

## Electronic Supplementary Information (ESI)

# Mesoporous Organosilica Nano-bowl with High DNA Loading Capacity - a Potential Gene Delivery Carrier

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## Experimental Section

### Materials

Tetraethyl orthosilicate (TEOS), (3-Aminopropyl)-triethoxysilane (APTES), 1, 2-Bis(triethoxysilyl)ethane (BTEE), cetyltrimethylammonium bromide (CTAB), ammonia solution (25 wt %), cetyltrimethylammonium tosylate (CTAT), triethanolamine (TEA), ammonium nitrate, toluene, ethanol, fluorescein isothiocyanate (FITC), 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT), sodium hydroxide, ethylenediaminetetraacetic acid (EDTA), Tris(hydroxymethyl)aminomethane (Tris), methylsulfoxide (DMSO), polyethylenimine (PEI, 25 kDa) and deoxyribonuclease I (DNase I) were purchased from Sigma Aldrich. Dulbecco's Modified Eagle's Medium (DMEM), fetal bovine serum (FBS), and phosphate buffered saline (PBS), trypsin, Tris Acetate-EDTA (TAE) buffers, and agarose were purchased from Gibco-BRL (Grand Island, U.S.A.). Gel Red (10000x in water) was purchased from Biotium, Australia. Gel loading dye blue (6x) was purchased from Biolab, Australia. Water used in all experiments was purified using a Milli-Q Plus 185 water purification system (Millipore, Bedford, MA) with a resistivity higher than 18 M $\Omega$  cm.

### Synthesis of silica nanospheres

Silica nanospheres (denoted as SS) with sizes around 160 nm were prepared by the Stöber method. Briefly, ethanol (20 mL), H<sub>2</sub>O (1 mL), and ammonia solution (25 wt %, 1 mL) were combined at 40 °C under stirring. Then, TEOS (1 mL) was added and stirred for another 8 h at 40 °C.

### **Synthesis of mesoporous organosilica nano-bowls**

The SS was then coated with an organosilica layer to obtain core-shell particle (denoted as SS@OS). The above Stöber reaction mixture (2 mL) was added to a solution containing water (75 mL), ethanol (5 mL), CTAB (150 mg) and ammonia (25 wt %, 1 mL). After stirring at room temperature for 0.5 h, a total of BTEE (30  $\mu$ L) dissolved in ethanol (3 mL) was added in 3 times at 10 min intervals. After reaction for another 24 h, the SS@OS product was collected by centrifugation and washed with ethanol and water for several times.

Mesoporous organosilica nano-bowl (denoted as Bowl) was obtained after removing the core part of the SS@OS by a hydrothermal-etching method.<sup>1</sup> The washed SS@OS was redispersed in a solution containing water (20 mL), Na<sub>2</sub>SO<sub>4</sub> (80 mg) by sonication. Then, the dispersion was adjusted to pH 3 with H<sub>2</sub>SO<sub>4</sub> (0.1 M) and stirred at room temperature for 24 h. Afterwards, the mixture was transferred into a sealed autoclave and heated at 180 °C for 10 h. The etched particles were centrifuged and washed with water for several times.

### **Synthesis of hollow mesoporous organosilica nanoparticles**

Hollow mesoporous organosilica nanoparticles (denoted as Hollow) were prepared according to the literature with slight modification.<sup>2</sup> The above Stöber silica solution was further mixed with H<sub>2</sub>O (20 mL), CTAB (150 mg) and ammonia solution (0.5 mL). Then, BTEE (0.5 mL) was added and the mixture was allowed to react for 6 h at 30 °C. The particles were collected by centrifugation and redispersed into Na<sub>2</sub>CO<sub>3</sub> aqueous solution (0.6 M). After stirring at 80 °C for 1 h, the hollow particles were centrifuged and washed with water for several times.

### **Synthesis of MCM-41 type nanoparticles**

MCM-41 type mesoporous silica nanoparticles (denoted as MCM41) were prepared according to the literature.<sup>3</sup> Sodium hydroxide aqueous solution (2 M, 0.7 mL) was added to water (96 mL) containing CTAB (0.2 g) at 80 °C. Then, TEOS (1 mL) was added under vigorous stirring. The mixture was allowed to react for 2 h at 80 °C and then centrifuged to get the precipitate. The white precipitate was washed with water for several times.

### **Synthesis of dendritic mesoporous silica nanoparticles**

Dendritic type mesoporous silica nanoparticles (denoted as Dendritic) were prepared according to the literature.<sup>4</sup> CTAT (0.48 g) and TEA (87 mg) was dissolved in water (25 mL) at 80 °C. Then, TEOS (4.9 mL) was added under vigorous stirring. The mixture was allowed to react for 2 h at 80 °C and then centrifuged to get the precipitate. The white precipitate was washed with water for several times.

### **Surfactant removal, amine functionalization and fluorescence labeling**

The surfactant in the above as-prepared product was removed by extraction for two cycles. In each cycle, the as-prepared powder (50 mg) was refluxed in ethanol solution (10 mL) containing ammonium nitrate (2 wt %) for 6 h. For amine functionalization, the extracted powders were refluxed in toluene (20 mL) containing APTES (50  $\mu$ L) at 90 °C for 12 h followed by washing sequentially with toluene and ethanol. The amine functionalized products are denoted with a prefix A for each type of particles. For example, A-Bowl stands for amine functionalized organosilica nano-bowls. For fluorescence labelling, A-Bowl (10 mg) were stirred in ethanol solution of FITC (5 mL, 10  $\mu$ g mL<sup>-1</sup>) in dark for 24 h at room temperature. The labelled nanoparticles were collected by centrifugation and washed with ethanol repeatedly until the supernatants were colourless.

### **Cell culture**

Human embryonic kidney 293 (HEK293) cells were purchased from American Type Culture Collection and grown in DMEM culture medium supplemented with 10% (v/v) FBS in a humidified 5% CO<sub>2</sub> atmosphere at 37 °C. For all experiments, cells were harvested by using 0.25% trypsin and resuspended in fresh medium before plating.

### **Cytotoxicity assay**

HEK293 cells were seeded into 96-well plates at a density of  $1 \times 10^4$  per well in culture medium (100  $\mu$ L) and grown for 24 h. Then, the medium was replaced by ones containing varied concentrations of nanoparticles. After 24 h incubation, the medium was replaced by fresh medium and 10  $\mu$ L of MTT (5.0 mg mL<sup>-1</sup> in PBS) was added to each well. After further incubation for 4 h, the medium was removed and 150  $\mu$ L of DMSO was added to each well to dissolve formazan crystals. Finally, the absorbance was determined using a micro-plate reader (Biotek, USA) at the wavelength of 595 nm. The viability of cells without nanoparticle treatment was assumed to be 100%. Data were expressed as mean  $\pm$  standard deviation (SD) of at least three independent experiments.

### **Plasmid DNA preparation**

The pEGFP-N1 plasmid DNA (pDNA, 4.7kb) expressing the enhanced green fluorescent protein (EGFP) was prepared in Escherichia coli DH5 $\alpha$  strain and extracted using a QIAGEN Plasmid Midi kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. The integrity and purity of the prepared pDNA was analyzed using 0.8% agarose gel electrophoresis and the pDNA concentration was determined using a nanophotometer (P300,

Implen GmbH). For microscopy imaging experiment, the pDNA was further labelled by fluorescent dye (YOYO-1) at a molar ratio of 1 molecular dye to 100 nucleic acid base pairs.

### **Gel retardation assay**

The binding capability of nanoparticles for pDNA was evaluated by agarose gel electrophoresis. Samples were prepared by mixing nanoparticles and pDNA (0.2 µg) at different weight ratios and incubated for 30 min at room temperature. After the addition of gel loading dye blue, the samples were electrophoresed through a 0.8% agarose gel containing gel red in TAE buffer solution at 60 V for 60-90 min. The resulting pDNA migration patterns were recorded under UV irradiation (G-BOX, SYNGENE). To evaluate the DNA protection capability, the nanoparticles and pDNA were mixed at a weight ratio of 20 and incubated for 30 min at room temperature. Then, 2.5 U of DNase I (0.5 U µL<sup>-1</sup>) in 10mM Tris-HCl, 2.5 mM MgCl<sub>2</sub>, 0.5 mM CaCl<sub>2</sub> solution (pH 7.6) was added and the mixture was further incubated at 37 °C for 30 min. The DNase I was inactivated by adding 500 mM EDTA (2 µL). The pDNA was then released from the complex by treatment with heparin (10 mg mL<sup>-1</sup>) for 30 min. Naked pDNA was also treated in the same procedure as the control.

### **In vitro transfection**

HEK293 cells were seeded into a 24-well plate with coverslips at the density of  $2 \times 10^4$  cells per well in culture medium (400 µL) 24 h before transfection. Prior to transfection, nanoparticle/pDNA complexes were freshly prepared by mixing pDNA (0.4 µg) and A-Bowl (8 µg) in water (20 µL). The mixture was incubated for 30 min at room temperature and then diluted with 400 µL DMEM media. The cells were incubated with this particle/pDNA suspension for 4 h and then the medium was replaced by DMEM containing 10% FBS. After 48 h, the transfection results was observed using a fluorescence microscope (Zeiss Axio Vert. A1) equipped with a 488 nm laser and the transfection efficiency was evaluated using a flow cytometry (Becton Dickinson) in FL1-H channel. For co-delivery of pDNA and chloroquine, A-Bowl (2 mg) was first incubated with chloroquine aqueous solution (200 µg mL<sup>-1</sup>, 1 mL) for 24 h, then the suspension was centrifuged and the precipitate was washed with water twice. The amount of loaded chloroquine was determined by the UV-vis absorption of super liquid at the wavelength of 329 nm before and after incubation.<sup>5</sup> Then, the chloroquine-loaded A-Bowl was mixed with pDNA following the process described above and used for subsequent transfection. For transfection mediated by PEI, pDNA (0.4 µg) in 50 µL DMEM was mixed with PEI (2 µg) in 50 µL DMEM. After incubation for 30 min at room

temperature, the mixture was diluted with 300  $\mu\text{L}$  DMEM and used for subsequent transfection. The delivery of pDNA and its subcellular location were also observed using a Leica SP5 confocal laser scanning microscope. HEK293 cells were fixed with 4 wt % formaldehyde at 16 h after transfection with the particle/YOYO-1-labelled pDNA complex. The cell nucleus and plasma membrane were stained with Hoechst 33258 ( $1 \mu\text{g mL}^{-1}$ ) and Alexa Fluor 594 Wheat Germ Agglutinin (WGA,  $5 \mu\text{g mL}^{-1}$ ) for 15 min at room temperature, respectively. After the staining, the cells were softly washed twice with PBS to remove excessive dye and incubated in PBS before imaging.

### **Material characterization**

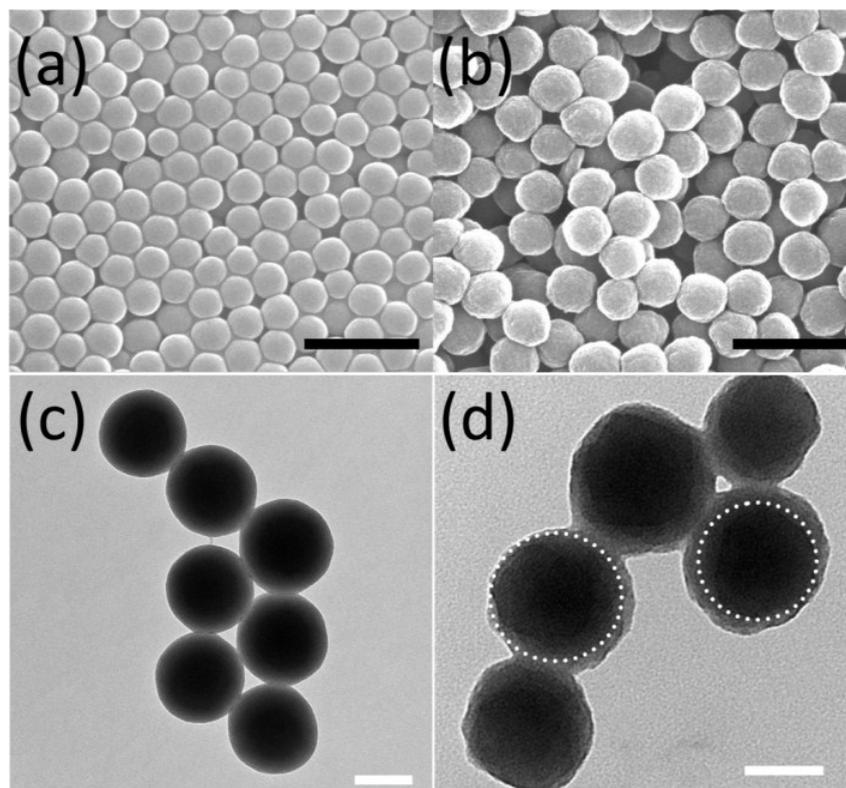
Transmission electron microscopy (TEM) images were taken with FEI Tecnai G2 operated at 120 kV. The samples were prepared by dispersing the powder in ethanol and then dropped on carbon film supported by a Cu grid and air dried. Scanning electron microscopy (SEM) observation was carried out on a FEI Quanta 450 FEG environmental emission scanning electron microscope. The samples were prepared by adding powder onto carbon tape and coated with platinum before SEM observation. The hydrodynamic size and zeta-potential of nanoparticles in aqueous dispersions were measured on a Malvern Zetasizer Nano ZS operated at a wavelength of 633 nm, where scattered light was collected at a fixed angle of  $173^\circ$ . Raman spectra were collected with HORIBA LabRAM HR Evolution with 633 nm laser. Fourier transform infrared (FTIR) spectra were recorded on a Thermo Scientific NICOLET 6700 FTIR spectrometer with a Diamond ATR (attenuated total reflection) crystal. For each spectrum, 64 scans were collected at a resolution of  $4 \text{ cm}^{-1}$  over the range  $400\text{-}4000 \text{ cm}^{-1}$ . UV-vis absorption spectra were recorded on a UV-2600 spectrophotometer (Shimadzu Corporation). Thermogravimetric analysis (TGA) and differential scanning calorimetry (DSC) was performed on a Mettler Toledo Thermogravimetric Analyser, using an air atmosphere ( $60 \text{ mL min}^{-1}$ ) with a heating ramp of  $20 \text{ }^\circ\text{C min}^{-1}$ . Nitrogen sorption measurement was carried out at  $-196 \text{ }^\circ\text{C}$  using a TriStar II surface area and porosity analyser (Micromeritics). Prior to the measurement, the samples were degassed at  $120 \text{ }^\circ\text{C}$  for 12 h.

### **References**

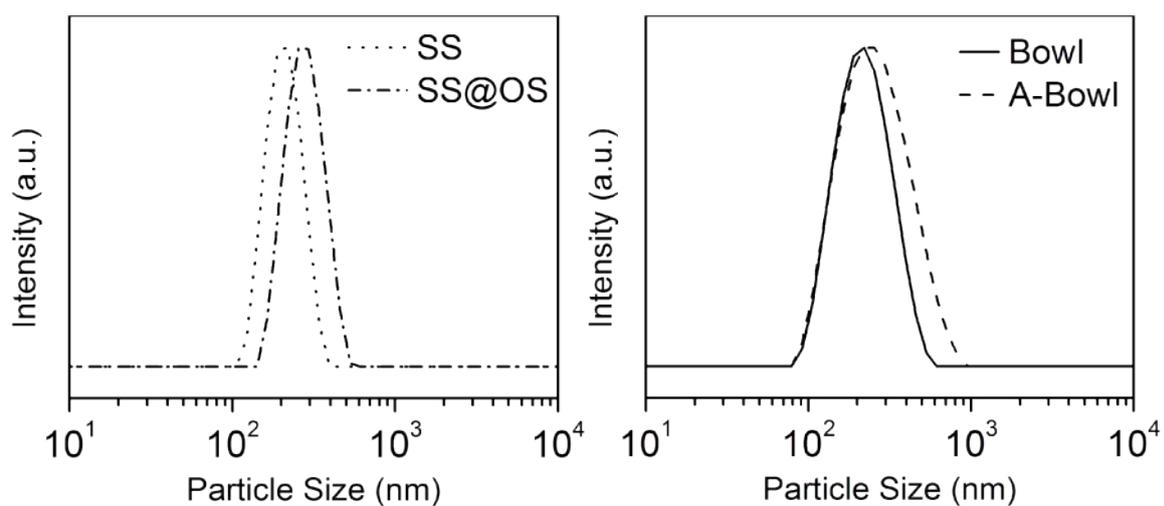
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2. Y. Chen, P. Xu, H. Chen, Y. Li, W. Bu, Z. Shu, Y. Li, J. Zhang, L. Zhang, L. Pan, X. Cui, Z. Hua, J. Wang, L. Zhang and J. L. Shi, *Adv. Mater.*, 2013, **25**, 3100.
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4. L. Xiong, X. Du, B. Y. Shi, J. X. Bi, F. Kleitz and S. Z. Qiao, *J. Mater. Chem. B*, 2015, **3**, 1712.
5. S. B. Hartono, N. T. Phuoc, M. H. Yu, Z. F. Jia, M. J. Monteiro, S. Z. Qiao and C. Z. Yu, *J. Mater. Chem. B*, 2014, **2**, 718.

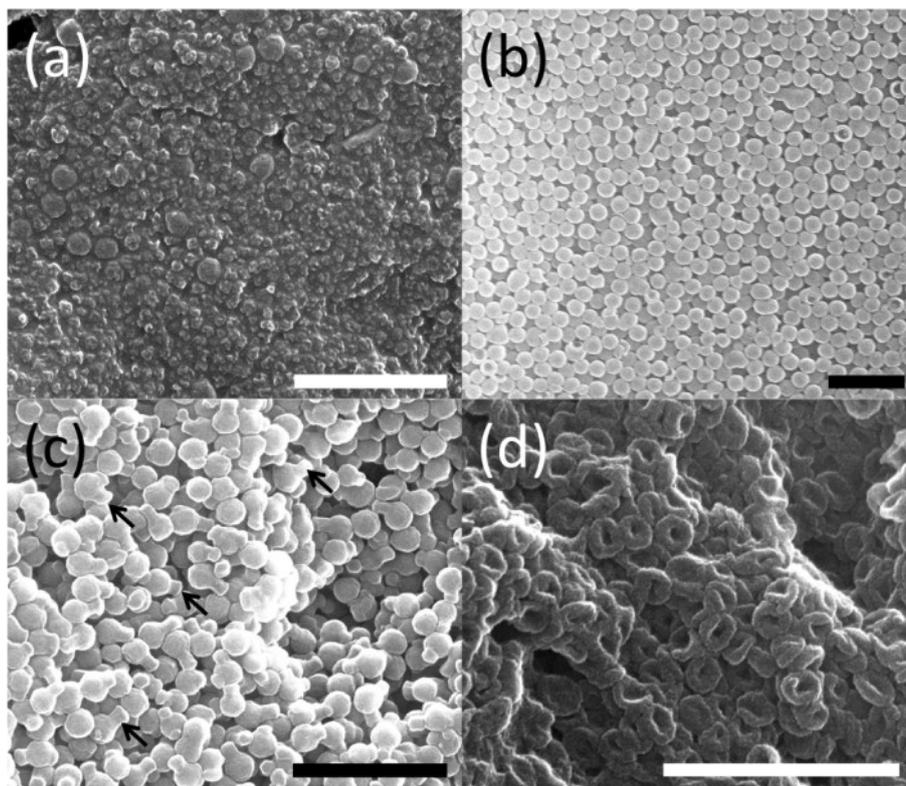
## Supporting Data



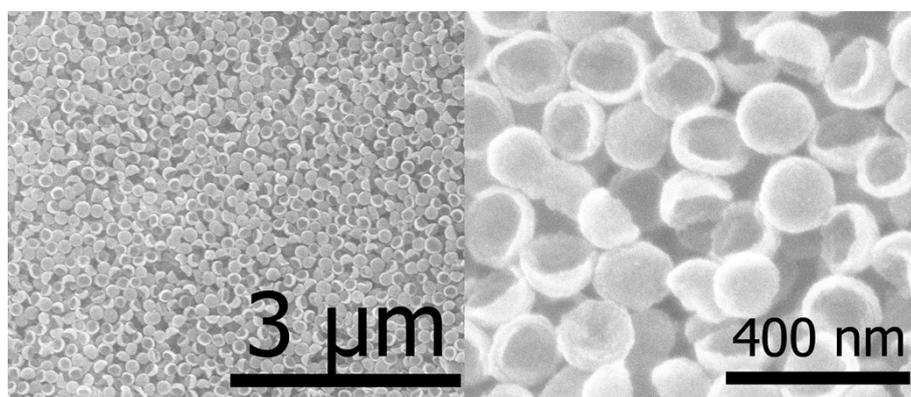
**Fig. S1** SEM (a, b) and TEM (c, d) images of SS (a, c) and SS@OS (b, d). The positions of two SS cores in SS@OS (d) are circled with dotted-line to illustrate the asymmetric coating of organosilica layer. The scale bars in (a, b) and (c, d) are 500 nm and 100 nm, respectively.



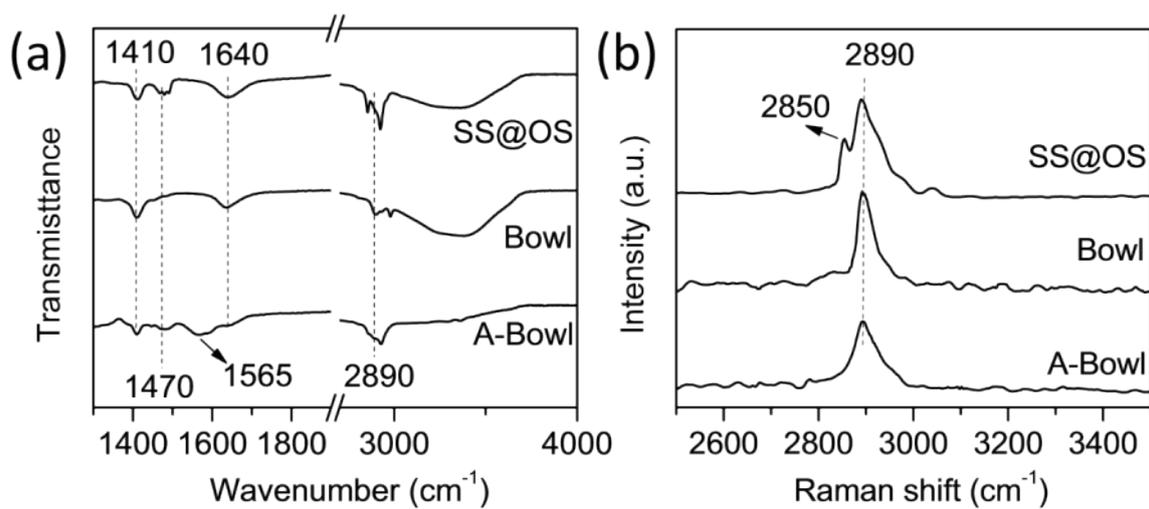
**Fig. S2** Hydrodynamic size distributions of nanoparticles dispersed in water.



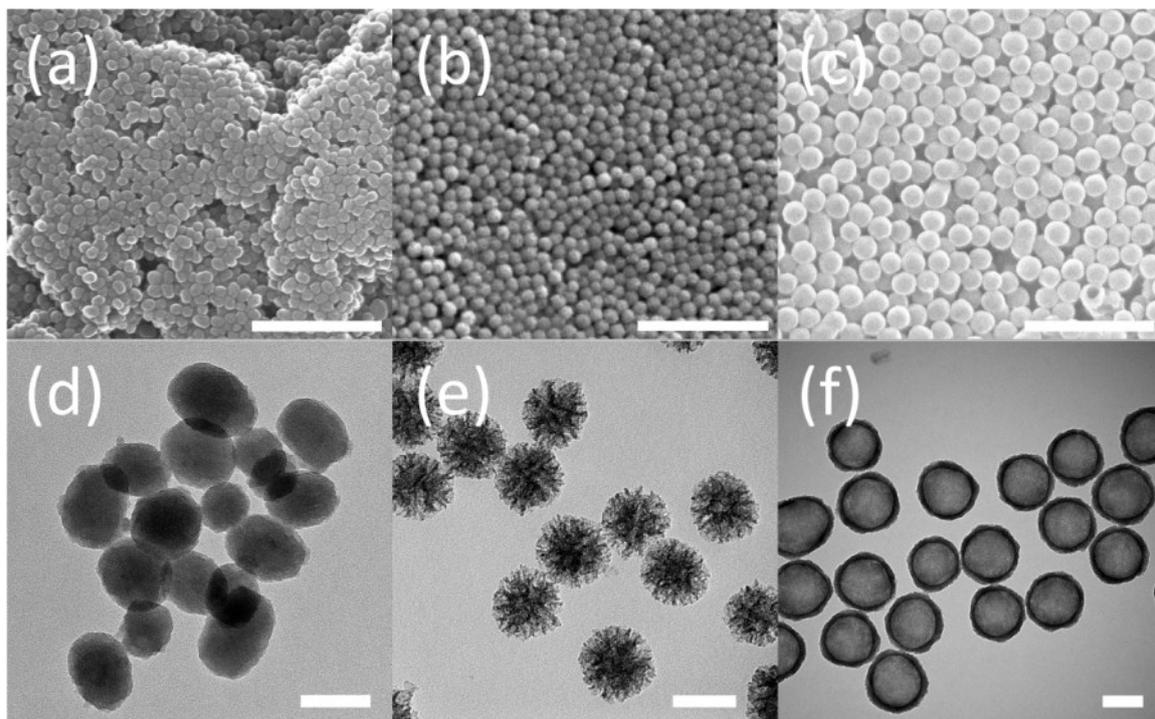
**Fig. S3** SEM images of products after hydrothermal treatment when the BTEE addition is (a) 10  $\mu\text{L}$  and (b) 50  $\mu\text{L}$ . SEM images of products when (c) 30  $\mu\text{L}$  BTEE is added over a period of 6 h, the Janus part is indicated with black arrow, (d) SS@OS is etched with 0.6 M  $\text{Na}_2\text{CO}_3$  at 60  $^\circ\text{C}$  for 6 h.



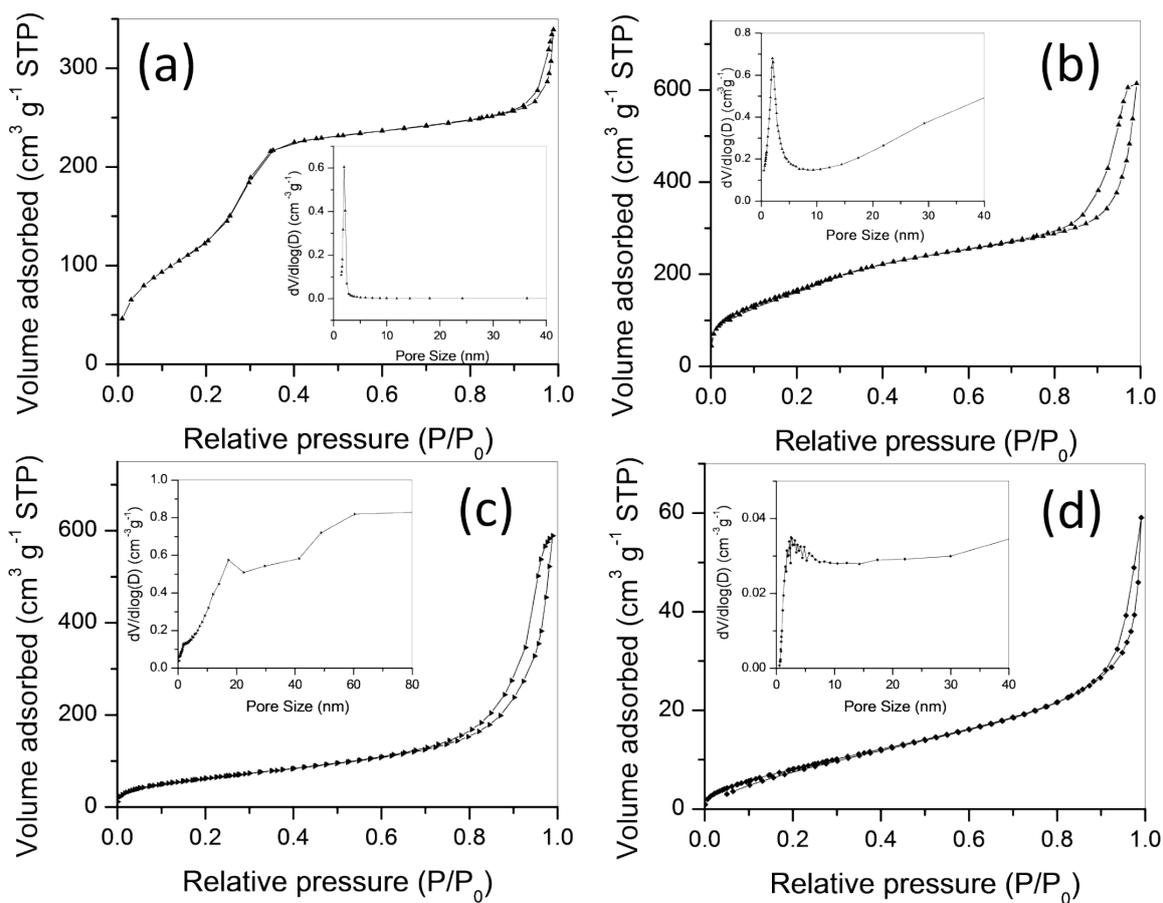
**Fig. S4** SEM images of amine functionalized mesoporous organosilica nano-bowls.



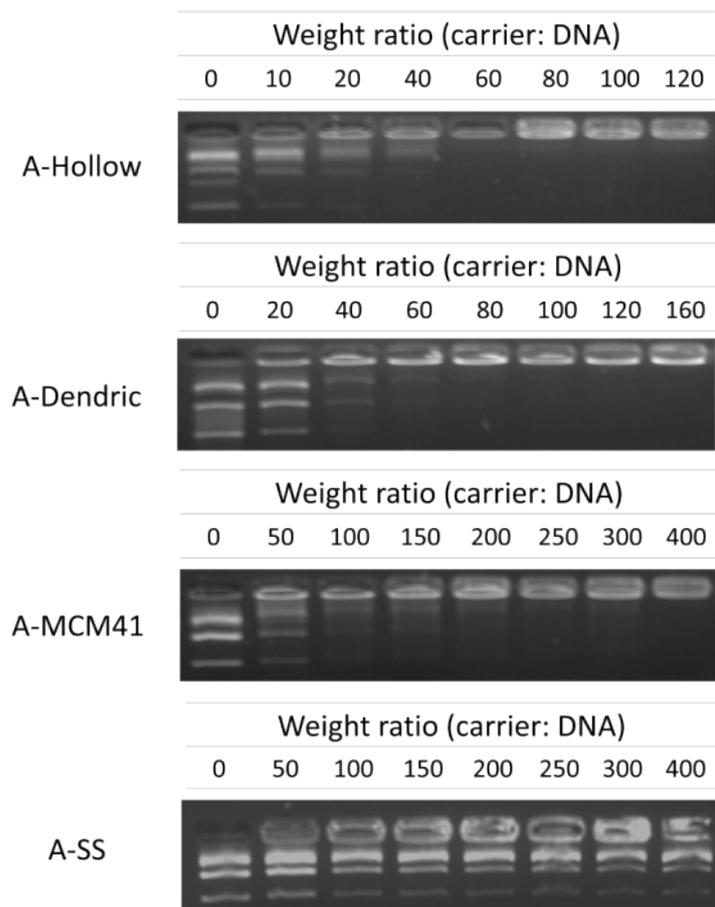
**Fig. S5** Enlarged (a) FTIR and (b) Raman spectra of mesoporous organosilica nano-bowls.



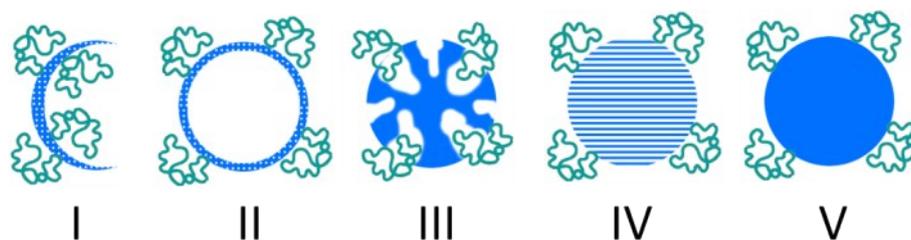
**Fig. S6** SEM (a, b, c) and TEM (d, e, f) images of A-MCM41 (a, d), A-Dendric (b, e) and A-hollow (c, f). The scale bars in (a, b, c) and (d, e, f) are 1  $\mu\text{m}$  and 100 nm, respectively.



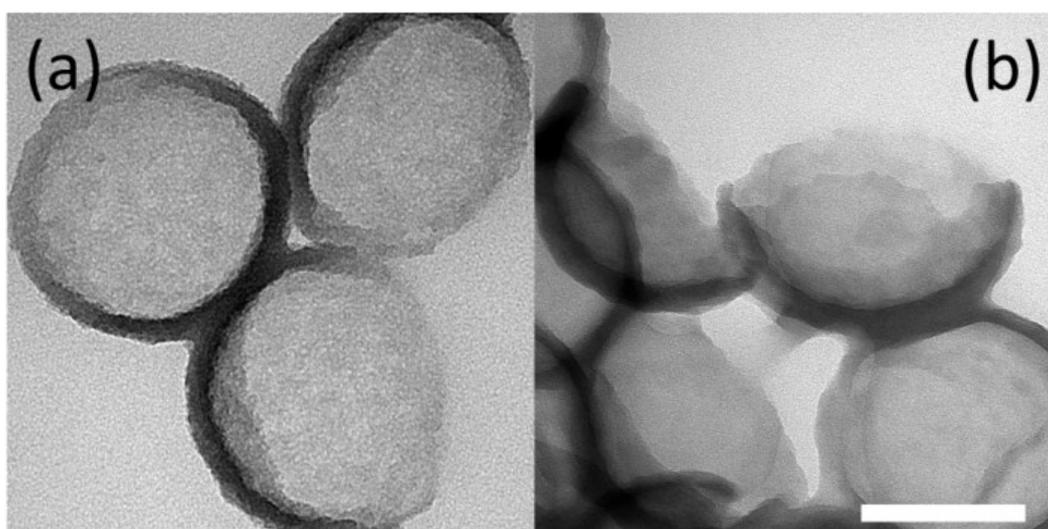
**Fig. S7** Nitrogen sorption and pore size distribution (insert) curves of (a) A-MCM41, (b) A-Hollow, (c) A-Dendric and (d) A-SS.



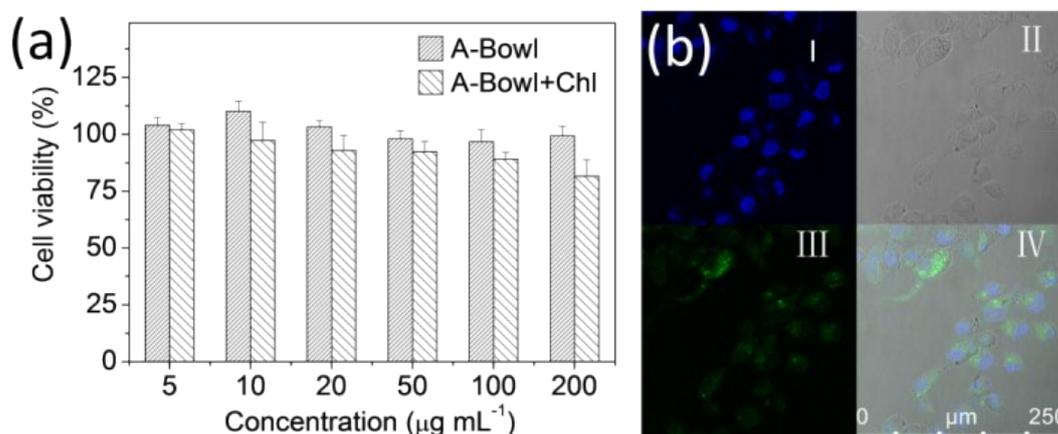
**Fig. S8** Gel retardation assays of nanoparticles/pDNA complex at different carrier/pDNA weight ratios.



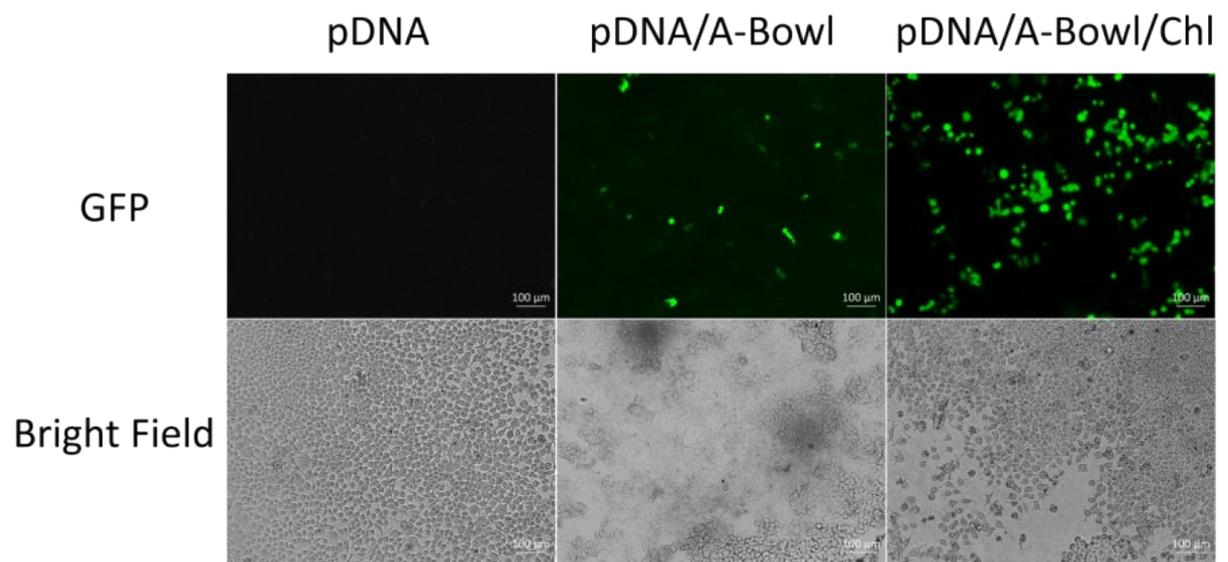
**Fig. S9.** Proposed loading status of plasmid DNA on various amine functionalized silica-based nanoparticles: ( I ) A-Bowl, ( II ) A-Hollow, ( III ) A-Dendric, ( IV ) A-MCM41, ( V ) A-SS.



**Fig. S10** TEM images of A-Bowl (a) before and (b) after loading of pDNA. The scale bar is 100 nm.



**Fig. S11.** (a) Viability of HEK293 cells after 24 h incubation with A-Bowl or A-Bowl containing 4 % chloroquine. (b) Confocal images of HEK293 cells after 4 h incubation with 20  $\mu\text{g mL}^{-1}$  A-Bowl. ( I ) cell nucleus (blue) labelled with Hoechst 33258; ( II ) differential interference contrast image; ( III ) A-Bowl particles (green) labeled with FITC; ( IV ) overlay image of ( I ), ( II ) and ( III ).



**Fig. S12.** Fluorescent and bright field images of HEK293 cells transfected with different transfection reagents.

**Table S1.** Hydrodynamic diameters of nanoparticles dispersed in water.

Sample	Average Particle Size (nm)	Polydispersity Index
SS	196	0.036
SS@OS	268	0.042
Bowl	207	0.178
A-Bowl	240	0.218

**Table S2.** Structure parameters of nanoparticles.

Sample	Surface Area <sup>a</sup> (m <sup>2</sup> g <sup>-1</sup> )	Pore Volume <sup>b</sup> (cm <sup>3</sup> g <sup>-1</sup> )	Pore Size <sup>c</sup> (nm)	Surface Amine <sup>d</sup> (mmol g <sup>-1</sup> )
Bowl	985	1.08	2.5	-
A-Bowl	876	0.97	2.1	0.81
A-MCM41	754	0.59	2.0	1.69
A-Dendric	234	0.91	17.2	1.24
A-Hollow	852	0.95	2.0	0.93
A-SS	31	0.09	-	0.11

a) Brunauer-Emmett-Teller (BET) specific surface area was calculated using N<sub>2</sub> adsorption data at a relative pressure P/P<sub>0</sub> range of 0.05-0.30. b) Pore size distribution was derived from adsorption branch of the isotherm using the Barrett-Joyner-Halenda (BJH) method. c) Pore volume was determined from the amount of N<sub>2</sub> adsorbed at the single point of P/P<sub>0</sub>=0.99. d) Surface amine group was calculated from the difference of weight loss in thermogravimetry between modified and unmodified samples.