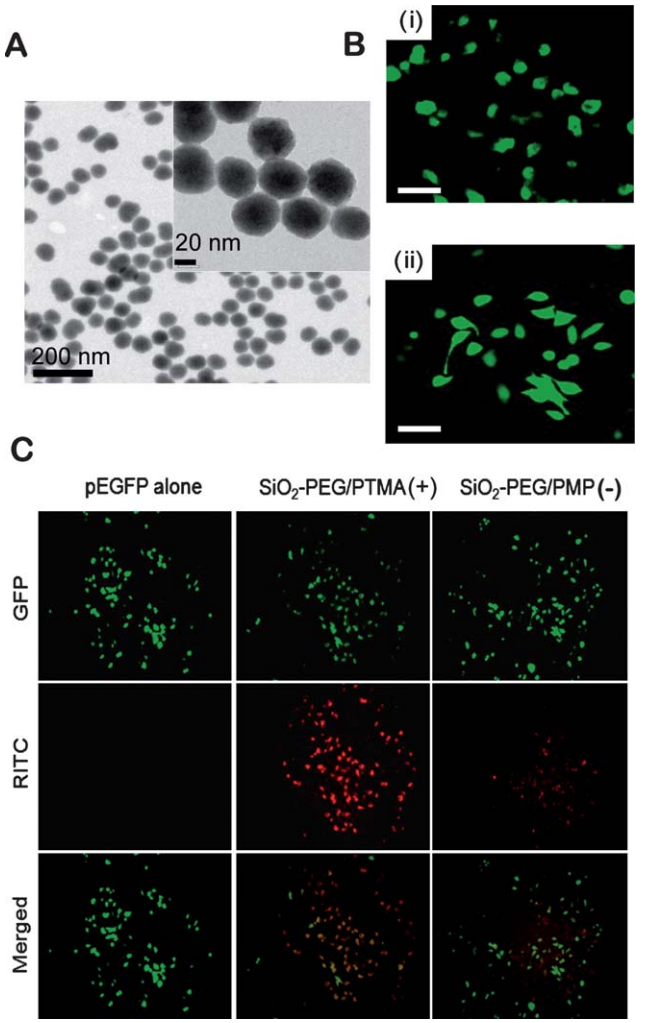

Fig. 2 Experimental results for electroporation of SiNPs-gene complex with different polarities. (A) TEM image of the SiNPs. The average size is 50 nm. Scale bar is 200 nm. (Inset) Close-up image. Scale bar is 20 nm. (B) Fluorescence images (GFP) of HeLa cells after 24 h incubation. (i) Cells electroporated with SiNPs/PEG-PTMA(+) and (ii) SiNPs/PEG-PMP(−). Scale bar is 30 μm. (C) Representative cell images after electroporation: (left) pEGFP alone, (middle) pEGFP in cationic SiNPs(RITC)-PTMA suspended solution, and (right) pEGFP in anionic SiNPs(RITC)-PMP. Green and red colors indicate GFP and RITC, respectively. The magnification is 200×. Scale bar is 100 μm.

genes could be diluted through unwanted aggregation with SiNPs at the cell surface in the mixtures. Based on these results, two assumptions can be made: First, the electrodynamic motion of pEGFP is accelerated by the same uptake direction of anionic SiNPs-PEG/PMP(−) under the electric field. Second, the opposite polarity of cationic SiNPs-PEG/PTMA(+) can considerably decrease the quantity of freely moving pEGFPs in solution.

However, for a lower gene concentration, *e.g.*, 10-times dilution of pEGFP, the SiNPs-PEG/PMP(−) had the highest transfection level (Fig. 4 and 5B). These results show that anionic SiNPs play a potentially significant role in assisting uptake of target genes in the presence of an electric field. For a freely moving pEGFP, even at a lower gene concentration level, the same polarity of anionic SiNPs might enhance the uptake

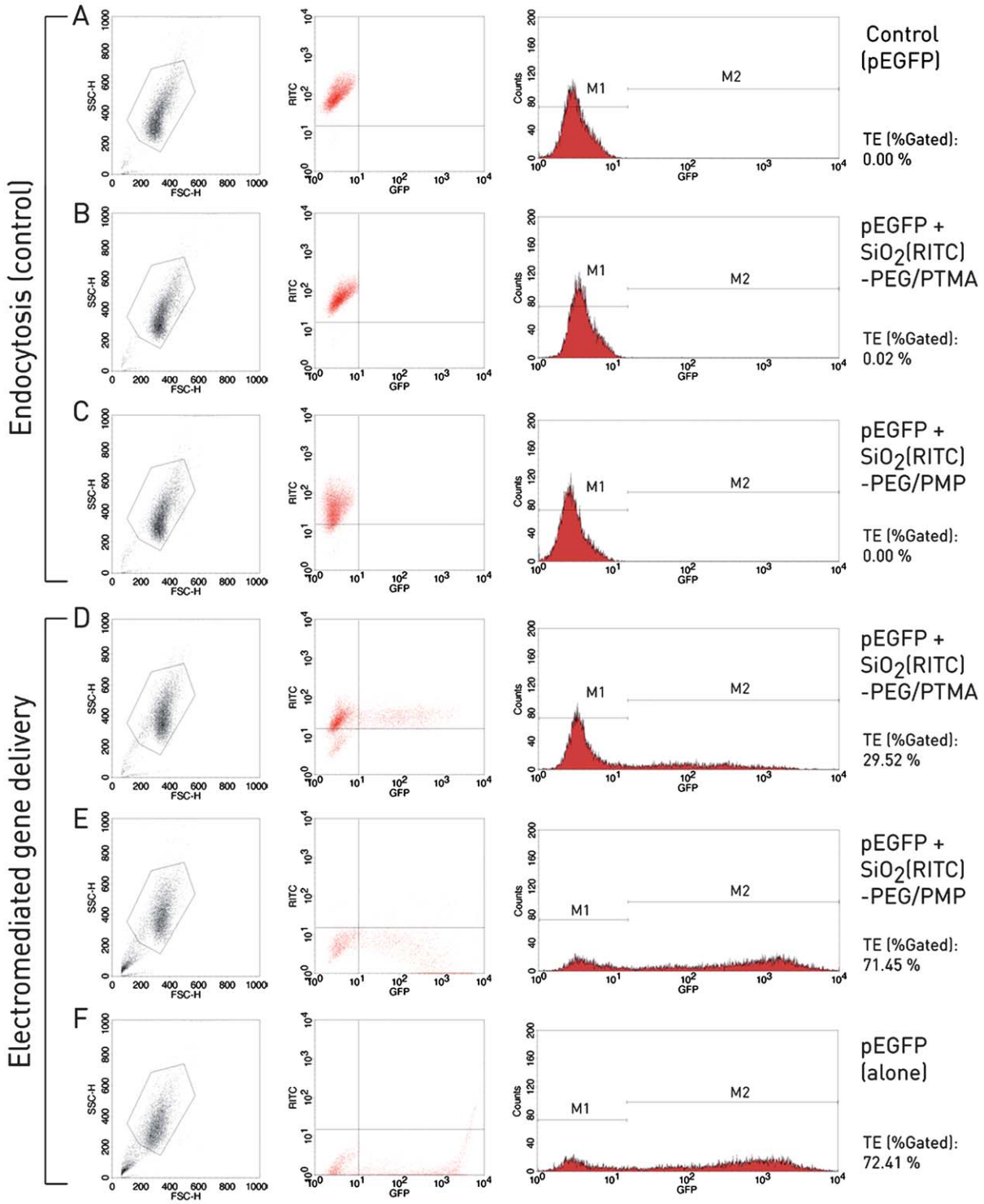

Fig. 3 Summary of the flow cytometry analysis of the transfection efficiency of pEGFP into HeLa cells *via* endocytosis and electroporation at a normal ratio of 0.05. The flow cytometry analysis of the transfection efficiency of pEGFP into HeLa in SiNPs (RITC)-PEG suspension (4.5 mg mL^{-1}): comparison of endocytosis (*A* to *C*) and electroporation (*D* to *F*). The 1st columns show the gated region (negative cells) of the sample. The 2nd columns show the EGFP-positive and the RITC-positive cells, respectively. The last columns show the histogram of counted EGFP-positive cells, *i.e.*, GFP transfection efficiency. M1: a marker for the GFP-negative cells, and M2: a marker for the GFP-positive cells. The electrotransfection efficiency of pEGFP into HeLa cells can be quantitatively obtained by counting the number of GFP-positive cells from the gated events of total events (10 000 cells).

directionality of the target genes. These results imply that anionic SiNPs are of great benefit to enhance the electrophorative uptake of negatively charged biological molecules such as DNA or RNA. This is in good agreement with the finding that carrier genes can enhance the delivery efficiency of target genes into the cells when used together.[32] In contrast, the cells with the

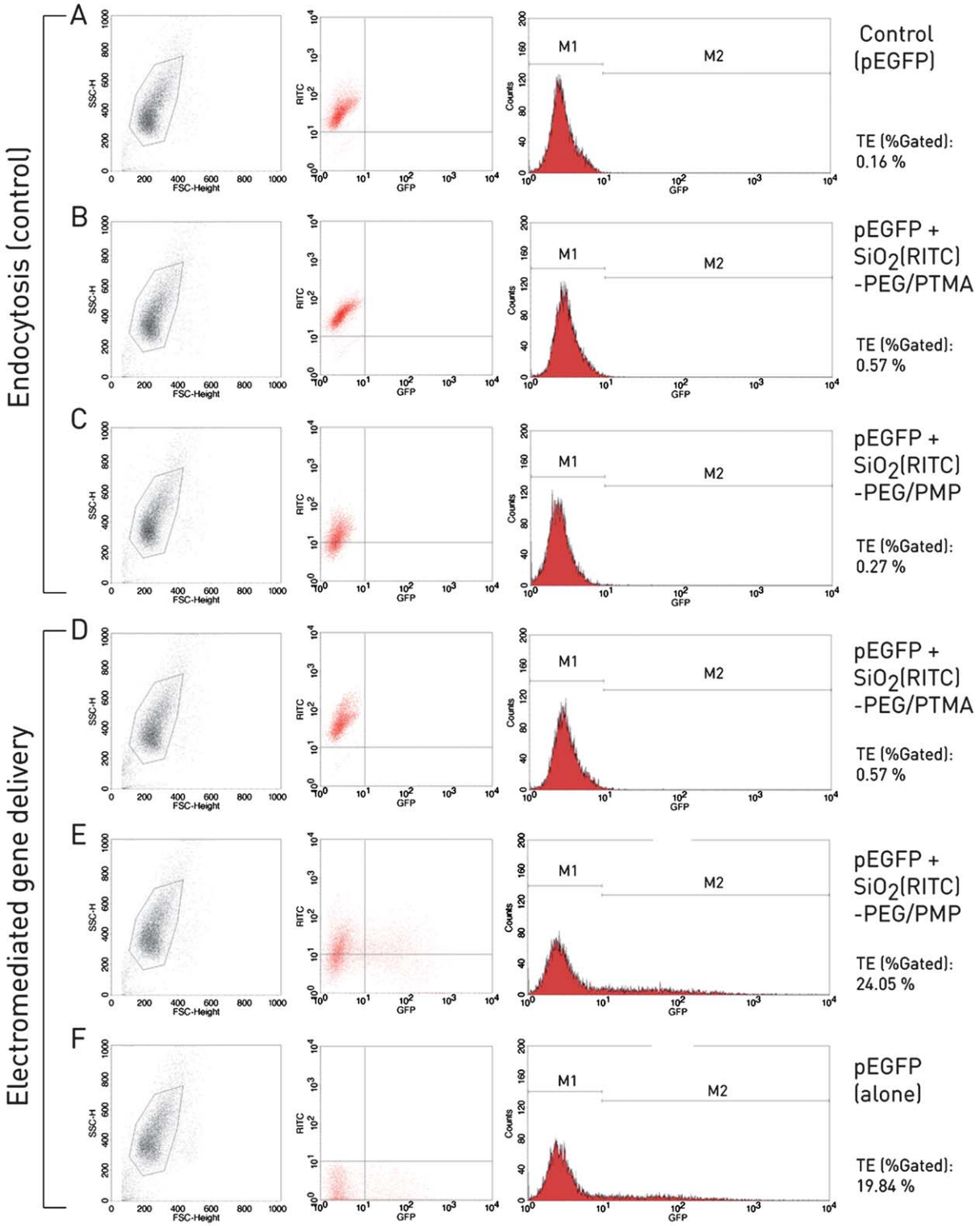

Fig. 4 Summary of the flow cytometry analysis of the transfection efficiency of pEGFP into HeLa cells *via* endocytosis and electroporation at a 10-times dilution ratio of 0.005. The flow cytometry analysis on the transfection efficiency of pEGFP into HeLa in SiNPs (RITC)-PEG suspension: comparison of endocytosis (*A* to *C*) and electroporation (*D* to *F*). The 1st columns show the gated region (negative cells) of the sample. The 2nd columns show the EGFP-positive and the RITC-positive cells, respectively. The last columns show the histogram of counted EGFP-positive cells, *i.e.*, GFP transfection efficiency. M1: a marker for the GFP-negative cells, and M2: a marker for the GFP-positive cells. The electrotransfection efficiency of pEGFP into HeLa cells can be quantitatively obtained by counting the number of GFP-positive cells from the gated events of total events (10 000 cells).

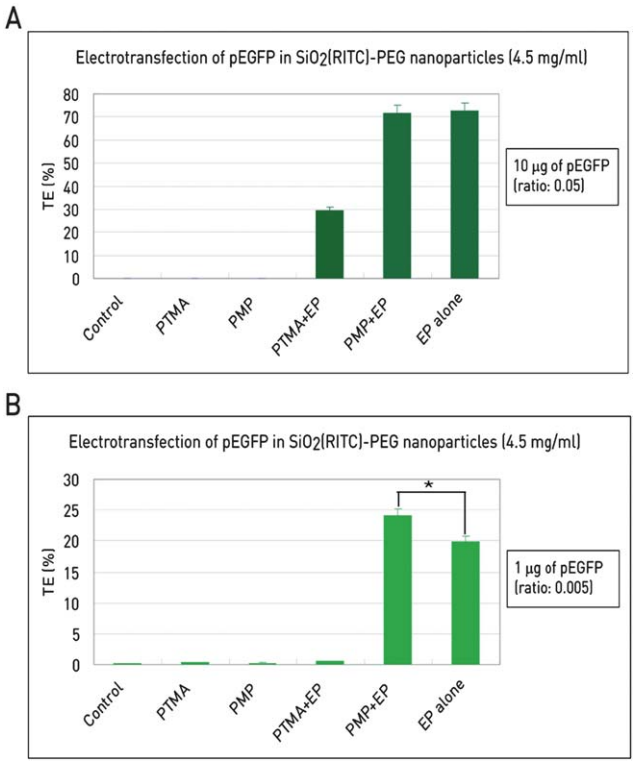

Fig. 5 Experimental results of electroporation in the presence of SiNPs. Comparison of the TE of pEGFP in SiNPs(RITC) suspension (4.5 mg mL^{-1}) under different pEGFP concentrations (A) for a normal ratio of 0.05 and (B) for a 10-times dilution ratio of 0.005, respectively. (*$p <$ 0.05).

SiNPs-PEG/PTMA(+) were less transfected and exhibited nonspecific bindings. These results indicate that most of the genes might be bound to the cationic SiNPs at a certain gene concentration, resulting in loss of free-moving genes. In the case of SiNPs with a smaller magnitude of zeta potential, the effect of the SiNPs-assisted environment on elctromediated gene uptake might be decreased. In our experiments, however, anionic SiNPs with a smaller magnitude of zeta potential could be used as gene transporters during electroporation. This implies that anionic SiNPs are potentially beneficial for initially increasing the gene uptake, resulting in greater transfection efficiency.

For further electroporation-based drug/gene therapy, however, more functional structures of SiNPs, such as semi-permeable and hollow or matrixed infrastructures, may be required. For clinical applications, the effects of nanoparticles on long-term cell viability should be thoroughly investigated, even if the cells might survive at a low dose of nanoparticles within a day after electroporation. In particular, the essential issue of toxicity or biocompatiblity of engineered nanoparticles should be addressed prior to electromediated gene delivery in future *in vitro*/*ex vivo* gene therapy associated with functional nanoparticles. Despite the seemingly endless debate on toxicity of nanoparticles within living cells or organs, cationic transfection reagents using the SiNPs for *in vitro*/*in vivo* gene delivery have been used. In previous reports, cationic SiNPs have been preferred to endocytosis by *in vivo* targeted gene delivery and *in vitro* gene condensation near the cells by gravity.[12,37–42] For most adherent cells, in particular, cationic SiNPs are useful as transfection enhancers, overcoming a surprisingly simple physical barrier, *i.e.*, a low gene concentration at the surface of the cell membrane. On the other hand, anionic SiNPs can be used to enhance gene delivery in hard-to-transfect cells such as suspension cells and stem cells that are not affected by gravity.

The delivery mechanism of the pEGFP-SiNPs complex is still unclear, but in the presence of an electric field, anionic SiNPs were preferred for electromediated gene delivery. This implies that electroporation-based gene delivery can be preferentially combined with anionic SiNPs.[39] It was previously reported that a combination of PEG-modified nanoparticles with electroporation showed huge potential for enhancing *in vivo* gene delivery.[43] In these experiments, in particular, cationic nanoparticles (+0.1 mV) can be changed to nanoparticle complexes with a negative zeta potential. This implies that anionic nanoparticles are potentially beneficial for electromediated gene delivery. Similarly, the concept of a platform to use gold nanoparticles to enhance electroporation in a microelectromechanical system (MEMS)-based device has been reported.[44] This approach showed the feasibility of a combination of MEMS and gold nanoparticles that can enhance the performance of electroporation in itself. In addition, a study on the stabilization of plasmid DNA *in vivo* by PEG-modified cationic gold nanoparticles and its gene expression by electric pulses has been reported.[43] In our work, however, we aimed to show that polarized SiNPs can enhance electromediated gene delivery, especially at a lower gene concentration.

Conclusions

In this study, we investigated the role of the polarity of engineered nanomaterials that can enhance electromediated gene delivery at a low gene concentration. In our experiments, we focused more on examining the feasibility of using anionic nanoparticles rather than cationic for electroporation. We found that anionic nanoparticles help to increase the uptake of intrinsically negatively charged genes in the same direction as the electric field. These results imply that the efficiency of gene delivery can be enhanced by the same polarity of gene carriers such as carrier DNA or RNA and engineered nanomaterials especially in microscale electroporation. We hope this finding will open up new vistas of advanced electroporation studies, especially at low sample concentrations. Furthermore, we believe that this approach will enable biologists and engineers to combine functional nanomaterials with electroporation for advanced genetic research.

Acknowledgements

The authors thank J. S. Kim for technical comments on nanoparticles (Biterials Inc., Korea), and Y. S. Shin for fruitful discussions on electroporation (California Institute of Technology, CA, USA). This research was supported by the Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2010-0005219). This study was also supported by a grant of the Korea Healthcare Technology

R&D Project, Ministry of Health & Welfare, Republic of Korea (A101987).

References

1 D. Luo and W. M. Saltzman, *Nat. Biotechnol.*, 2000, **18**, 33.
2 J. W. Yockman and D. A. Bull, *Adv. Drug Delivery Rev.*, 2009, **61**, 863.
3 S. Diez and G. Navarro, *J. Gene Med.*, 2009, **11**, 38.
4 F. I. Clanchy and R. O. Williams, *Expert Opin. Biol. Ther.*, 2008, **8**, 1507.
5 Y. Gao, Z. Zhang, L. Chen, W. Gu and Y. Li, *Int. J. Pharm.*, 2009, **371**, 156.
6 P. Midoux, G. Breuzard, J. P. Gomez and C. Pichon, *Curr. Gene Ther.*, 2008, **8**, 335.
7 P. Kortesuo, M. Ahola, S. Karlsson, I. Kangasniemi, A. Yli-Urpo and J. Kiesvaara, *Biomaterials*, 2000, **21**, 193.
8 C. Kneuer, M. Sameti, U. Bakowsky, T. Schiestel, H. Schirra, H. Schmidt and C. M. Lehr, *Bioconjugate Chem.*, 2000, **11**, 926.
9 C. Kneuer, M. Sameti, E. G. Haltner, T. Schiestel, H. Schirra, H. Schmidt and C. M. Lehr, *Int. J. Pharm.*, 2000, **196**, 257.
10 M. Sameti, G. Bohr, M. N. Ravi Kumar, C. Kneuer, U. Bakowsky, M. Nacken, H. Schmidt and C. M. Lehr, *Int. J. Pharm.*, 2003, **266**, 51.
11 S. J. Russell, *Nat. Biotechnol.*, 2003, **21**, 872.
12 M. N. V. R. Kumar, M. Sameti, S. S. Mohapatra, X. Kong, R. F. Lockey, U. Bakowsky, G. Lindenblatt, H. Schmidt and C.-M. Lehr, *J. Nanosci. Nanotechnol.*, 2004, **4**, 876.
13 C. Graf, S. Dembski, A. Hofmann and E. Ruhl, *Langmuir*, 2006, **22**, 5604.
14 C. R. Vestal and Z. J. Zhang, *Nano Lett.*, 2003, **3**, 1739.
15 T. Tago, T. Hatsuta, K. Miyajima, M. Kishida, S. Tashiro and K. Wakabayashi, *J. Am. Ceram. Soc.*, 2002, **85**, 2188.
16 F. Grasset, N. Labhsetwar, D. Li, D. C. Park, N. Saito, H. Haneda, O. Cador, T. Roisnel, S. Mornet, E. Duguet, J. Portier and J. Etourneau, *Langmuir*, 2002, **18**, 8209.
17 C. Barbe, J. Bartlett, L. Kong, K. Finnie, H. Q. Lin, M. Larkin, S. Calleja, A. Bush and G. Calleja, *Adv. Mater.*, 2004, **16**, 1959.
18 T. Nann and P. Mulvaney, *Angew. Chem., Int. Ed.*, 2004, **43**, 5393.
19 H. Ow, D. R. Larson, M. Srivastava, B. A. Baird, W. W. Webb and U. Wiesner, *Nano Lett.*, 2005, **5**, 113.
20 H. C. Moeller, M. K. Mian, S. Shrivastava, B. G. Chung and A. Khademhosseini, *Biomaterials*, 2008, **29**, 752.
21 K. Y. Suh, A. Khademhosseini, S. Jon and R. Langer, *Nano Lett.*, 2006, **6**, 1196.
22 O. C. Farokhzad, S. Jon, A. Khademhosseini, T. N. Tran, D. A. Lavan and R. Langer, *Cancer Res.*, 2004, **64**, 7668.
23 P. Kim, H. E. Jeong, A. Khademhosseini and K. Y. Suh, *Lab Chip*, 2006, **6**, 1432.
24 D. Luo and W. M. Saltzman, *Gene Ther.*, 2006, **13**, 585.
25 M. B. Fox, D. C. Esveld, A. Valero, R. Luttge, H. C. Mastwijk, P. V. Bartels, A. van den Berg and R. M. Boom, *Anal. Bioanal. Chem.*, 2006, **385**, 474.
26 W. G. Lee, U. Demirci and A. Khademhosseini, *Integr. Biol.*, 2009, **1**, 242.
27 Y. Zhan, J. Wang, N. Bao and C. Lu, *Anal. Chem.*, 2009, **81**, 2027.
28 Y. S. Shin, K. Cho, J. K. Kim, S. H. Lim, C. H. Park, K. B. Lee, Y. Park, C. Chung, D. C. Han and J. K. Chang, *Anal. Chem.*, 2004, **76**, 7045.
29 J. A. Kim, K. Cho, Y. S. Shin, N. Jung, C. Chung and J. K. Chang, *Biosens. Bioelectron.*, 2007, **22**, 3273.
30 W. G. Lee, H. Bang, H. Yun, J. Min, C. Chung, J. K. Chang and D. C. Han, *Lab Chip*, 2008, **8**, 224.
31 J. A. Kim, K. Cho, M. S. Shin, W. G. Lee, N. Jung, C. Chung and J. K. Chang, *Biosens. Bioelectron.*, 2008, **23**, 1353.
32 J. A. Kim, W. G. Lee and N. C. Jung, *Bioelectrochemistry*, 2010, **78**, 186.
33 Y. Lu, Y. Yin, B. T. Mayers and Y. Xia, *Nano Lett.*, 2002, **2**, 183.
34 A. M. Kalsin and B. A. Grzybowski, *Nano Lett.*, 2007, **7**, 1018.
35 T. D. Xie and T. Y. Tsong, *Biophys. J.*, 1992, **63**, 28.
36 J. H. Kim, J. S. Kim, H. Choi, S. M. Lee, B. H. Jun, K. N. Yu, E. Kuk, Y. K. Kim, D. H. Jeong, M. H. Cho and Y. S. Lee, *Anal. Chem.*, 2006, **78**, 6967.
37 K. W. Leong, H. Q. Mao, V. L. Truong-Le, K. Roy, S. M. Walsh and J. T. August, *J. Controlled Release*, 1998, **53**, 183.
38 D. Luo and W. M. Saltzman, *Nat. Biotechnol.*, 2000, **18**, 893.
39 D. Luo, E. Han, N. Belcheva and W. M. Saltzman, *J. Controlled Release*, 2004, **95**, 333.
40 R. A. Gemeinhart, D. Luo and W. M. Saltzman, *Biotechnol. Prog.*, 2008, **21**, 532.
41 I. Roy, T. Y. Ohulchanskyy, D. J. Bharali, H. E. Pudavar, R. A. Mistretta, N. Kaur and P. N. Prasad, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, **102**, 279.
42 D. R. Radu, C. Y. Lai, K. Jeftinija, E. W. Rowe, S. Jeftinija and V. S. Lin, *J. Am. Chem. Soc.*, 2004, **126**, 13216.
43 T. Kawano, M. Yamagata, H. Takahashi, Y. Niidome, S. Yamada, Y. Katayama and T. Niidome, *J. Controlled Release*, 2006, **111**, 382.
44 C. P. Jen, Y. H. Chen, C. S. Fan, C. S. Yeh, Y. C. Lin, D. B. Shieh, C. L. Wu, D. H. Chen and C. H. Chou, *Langmuir*, 2004, **20**, 1369.

