Molecular Recognition Enables Nanosubstrate-Mediated Delivery of Gene-Encapsulated Nanoparticles with High Efficiency **

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1. Determination of gene-transfection of cells

Gene transfection of cells was determined with the use of a Nikon TE2000S inverted fluorescent microscope with a CCD camera (Photomatrix, Cascade II), X-Cite 120 mercury lamp, automatic stage, and filters for two fluorescent channels (i.e., W1: 350nm and W2: 485 nm). A FocalCheck fluorescence microscope test slide from Invitrogen was used for light source emission calibration. The exposure time was 0.2 sec. At the same exposure time, the EGFP-expression levels of the individual cells were quantified by MetaMorph (Molecular Devices, Version 7.5.6.0) using the Multi-Wavelength Cell Scoring module. The total pixels on a given cellular footprint were integrated for quantification. The background of each image was selected with the spot without cells and then its intensity was compared to the spot with cells. The auto-fluorescence signals in non-transfected cells were also quantified using the aforementioned protocol. A “transfected cell” is defined to have EGFP-expression levels at least 4 times higher than the averaged auto-fluorescence signals observed in non-transfected cells (Fig. S1).

![Microscopic images](image)

**Figure S1.** The microscopic images of (a) EGFP transfected cells and (b) non-transfected U87 cells.
2. Control experiments of the host guest recognition of adamantane and cyclodextrin

To examine the host guest recognition between adamantane (Ad) and cyclodextrin (CD), we performed two control experiments. In the first experiment, excess (2-carboxyethyl)-β-cyclodextrin (40 μg/mL) - which can outcompete the SNP in binding to SiNWS was added to the cell media before SNP treatment. In the second experiment, the surface of SiNWS was modified with dodecyl group instead of adamantyl group. As shown in Fig. S2, no GFP signal was observed from these two control experiments. These results suggest that the Ad/CD recognition is critical for the NSMD platform to achieve the highly efficient gene delivery presented in the manuscript.

![Microscope images of U87 cells on Ad modified SiNWS, Ad modified SiNWS which were treated with excess CD before SNP treatment, and dodecyl group modified SiNWS.](image)

**Figure S2.** Microscope images of U87 cells on Ad modified SiNWS, Ad modified SiNWS which were treated with excess CD before SNP treatment, and dodecyl group modified SiNWS.
3. SEM study on the interaction between cells and SINWS

Three different cells (adherent cell: U87 glioblastoma cells, suspension cell: human white blood cell, WBC, primary cells: mouse embryonic fibroblast, MEF) were selected and incubated with SINWS. SEM images of these cells were taken before and after the treatment of pEGFP<sub>$\odot$</sub>SNPs (50 ng plasmid/mL). We prepared samples of the cell-attached substrate for SEM observation by a standard procedure. Cells were cultured on Ad-SINWS overnight to ensure their settlement onto Ad-SINWS. DNA<sub>$\odot$</sub>SNPs were then added to the medium, and the resulting solution was incubated for 20 min at 37°C (5% CO₂). The cells were washed with PBS twice and fixed with 4% glutaraldehyde solution (4°C, 1 h). Cells were then post-fixed in 1% osmium tetroxide for 1 h with 1% tannic acid, as a mordant. Samples were dehydrated through a series of alcohol concentrations (30%, 50%, 70%, and 90%), stained in 0.5% uranyl acetate, and further dehydrated (100% alcohol). The final dehydration was in hexamethyldisilazane (HMDS), followed by air drying. Once dry, the samples were sputter coated with gold before examination with a Hitachi S800 field emission SEM at an accelerating voltage of 10 keV. The interactions between the three types of cells and Ad-SINWS are visualized in Fig. S3. In addition, the suspension cells were also able to contact and interact with the nanostructured substrates as the results of gravitation and Brownian motion. An insignificant difference in terms of cell morphologies was identified before/after DNA<sub>$\odot$</sub>SNP delivery.

![Figure S3](image-url)  
**Figure S3.** SEM images of various cells including adherent (U87), suspension (human WBC), and primary cell (MEF) on
SiNWS before and after the DNA\textendash SNP treatment.

4. Intensities of EGFP transfected by different platforms
The intensities of GFP by using different transfection platforms were quantified by the mean relative fluorescence intensity. 12 h after administration of pEGFP\textendash SNPs (50 ng plasmid/mL), U87 cells seeding on the Ad-SiNWS were fixed and stained with DAPI. The relative fluorescence intensities of EGFP (EGFP signal intensity divided by background signal intensity) were collected for cells transfected with the different platforms including pEGFP\textendash SNPs, RGD-jet-PEI, Lipo-2000 and NSMD. The mean value of the EGFP intensities was then obtained and summarized (Fig. S4). As illustrated in Fig. S4, the U87 cells transfected by the NSMD platform expressed GFP signals an average of 4.3 to 7.2 times stronger than those observed for U87 cells treated by Lipo-2000, RGD-jet-PEI, or pEGFP\textendash SNPs alone.

![Graph showing EGFP expression intensities for different platforms](image)

**Figure S4.** The intensity of expressed EGFP signals of U87 cells transfected by the pEGFP\textendash SNPs alone, RGD-jet-PEI, Lipo-2000, and NSMD platform, respectively.

5. Transfection performance of NSMD for repeatedly treatment
To explore the feasibility of recycling Ad-SiNWS for multiple rounds of delivery, we repeatedly used the same Ad-SiNWS in more than 10 cycles of transfection studies. In each cycle, the cells (U87) were seeded on the same cleaned Ad-SiNWS chip and administered with pEGFP\textendash SNPs. We rinsed the Ad-SiNWS with ethanol after each transfection cycle. Ethanol
can effectively disassemble DNA<sup>SNPs</sup>, leaving no DNA<sup>SNPs</sup> on the Ad-SiNWS for the next round of transfection study. The treated U87 cells were then investigated under the fluorescent microscope to give out the transfection efficiency. During the 10 cycles of transfection studies, each batch of cells was transfected successfully without observing compromised delivery performance (Fig. S5).

![Graph showing transfection efficiency](image)

**Figure S5.** Transfection efficiency of pEGFP in U87 cells on the same repeatedly used Ad-SiNWS chip for 10 repeated cycles.

6. **Gene transfection of cell with various types of DNA<sup>SNPs</sup>.**

DNA<sup>SNPs</sup> with three different types of genes were individually treated on U87 cells. After the cells fully attached onto Ad-SiNWS (for 12 h), 100 nm DNA<sup>SNPs</sup> (50 ng plasmid/mL) were introduced into individual chambers and co-incubated with the attached cells for a designated period of time. The chambers were then washed with PBS and the cells were immediately fixed with 2% PFA and stained with DAPI. Transfection performances of individual genes were quantified using microscopy-based image cytometry. As shown in Fig. S6, no over-spilled signals were observed in individual studies.
7. Time-dependent transduction of DNA-SNPs into cells on different substrates

In order to understand the dynamics of DNA-SNPs transduction into cells on different substrates, we employed real-time fluorescence microscopy to monitor the uptake of Cy5 labeled DNA-SNPs by U87 cells.

NHS-Cy5 reacted with the amine group of CD-PEI resulting in Cy5 labeled CD-PEI, which was utilized to form Cy5 labeled DNA-SNPs (50 ng plasmid/mL). To a solution of CD-PEI (1.7 mg, 0.1 µmol, 1.0 equiv.) in PBS buffer solution (pH 7.2), Cy5-NHS (0.8 mg, 1.0 µmol, 10.0 equiv.) was added. The reaction mixture was stirred at room temperature for 2 h. After the reaction completed, the mixture was purified by dialysis (Slide-A-Lyzer® dialysis cassette, MWCO 3 kD) against DI water overnight and lyophilized to yield Cy5-CD-PEI which is ready for preparation of DNA-SNPs. Cy5 labeled DNA-SNPs were synthesized with the same method which is described in the procedure section in the manuscript.

The DNA-SNPs grafted onto the Ad-SiNWS showed the highest cell-uptake efficiency when compared to the other three substrates (i.e., silicon nanowire substrate (SiNWS), Ad-grafted flat silicon (Ad-SiFlat), and flat silicon (SiFlat)). The fluorescence intensity, reflecting the quantity of the Cy5 labeled DNA-SNPs by cells, was saturated ca. 3-4 h (Fig. S7,
The typical fluorescence micrographs of the Cy5 labeled DNA⊂SNPs uptake by U87 cells is shown in Fig. S7 (right) and Fig. S8. Given the outstanding delivery performance observed for our NanoSubstrate-Mediated Delivery (NSMD) platform, significant quantities (6-10 fold higher Cy5 signals) of Cy5-labeled DNA⊂SNPs were delivered into the Ad-SiNWS immobilized cells in contrast to the control studies.

**Figure S7.** Time-dependent Cy5 labeled DNA⊂SNPs uptake by U87 cells on different silicon substrates including Ad-SiNWS, SiNW, Ad-Siflat, and Siflat substrates (left). Error bars are obtained from triplicated experiments. Fluorescence micrographs of the Cy5 labeled DNA⊂SNPs uptake by U87 on different silicon substrates for 4 h (right). The cell nuclei were stained with DAPI (blue).

**Figure S8.** Micrograph of U87 cells on Ad-SiNWS which obtained after 1h from the Cy5 labeled DNA⊂SNPs treatment. Each image represents DAPI (left), Cy5 labeled DNA⊂SNPs (middle), and their merged images (right).

The influence of timing of DNA⊂SNPs treatment on gene delivery efficiency was also investigated. DNA⊂SNPs was first introduced onto flat Si chips (Ad-Siflat) and then U87 cells were settled onto the chips. As shown in Fig. S9, U87 cells
showed non-detectable fluorescent signals (as the result of poor transfection performance), which are similar to those shown in Fig. 4b.

**Figure S9.** Fluorescent micrographs of U87 cells, which were on the DNA–SNP-grafted Si₃ nitride.

8. **Cell viability assay**

The dosage-dependent cell viability on the NSMD platform was assessed by the MTT assay. U87 (5 × 10⁴ cells/chamber) cells were seeded into a 2-well chamber slide containing Ad-SiNWS, and 100-nm DNA–SNPs with different concentrations of plasmids (from 20 ng plasmid/mL to 1000 ng plasmid/mL), added into the culture medium, and incubated with cells for 24 h. After incubation, Cell Titer-Blue (100 μL) was added into each well and incubated for 3 h. The chamber slide was then placed on a shaking table (150 rpm for 5 min) to thoroughly mix the solution, and then fluorescence intensities were measured with excitation at 535 nm and emission at 585 nm. As shown in Fig. S10, there were no significant fluorescence intensity differences between the treated and non-treated cells, which suggested negligible cell disruption and toxicity caused by NSMD platform.

**Figure S10.** Cell viability on the NSMD platform was assessed by the MTT assay. Different dosages of 100-nm DNA–SNPs
were used to treat the U87 cells for 24 h for MTT assay. Error bars are obtained from triplicate experiments.

9. In vivo gene expression test with Ad- modified flat Si chip

The mouse, which was implanted with Ad modified flat Si chip (Ad-Si\textsubscript{flat}) on its left side, was imaged after the pGL3⊂SNPs injection. The experiment was performed following the same conditions as the one shown in Fig. 5. However, unlike Ad-SiNW in Fig. 5, no luminescent signal was observed at the site with Ad-Si\textsubscript{flat} (Fig. S11a). Also, as shown in Fig. S11b, no signal increase was observed at the site with Ad-Si\textsubscript{flat} compared to the opposite site without Ad-Si\textsubscript{flat}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig11.png}
\caption{\textbf{Figure S11.} \textbf{a)} Optical images of luciferase expression of a mouse implanted with Ad-Si\textsubscript{flat} in the presence of luciferase reporter plasmid-encapsulated SNPs (pGL3⊂SNPs; 200 ng/site) at designated time points. \textbf{b)} Continuous monitoring of the luciferase expression in \textit{in vivo} Ad-Si\textsubscript{flat} treated-mouse along with control studies (w/o Ad-Si\textsubscript{flat}). The differences in total flux at region of interest (ROI) were calculated by subtracting the total flux value of background from those of ROI.}
\end{figure}