Supporting Information

Silica nanoparticle supported lipid bilayers for gene delivery

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Materials. Unmodified silica nanoparticles (8-130 nm) were obtained from Silco2 Inc. in 30% suspension. Silica nanoparticles with green fluorescence emissions were purchased from G.Kisker. All phospholipids were purchased from Avanti Polar Lipids Inc. (Alabaster, AL). Cholesterol was purchased from Aldrich. Hoechst 33342, ProLong Gold mounting agent, and mammalian cell viability assay kit were purchased from Invitrogen (Carlsbad, CA). Chinese Hamster Ovary (CHO) cells and cell culture related chemicals and media were purchased from American Type Culture Collection (ATCC). The pCMV-DsRed plasmid that contains a gene encoding for the DsRed fluorescent protein was purchased from Clontech. The plasmid DNA labeling kit for covalently labeling Cy5 was purchased from Mirus.

Preparation of liposomes: Phospholipids were dissolved in chloroform at concentrations of 10–25 mg/mL. Aliquots were dispensed into scintillation vials so that each vial contained 2.5 mg lipids. Some lipids were mixed with a small fraction (2 %) of Texas Red-labeled DHPE. The chloroform in the vials was evaporated under a nitrogen flow in a fume hood and lipid films were formed. The vials were then stored in a vacuum oven at room temperature overnight to remove any residual chloroform. The samples were frozen at -20^{o}C before use. To prepare liposomes, the vials were brought to room temperature and rehydrated by adding 1 mL of buffer A (10 mM MOPS, pH 7.0, 60 mM NaCl) with occasional shaking for at least 1 hr, forming a cloudy lipid suspension. The suspension was extruded with a mini-extruder purchased from Ananti Polar Lipids following standard protocols. A membrane with pore diameter of 100 nm was used and at least ten extrusion cycles were performed. The resulting clear liposomes were stored in a new vial at 4 ^{o}C.

Preparation of supported bilayers. The silica particles came with 30% solid and were stabilized by base from the vendor. Therefore, the particles were first diluted 3x with DI water and neutralized with 1 M HCl. For 30 to 130 nm particles, 10 μL of each particle was transferred to a small centrifuge tube and liposomes were added until a stable suspension was obtained. The particles were centrifuged at 6000 rpm for 2 min and the supernatant was removed. The supported bilayers were washed once with PBS and dispersed in PBS at a concentration of 25 mg/mL.

Agarose gel electrophoresis. Agarose (0.5 g) was dissolved in 50 mL 0.5X TBE buffer and heated with microwave. After the solution cooled to ~60 °C, 10 μL 10 mg/mL ethidium bromide was added and the agarose sol was cast to make a gel. 1.7 μL 10% silica nanoparticles were mixed with 80 μL 2.5 mg/mL DOTAP in 0.5X PBS by manual pipetting. The mixture formed a stable suspension at room temperature. After incubation for 1 hr, the particles were centrifuged at 5000 rpm for 3 min and the supernatant was removed. After washing with PBS, the supported bilayers were dispersed in 50 μL PBS. Therefore, 1 μL of such solution contains 40 μg silica.

Supported bilayers with mass of 10, 4, 1, and 0.5 μg were mixed with 0.25 μg plasmid DNA. The mixtures were allowed to sit at room temperature for 30 min. Equal volume of loading buffer (30% glycerol, 0.0025% bromophenol blue) was added to each tube and the samples were loaded to the gel. After running at 6 V per centimeter for 70 min in 0.5X TBE, the gel was scanned with a fluorescence image scanner (Fuji).

Transfection. The DsRed plasmid was transformed into competent HB101 E. Coli cells using standard heat shock protocols. The transformed cells were selected on a LB plate with kanamycin (50 μg/ml) and plasmid DNAs were extracted with standard mini-prep protocols (Qiagen). To transfect CHO cells, 1 μg of the plasmid was dissolved in 500 μL serum free F-12K media. In another tube, silica nanoparticle supported bilayers were dispersed in 500 μL of F-12K media. These two tubes were combined, mixed, and incubated for 5 min. Cells were cultured in 12-well plates to ~70% confluency. The old media was removed, and new media with the complex of supported bilayers and plasmid DNA was added. The cells were incubated at 37 °C for 4 hrs. The media was then removed and replaced with fresh media containing antibiotics and 10% fetal bovine serum. The cells were then allowed to grow for another 24 hrs in the incubator.

Flow cytometry. After removal of the media and washed twice with PBS, the CHO cells treated with transfection agents were treated with 500 μL of trypsin/EDTA solution (0.25% trypsin, 0.53mM EDTA in Hanks' Balanced Salt Solution (HBSS), modified without calcium chloride or magnesium chloride) at room temperature for ~5 min. The detached cells were then transferred to 500 μL serum containing F-12K media for flow cytometry studies. The flow cytometry measurement was performed on a FACScalibur instrument. The excitation laser was at 488 nm and the red emission of DsRed protein was monitored.

Cy5-labeling of plasmid DNA. A kit from Mirus was used for labeling the plasmid DNA with Cy-5. The plasmid DNA (5 μg) was dispersed in 47.5 μL of solution containing 1X reaction buffer provided in the kit. 2.5 μL of the reactive dye was added and the tube was kept at 37 °C for 1 hr. The solution was diluted to 170 μL with water and 30 μL of 3 M NaCl and 400 μL 200 proof ethanol were added for ethanol precipitation. The tube was kept at -20 °C for 30 min and centrifuged at 15000 rpm for 10 min. A DNA pellet with characteristic Cy5 dye color was at the bottom of the tube. After removal of the supernatant, the pellet was washed with 70% ethanol and finally dispersed in 20 μL water. The DNA concentration was measured by UV absorption at 260 nm. The labeling efficiency was determined to be about 10 Cy-5 fluorophores for each plasmid DNA.

Confocal fluorescence microscopy. The cells were grown on glass coverslips and were incubated with supported bilayer/plasmid DNA complex at 37 ^{o}C for 2 or 12 hrs. The silica nanoparticles (50 nm or 200 nm diameter) contained a green emission fluorophore, the DOTAP/cholesterol lipid was either labeled with 2% Texas red-DHPE or non-labeled. The plasmid DNA was labeled with Cy-5. After the incubation, free transfection agents were washed away by three washes (five minutes each with shaking) with PBS. The cells were stained with Hoechst 33342, fixed with 3.6% paraformaldehyde for 20 min at room temperature, and mounted with ProLong Gold mounting agent. A Zeiss LSM 510-META confocal system mounted on an inverted (Axiovert 100) microscope was used for imaging. The images were collected with a 63X oil immersion objective.

Transfection in the presence of DNase I. To the 500 μL of media with DNA, add 5 μL (10 units) of DNase 1 and allow it to incubate at room temperature for 15 min before adding the 50 nm protocell (10 μg with 50% DOTAP and 50% cholesterol). In this case, almost no transfected cells can be found (Figure S1B). Otherwise, mix DNA and protocells first, incubate for 5 min before adding the same amount of DNase I and high transfection efficiency was still obtained (Figure S1A). This experiment demonstrates that supported bilayers can effectively protect DNA from nuclease degradation.

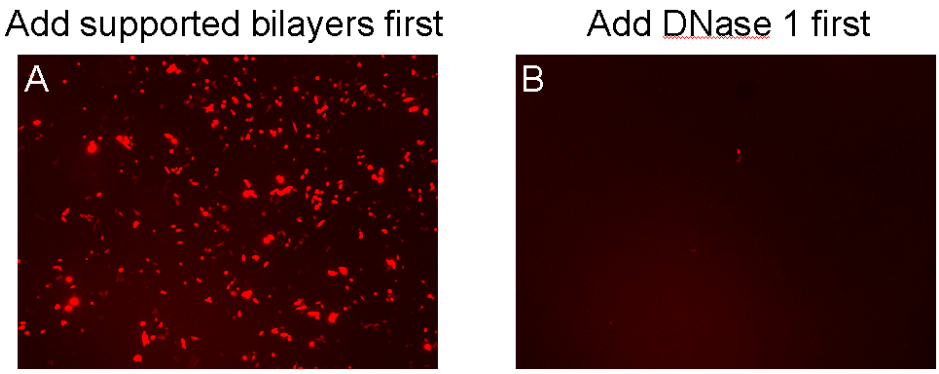

Figure S1. Epifluorescence images of CHO cells transfected in the presence of DNase 1. (A) the plasmid DNA and supported bilayers were mixed first then the DNase was added. (B) the DNase was added before adding the supported bilayers.

Transfection with lipofectamine. The vendor's procedure was followed for the transfection using lipofectamine. 4 μL of lipofectamine 2000 and 1 μg of the plasmid DNA were separately dispersed in 500 μL of serum free culture media and then mixed for transfection.

Cell viability assays. CHO cells were cultured in 12 well plates to ~70% confluence. After incubating with various supported bilayers (10 μg silica) for 24 hrs, the cells were washed with PBS. Cell viability was assayed with calcein AM and ethidium homodimer (kit purchased from Invitrogen) following the vendor's instruction. Briefly, to 12 mL PBS buffer, 10 μL of 2 mM ethidium homodimer and 2.5 μL of 4 mM were added and mixed. 500 μL of this working solution was added to each well. After 20 min at room temperature, the cells were washed with PBS and observed with the inverted fluorescence microscope with a 4X objective. In each field of view, there were ~7000 cells. The dead cells (red dots) were counted from three random regions in three independent samples.

Zeta-potential measurement. The measurements were performed on a Zetasizer Nano dynamic light scattering instrument (Malvern) at 25 °C. The particles were centrifuged to remove free lipids or silica particles and dispersed at a concentration of about 1 mg/mL in 0.6 mL PBS. The particles were then transferred to a disposable cuvette for zeta-potential measurement.