Supporting Information

Silica-based Gene Reverse Transfection: Upright Nanosheet Network for Promoted DNA Delivery to Cell

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1. Experimental Details

1.1. Materials

Polished p-type silicon wafer was purchased from Shin-Etsu chemical Corp. (Japan). Fluorescein-labeled Plasmid DNA (2.7 kb double stranded) was purchased from Mirus Bio Corp. Cationic lipid-based transfection reagent, LyoVec, pDNA/LyoVec complex and human embryonic kidney cell lines, HEK293XL/null were purchased from Invivogen (USA). Other reagents, NaBH₄, ethanol dehydrated methanol, 4-nitrobenzaldehyde, hydrazine, NH₄-HF₂ 28%NH₃-H₂O and NaOH were purchased from Wako Pure Chemicals (Japan). Water used in experiments was purified by using a Purelab Prima system to a resistivity of 18.2 MΩ cm⁻¹.

1.2. Preparation of the silica film

Silica thin film with 500 nm thickness was formed on one side of a polished Si(001) wafer (4 inch diameter) or glass slide (75 × 25 mm) by magnetron sputtering deposition. The silicon wafer or glass slide was thoroughly cleaned and spun dry before being loaded in the sputtering system. A manufacturing-scale apparatus, Shibaura Mechatronics (OCTAVA-II), was used for sputter deposition. A high purity (99.99%) silica target was used with pure Ar gas.

The silicon wafer or glass slide with 500 nm thick silica layer was cut into 1×1 cm pieces for convenience. These were then immersed in NaBH₄ solution (1.4 mM, 3ml) in a 20 mL capacity teflon-lined steel autoclave and incubated at 75 °C for 1~6 hours. After the reaction, the pieces were rinsed with pure water and dried under vacuum at room temperature. The process was also tried using other solutions of NaOH (1mM), NH₃-H₂O(10%), NH₄-HF₂ (5%) and hydrazine (1%).

1.3. Surface modification of silica film
The wafer substrates with silica films composed of upright-sheets were silanized with 2% aminopropyltriethoxysilane (APTES) in ethanol for 12 hours, resulting in amine-terminated surfaces. After washing with ethanol and subsequently by pure water, the substrates were dried at room temperature.

The amine group content of the silica films was evaluated by reacting with 4-nitrobenzaldehyde (NBZ). NBZ (5 mg), acetic acid (0.01 mL) and 4-Å molecular sieve (0.5 g) were mixed in dehydrated methanol (10 mL). The silica films on the silicon substrates were immersed in the resulting solution at 50 °C for 3 hours. The substrates were then sonicated in methanol for 1 minute and washed with methanol. After repeating this procedure 3 times, the substrates were dried under vacuum. Finally, the reacted NBZ on the silica films was cleaved by rinsing the surface with 4 mL water containing 50μL acetic acid. The concentration of the cleaved NBZ was measured using a UV-vis spectrophotometer.

1.4. DNA adsorption on silica films

Fluorescein-labeled plasmid DNA 0.5 μg/μL was diluted to 0.25 μg/μL in 50 mM Tris-HCl pH 7.0 buffer solution. The plasmid DNA (0.25 μg/μL, 5 μL) was dropped on the surface of the substrates with silica films and incubated in a moisture chamber for 3 hours at room temperature. The non-bound DNA was removed by immersing the sample substrate in Tris-HCl pH 7.0 buffer solution (50 mM, 5 ml) for three minutes and repeated twice. Fluorescein-labeled plasmid DNA adsorption on silica films was observed by fluorescence microscopy with bandpass filter (BP 527/30nm) and 480/40 nm excitation. For quantitative determination, the amount of plasmid DNA bound to silica films was obtained by determination of the residual plasmid (unbound DNA) in the solution recovered after incubation using a NanoDrop 2000 spectrophotometer at 260 nm.
1.5. Cell culture

Human embryonic kidney cell lines, HEK293XL/null cells were grown in DMEM medium supplemented with 10% (v/v) FBS, 50 U/mL penicillin, 50 μg/mL streptomycin, 100 μg/mL normocin, and 2 mM L-glutamine at 37°C in a moisture chamber containing 5% CO₂.

1.6. Plasmid DNA transfection to mammalian cells on silica films

The sample substrates were cleaned by immersing in 70% (v/v) ethanol solution for 2 hours and dried under vacuum. 100 μL pDNA/LyoVec complex in 25 mM Tris-HCl pH 7.0 buffer solution was dropped on the sample substrates and incubated in a 12-well tissue culture plate for 30 min at room temperature. The sample substrates were then washed three times with 3 ml 50 mM Tris-HCl pH 7.0 buffer solution. 1 ml HEK293XL/null cell suspension (1.0 × 10⁵ cells/mL) was added to each well of the tissue culture plate and incubated in 5% CO₂ at 37°C for 48 hours. Cell nuclei were stained with Hoechst 3342 before testing transfection expression of the sample substrates.

For evaluation of transfection efficiency on silica films, we transfected pDNA/LyoVec complex in HEK293XL/null cells following the company recommended solution-based transfection protocol as follows. LyoVec standard solution-based transfection method: 1 ml HEK293XL/null cell suspension (1.0 × 10⁵ cells/mL) were seeded on a 12-well culture plate, and then 50 μL pDNA/LyoVec complex solution was added directly to each well and incubated in 5% CO₂ at 37°C for 48 hours.

The transfection efficiency was calculated by counting the percentage of GFP expressing cells in the total cells. Total cells were about 500 to 600 cells in 7 independent experiments. For further details of the transfection efficiency measurement are as follows: the transfected HEK293XL/null cell was observed by fluorescence microscopy (DM2500 Fluo/DIC, Leica, Germany) 48 hours after post-transfection. The fluorescence images of cells on silica films were obtained to analyze the transfection efficiency. Expressed GFP in cells was imaged with
bandpass filter (BP 527/30nm) with 480/40 nm excitation. Cell nuclei were stained with Hoechst3342 in order to ascertain the number of total cells, and obtained by Hoechst3342 using a longpass filter (LP 425nm) with 340-380 nm excitation. Data was saved in 8 bit Tiff image format and then processed to threshold and merge two fluorescence image derived from GFP and cell nuclei using Image-J software (version 1.44o, National institutes of Health, USA). Maximum entropy threshold was applied to distinguish between GFP expressing cell and non-expressing cells. After threshold, the fluorescence images derived from GFP and cell nuclei were merged and the number of GFP expressing cells and total cells were counted. The transfection efficiency result was compared with those obtained using the solution-based LyoVec standard transfection protocol.

1.7. Characterizations

FTIR spectra were measured on a Nicolet Nexus 670 FTIR instrument. Scanning electron microscopy (SEM) and scanning transmission electron microscopy (STEM) were performed using a Hitachi S-4800 using accelerating voltages 10 kV and 30 kV for SEM and STEM, respectively. Energy dispersive X-ray analysis (EDX) was measured using a Horiba EDX attachment of the Hitachi S-4800 FE-SEM at high probe current with 15 kV accelerating voltage. High-resolution transmission electron microscopy (HR-TEM) was performed by using a JEOL-JEM2000 operating at 200 kV. A Shimadzu UV-vis spectrophotometer (Model: Shimadzu UV-3600) was used to measure the absorption spectra. Fluorescence images were acquired using an upright fluorescence microscope (DM2500 Fluo/PH; Leica microsystems, Germany).
2. Additional Data

**Figure S1.** (A) STEM and (B) TEM image of silica film composed of dense upright-sheets detached from the substrate surface by ultrasonication, (C) the EDX spectrum of the silica film composed of dense upright-sheets.

**Figure S2.** N$_2$ adsorption-desorption at 77K isotherms of the silica film composed of dense upright-sheets. The inset curve is the pore size distribution.
**Figure S3.** SEM images of silicon wafer with 500 nm thick silica layer after 2 hours reaction in 1mM NaOH solution at 75°C. The inset is the cross sectional image of the wafer piece. The silica was consumed without regrowth of silica.

**Figure S4.** SEM images of silicon wafer with 500 nm thick silica layer after 2 hours reaction in 10% NH₃-H₂O solution at 75°C for 2 hours. The inset is the cross sectional image of the wafer piece. Silica was not dissolved in the process.
Figure S5. SEM images of silicon wafer with 500 nm thick silica layer after 2 hours reaction in 1% hydrazine solution at 75°C. The inset is the cross sectional image of the wafer piece. Silica was not dissolved in the process.

Figure S6. SEM images of silicon wafer with 500 nm thick silica layer after 2 hours reaction in 5% NH$_4$-HF$_2$ solution at 75°C. Silica layer was completed dissolved and left silicon surface on the top. There was not regrowth silica found in this process.
**Figure S7.** SEM images of glass slide with 500 nm thick silica layer after (A) 2 hours and (B) 6 hours reaction in NaBH₄ solution at 75°C. The inset image is indicated standing sheets on the top surface.

**Figure S8.** FTIR spectra (A) before and (B) after the aminosilylation of the silica film.
Figure S9. Fluorescence microscopy images of DNA immobilization on (A) planar silica film, (B) silica film composed of loose networked upright sheets, and (C) silica film composed of dense networked upright sheets.