In Situ Investigation of Uptake Phenomena of Biological Molecules and Silica Nanoparticles into Mammalian Cells in Microstructures

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ABSTRACT

Nanoparticle researches have been expedited with an incredible paradigm shift from basic research groups to clinical laboratories and companies. Such multidisciplinary efforts require a full understanding of basic mechanisms, in particular, when applied to practical applications of human gene therapy and medicine. These trends consistently promote these nanoparticle researches to be expanded to more applications: electrophoretic and endocytic uptake. In this study, we investigate real-time uptake phenomena of nanoparticles and biological molecules into cells at single-cell level within microstructures during electroporation. These findings would unveil another potential of silica nanoparticles in electroporation using microstructures. Furthermore, another strong advantage of electroporation devices using microstructures is discussed.

Keywords: electroporation, electrotransfection, mammalian cells, silica nanoparticles, microstructure

1 INTRODUCTION

Electroporation is a powerful gene transfection method to deliver foreign biological molecules such as DNA and protein into living cells with electric pulsations [1]. However, conventional cuvette-type electroporation devices generally suffer from poor transfection efficiency and cell survivability. Recently a novel electrotransfection method using various microchannels has been developed [2-4]. The uniform electric field inside the narrow microchannels and few side reactions owing to the small area of electrodes are useful to enhance transfection efficiency and cell survivability. One of the advantages of microchannel-type electroporation is a big capability for *in situ* visualization of uptake phenomena of molecules into cells, which makes it possible to profoundly investigate the basic mechanisms of DNA transfer and intracellular response to the external electric pulses. In this study, we investigated *in situ* uptake phenomena of several foreign materials into living cells at single cell level in a microchannel during electroporation. These results would give a big help to clearly understand basic uptake mechanisms of foreign molecules during electroporation. A future potential and commercialization of the electroporation devices using microstructures such as microchannel and capillary are finally discussed [5].

2 MATERIALS AND METHOD

In the present study, many experimental observations at single-cell level were made to explore the uptake mechanism of small and macro molecules into mammalian cells using transparent microchannels equipped on an electroporation tool kit.

2.1 Preparation of cells and uptake materials

Cell line

HeLa cells were tested to observe real-time uptake phenomena in microchannel-type electroporation. All of the cells were cultured and seeded on the culture flask two days before the experiment. The cells were detached with the Trypsin-EDTA (Sigma-Aldrich, USA) and washed using Dulbecco's Phosphate Buffered Saline (DPBS, 1×) without calcium and magnesium. Then, the cells were centrifuged and resuspended in the DPBS buffer on the final concentration of 5×10^6 to 1×10^7 cells/ml.

Uptake materials

Several image analyses on directionality and polarity of uptake materials were performed such as propidium iodide (PI), RNA, DNA and fluorescent dye-doped silica nanoparticles (SiNPs). The fluorescence and TEM images of the synthesized SiNPs are shown in Fig.1.

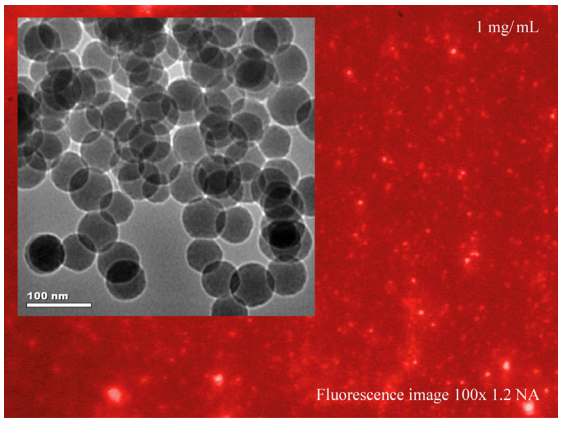


Figure 1: Fluorescence and TEM images of the synthesized SiNPs

In particular, two classes of SiNPs were used to examine the feasibility of SiNPs such as 30-nm propidium iodide (PI)-doped core SiNPs, and 50-nm and 70-nm dextran tetramethylrhodamine (TMR)-doped core-shell SiNPs for cell electropermeablization. These SiNPs are a versatile material for practical molecular and cellular applications which require size-controlled, mono-disperse, bright nanoparticles for specific conjugations to biological macromolecules [6].

2.2 Construction of microchannel-type electroporation device

All the experiments were conducted on a home-made electroporation tool kit using a simple rectangular poly (dimethylsiloxane) (PDMS) microchannel for a real-time observation of uptake phenomena at single-cell level.

Microchip fabrication

The microchip contains a single microchannel (400 µm wide and 50 µm, and 2 cm long) and two inlet and outlet reservoirs. The microchip was fabricated using the replica molding method. The microchannel patterns were fabricated by MEMS based soft-lithography using chrome photo mask. The fabrication process of the microchip was described in our previous work [2].

Electroporation setup

The experimental setup for the electroporation was made up of a homemade pulse generator, two *Pt* wire electrodes, and an electrode holder onto an inverted fluorescence microscope (IX71, Olympus, Japan) equipped with a 100-W mercury lamp and an oil-immersion objective (100× and NA1.2). Real-time uptake phenomena could be observed directly via the overall electroporation tool kit. The setup of microchannel-type electroporation device is shown in Fig. 2.

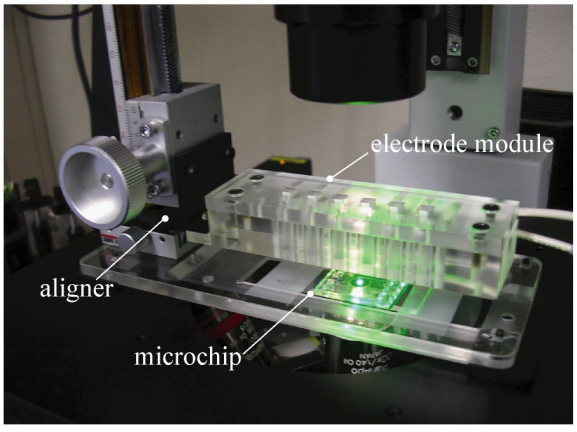


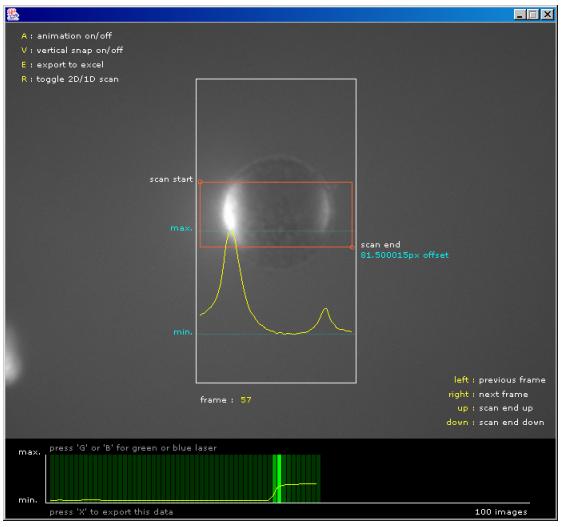
Figure 2: Photograph of a home-made electroporation tool

In our experiments, the optimized electrical conditions of DNA uptake for HeLa cells are following: electric field strength is 335V/cm, pulse type is square-wave pulse, pulse duration is 35 ms, pulse trigger is 2 times, and pulse period is 1 s, respectively. All images (640×480 pixel) were acquired by a 12-bit cooled CCD camera (Cooke, U.S.A.) at 15 frames/s. All the images were sequentially recorded and quantitatively analyzed with a fluorescence intensity profiler built in an interactive *JAVA* program (hereafter we call it *WG* profiler).

3 RESULTS AND DISCUSSION

In our experiments, real-time uptake phenomena of several exogenous molecules into cells were directly observed at single-cell level and quantitatively analyzed by using *WG* profiler as seen in Fig. 3. The uptake directionality of biological molecules is strongly dependent on the net charge and size of them.

a



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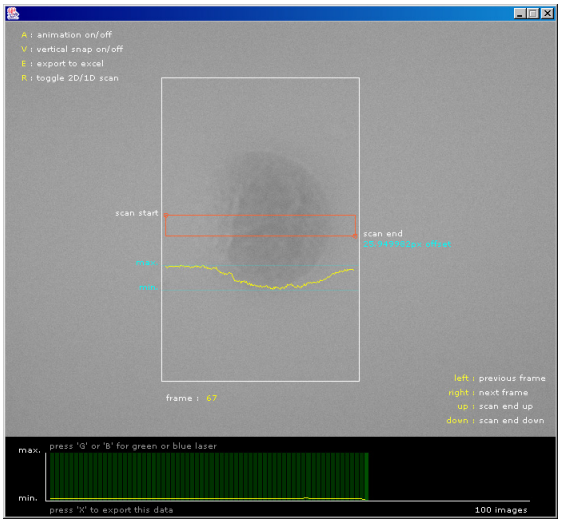

NSTI-Nanotech 2007, www.nsti.org, ISBN 1420061836 Vol. 2, 2007

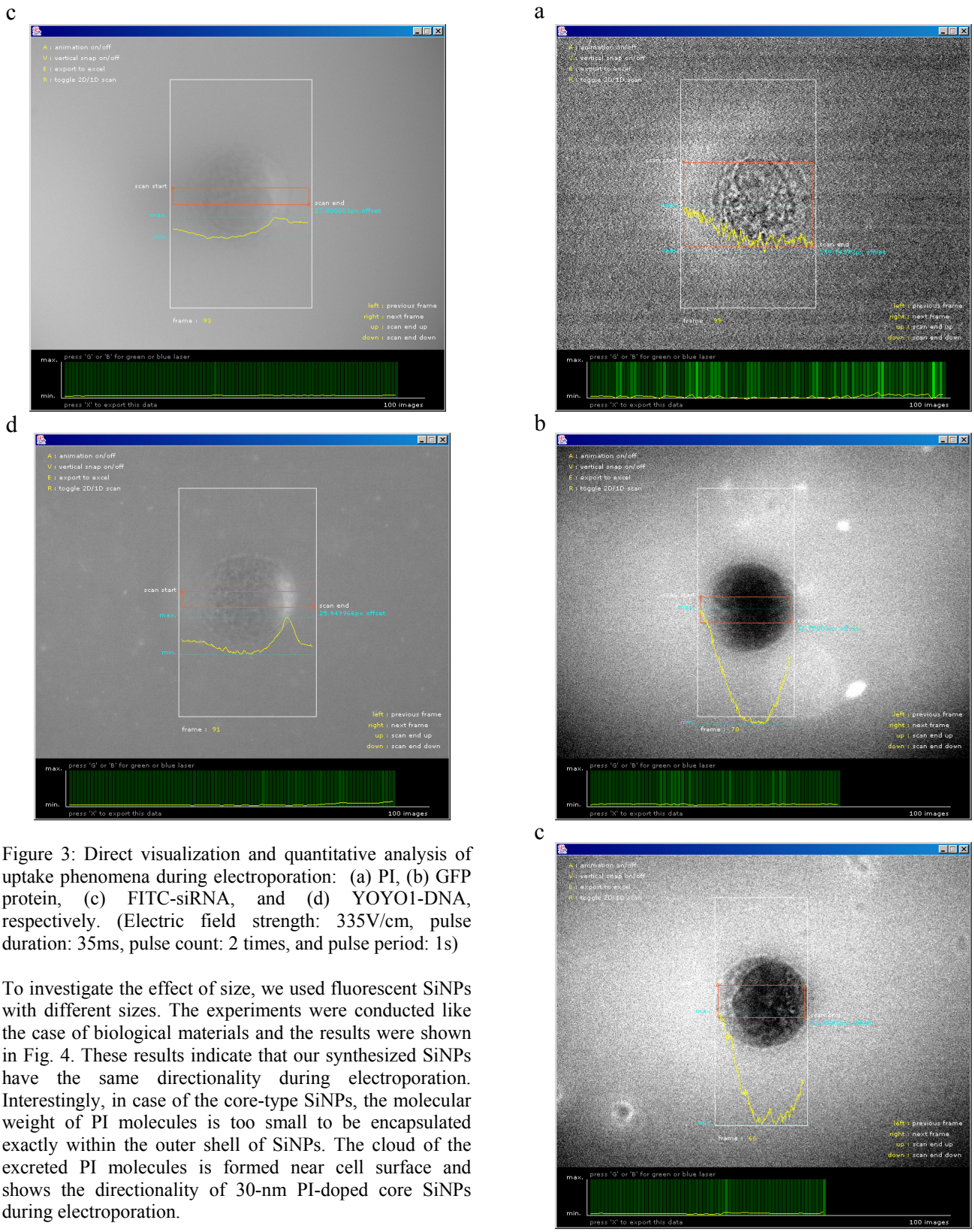

Figure 3: Direct visualization and quantitative analysis of uptake phenomena during electroporation: (a) PI, (b) GFP protein, (c) FITC-siRNA, and (d) YOYO1-DNA, respectively. (Electric field strength: 335V/cm, pulse duration: 35ms, pulse count: 2 times, and pulse period: 1s)

To investigate the effect of size, we used fluorescent SiNPs with different sizes. The experiments were conducted like the case of biological materials and the results were shown in Fig. 4. These results indicate that our synthesized SiNPs have the same directionality during electroporation. Interestingly, in case of the core-type SiNPs, the molecular weight of PI molecules is too small to be encapsulated exactly within the outer shell of SiNPs. The cloud of the excreted PI molecules is formed near cell surface and shows the directionality of 30-nm PI-doped core SiNPs during electroporation.

Figure 4: Direct visualization and quantitative analysis of uptake phenomena during electroporation: (a) 30-nm core

PI-doped, (b) 50-nm core TMR-doped, and (c) 70-nm core-shell TMR-doped SiNPs, respectively. The camera mode is long exposure, gain is normal, trigger mode is auto, and the exposure time is 5 ms. (Electric field strength: 335V/cm, pulse duration: 50 ms, pulse count: 2 times, and pulse period: 1s).

Additionally, we examined the change of cell size due to electroporation by using mono-disperse, size-controlled SiNPs as shown in Fig. 5. These results indicate that the feasibility of SiNPs as a potential indicator of electropore formation during electroporation. In our experiments, the sizes of SiNPs were measured by the TEM showing 30 nm, 50 nm, and 70 nm in a dry form. Normally, hydrodynamic diameters may get larger than those in a dried state with 15% increase.

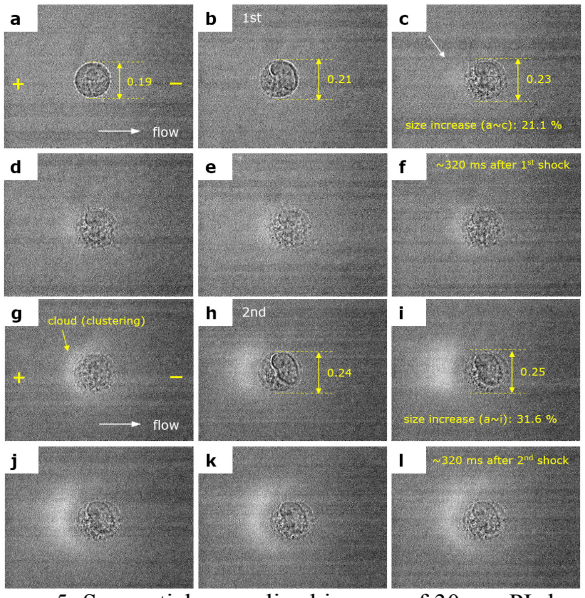

Figure 5: Sequential normalized images of 30-nm PI-doped core-type SiNPs uptake into HeLa cells. Two electrical pulses are given at the time [a] and [h], respectively. The white arrow means the direction of a trivial hydrostatic flow due to a small siphoning effect. (Electric field strength: 335V/cm, pulse duration: 50 ms, pulse count: 2 times, and pulse period: 1s)

4 DISCUSSION

Undoubtedly, the SiNPs are very useful for advanced genetic researches as a nonviral transfection method especially for targeted gene delivery. Here the SiNPs alone may be meaningless without a proper combination of multifunctional elements. Although the SiNPs were used only as uptake materials for direct visualization in this study, these nanoparticles might play a significant role in exploring the electroporation mechanism: a pore-size indicator, a gene transporter, a drug container and an intracellular monitoring marker in future gene/drug delivery.

In the previous section, we also visualized a basic electrophoretic uptake of the fluorescent SiNPs into cells via microchannel-type electroporation. These works may become very difficult thing in case of the cuvette-type electroporation. In our case, microstructure worked best. Such our effort, if somewhat reckless, to the miniaturization of effective region of electroporation could be realized through a novel concept of 'microcapillary'. More recently, we commercialized a novel electroporation device using a microcapillary [5]. Indubitably, this method remarkably reduces various harmful side-effects such as water dissociation, local pH change, and metal ion generation; otherwise these factors could decrease cell viability severely. A complementary combination of biocompatible silica nanoparticles and microstructures is expected to bring a synergy effect into future electromediated gene delivery.

5 CONCLUSIONS

Nanoparticle researches are indeed becoming one of the promising cores of future nanotechnology and nanoscience. However, practical realization of these studies into biotechnology companies starts toddling and is applicable to animal. Such efforts of commercialization first require several fundamental observations from complementary approaches. In this study, we tried to explore the basic uptake phenomena of biological molecules and silica nanoparticles into living cells within microchannel during electroporation. These results imply that a full knowledge of these uptake phenomena would be helpful to practical applications of future gene or cell therapy, especially for the hard-to-transfect cells.

ACKNOWLEDGMENT

The authors specially thank Y. S. Shin at California Institute of Technology for helpful suggestions. This work was supported by a grant of the Korea Health 21 R&D Project, Ministry of Health & Welfare, Republic of Korea (02-PJ10-PG4-PT02-0042).

REFERENCES

[1] D. Luo and W. M. Saltzman, Nat. Biotechnol., 18, 33, 2000.
[2] Y. S. Shin et al., Anal. Chem., 76, 7045, 2004.
[3] H.-Y.Wang and C. Lu, Anal. Chem., 78, 5158, 2006.
[4] K.-S. Huang et al., Lab Chip, 7, 86, 2007.
[5] Refer to the website of www.microporator.com.
[6] J. K. Jaiswal et al., Nat. Biotechnol., 21, 47, 2003.