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Reverse Electroporation with Carbon Nanotubes-loaded Electrode for Highly Efficient Gene Transfer

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1. Introduction

Site-specific gene transfer into adherent cells on a substrate, termed transfection array, has been intensively investigated, because it holds great promise as a tool for post-genome research. In the transfection array technique, nucleic acids, such as within plasmid and viral DNA or as short interference RNA (siRNA), were immobilized on a substrates, and then transfected into cells through gene transfer methods including usage of a virus carrier, endocytosis, electropermeation, and magnetic force. With the exception of our reverse electroporation method, non-viral transfection arrays were fabricated based on the endocytosis of cells utilizing cationic-liposomes, cationic polymers and their complexes with nucleic acids. Several problems remain to be solved, especially the low transfection efficiencies. High transfection efficiency is indispensable for high throughput analyses because the adherent cell number on a single spot decreases as the overall spot density increases on the transfection array. The formation of fine pores on the cell membrane with cell damage decreased is important to increase the efficiency of gene deliver in reverse transfection.

Carbon nanotubes (CNTs) have the unique properties of their nano-size, high aspect ratio, strong mechanical strength, and electric conductivity; therefore CNTs have attracted much attention not only in the field of electronics, but also in the biomedical sciences. Reverse electroporation using an electrode modified with CNTs could become a promising transfection array because of their excellent compatibility with cells and biomacromolecules, and the field emission formed from the tip of the CNTs.

In this study, we evaluated the transfection efficiency in using the reverse electroporation with a CNT-loaded electrode, and site-specific gene transfer was performed on the CNT-loaded electrode.

2. Experimental

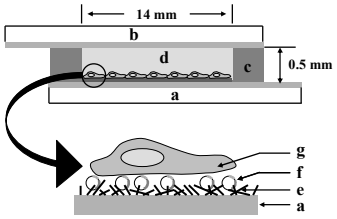
Preparation of a CNT-loaded electrode

A self-assembled monolayer (SAM) of carboxylic acid-terminated alkanethiol (COOH-SAM) was formed on the gold-evaporated glass plate using a 1 mM ethanol solution of 11-mercaptoundecanoic acid. For site-specific gene transfer, a SAM of methyl-terminated alkanethiol (CH_3-SAM) having COOH-SAM region (ϕ = 1 mm, 5 x 5 dots) was formed in photolithographical method. Branched polyethyleneimine (B-PEI) with an average molecular weight of 25000 was adsorbed on the obtained SAM surfaces using its Dulbecco's phosphate buffered saline (PBS) solution (pH 7.4) at 10 mg/mL. Crude CNTs (Multi-walled carbon nanotubes, average diameter: 1.2-20 nm, range of length: 100-2000 nm, layer: 5-20 layers) were functionalized with sonication in a nitric-sulfuric acid solution (1:3 by volume). After the purification, the dry functionalized CNTs were obtained by lyophilization. The functionalized CNTs were adsorbed on the B-PEI-adsorbed surfaces using its MilliQ water suspension at 0.1 mg/mL.

The loading process of CNTs and subsequent adsorption of plasmid DNA on the electrode were evaluated by a surface plasmon resonance (SPR) apparatus. The electrode surfaces were characterized by X-ray photoelectron spectroscope (XPS) and Fourier transform infrared reflection-absorption spectroscopy (FTIR-RAS).

Transfection of plasmid DNA into adherent cells

Plasmid DNAs, encoding an enhanced green fluorescent protein (EGFP) (pEGFP-C1, 4.7 kbp) and a *Discosoma sp.* red fluorescent protein (DsRed), were transfected into adherent human embryonic kidney (HEK293) cells on the CNT-loaded electrode by applying an electric pulse. The pEGFP-C1 was adsorbed electrostatically onto the electrode surface in PBS solution at a given concentration (Scheme 1). For site-specific gene transfer, PBS solutions (5 μg/mL) of either pEGFP-C1 or pDsRed-C1 were applied to the COOH-SAM spots by manual pipetting. The cells were seeded on the CNT-loaded electrode and cultured at 37 ºC under a 5% CO_2 atmosphere for 24 h using minimal essential medium (MEM) containing 10% heat-



Scheme 1. Systematic representation of the apparatus of an electro-stimulated transfection array modified with carbon nanotubes. (a) CNT-loaded electrode (cathode), (b) counter electrode (anode), (c) silicone ring, (d) PBS (4 ºC), (e) CNTs, (f) plasmid DNA, (g) cell.

inactivated fetal bovine serum (FBS). After incubation, the MEM was replaced with PBS, and a single pulse was applied between an electrode with adherent cells and a counter electrode (gold-evaporated glass plate) at the pulse strength of 240 V/cm for duration of 5 or 10 msec at 4 ºC. After the pulse application, the cells were cultured in MEM for additional 48 h. Cells expressing the EGFP were observed using an epifluorescence microscope. The fraction of cells expressing the EGFP was quantitatively evaluated by the fluorescent-activated cell sorting (FACS).

3. Results and discussion

Shifts in the surface plasmon resonance (SPR) angle were clearly observed during procedures resulting in the adsorption of the cationic B-PEI onto anionic COOH-SAM surface and during the subsequent loading of the anionic CNTs (Fig. 1). Direct exposure of the anionic CNTs suspension to the anionic COOH-SAM surface also results in SPR angle shift, but of lesser magnitude (results not shown). Thus, a large quantity of CNTs could be loaded onto the B-PEI adsorbed surface, but not onto the anionic COOH-SAM surface. As predicted, the anionic CNTs were adsorbed onto the cationic electrode mainly through electrostatic interaction. Due to electrostatic repulsion, the negatively charged plasmid could not adsorb onto the anionic CNTs. The negatively charged plasmid DNA might be attached on the positively charged B-PEI surfaces remaining between adsorbed CNTs.

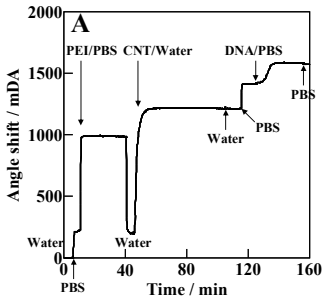


Fig. 1. Real-time observation of sequential deposition of B-PEI, CNT and DNA on COOH-SAM surface by the surface plasmon resonance method.

Fig. 2 shows the phase contrast and fluorescent microscopic images of HEK293 cells on the electrodes with or without CNTs 48 h after the pulse application. The cells on the CNT-loaded electrode expressed EGFP more efficiently than those on the electrodes lacking CNTs. From the quantitative analysis of the fraction of cells expressing EGFP by the FACS measurement, the EGFP expression efficiency of the cells on the CNT-loaded electrode increased to 55%, while that on the electrodes without CNTs was 30%. This transfection efficiency by reverse electroporation on the CNT-loaded electrode was distinctively higher than that by reported reverse transfection methods based on the cell endocytosis. The electric field is expected to localize at the CNT tip and thus it could make fine pores on the cell membrane when electric pulse was applied.

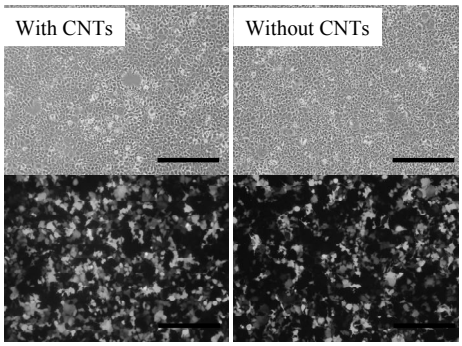


Fig. 2. Phase contrast (upper) and fluorescent (lower) microscopic images of HEK293 cells adhered on pEGFP-C1 adsorbed electrodes with or without CNTs at 48 h after an electro-pulse application. Scale bar: 500 μm.

For site-specific gene transfection, spots with plasmid DNA were arrayed on a CNT-loaded electrode. Forty-eight hours after the pulse application to cells on the plasmid DNA-arrayed electrode, fluorescent microscopic images were acquired (Fig. 3). While cell adhesion occurred over the whole area (phase contrast images were not shown), the EGFP and DsRed expression in an array fashion was observed with high efficiency and with no contamination between the spots.

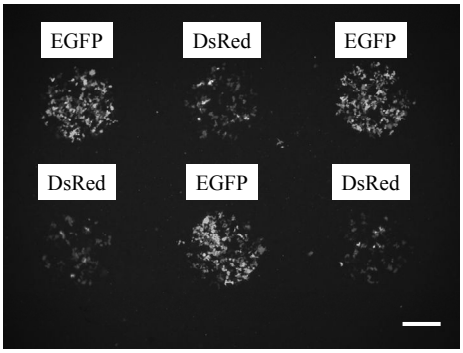


Fig. 3. Fluorescent microscopic image of HEK293 cells transfected with pEGFP-C1 or pDsRed-C1 in an arrayed fashion on a CNT-loaded electrode. Scale bar: 500 μm.

4. Conclusions

A CNT-loaded electrode was prepared using the electrostatic interaction between the functionalized CNTs and cationic electrode surface. Cells on the CNT-loaded electrode were transfected with plasmid DNA by applying electro-pulses and they efficiently expressed the proteins site-specifically introduced by the plasmid. These results indicate that reverse electroporation with CNT-loaded electrodes could be a promising tool for post-genome investigations.

Acknowledgements

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