Supplementary Information for

**Shaping Nanoparticles with Hydrophilic Compositions and Hydrophobic Properties as Nano-carriers for Antibiotic Delivery**

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Experimental section

Nanoparticles Preparation

The method for the preparation of MHS was adopted from Chen et al.\(^1\) Monodipersed Stöber silica particles were prepared and then a mesoporous silica layer was coated on the outer surface. Subsequent alkaline etching of the silica core in Na\(_2\)CO\(_3\) aqueous solutions was conducted to produce MHS. Typically, ethanol (35.7 ml), deionized water (5 ml) and ammonia solution (1.57 ml) were mixed under magnetic stirring at 30 °C. Tetraethyl orthosilicate (TEOS, 3 ml) was rapidly added into the mixture under magnetic stirring. After stirring for 2 h, premixed TEOS (2.5 ml) and trimethoxy(octadecyl)silane (1.5 ml) were rapidly poured into the mixture and stirring was continued for another 2 h. The product was collected by centrifugation and dispersed in 0.6 M Na\(_2\)CO\(_3\) solution (100 ml) at 80 °C under stirring for 4 h. The products were further washed thoroughly with water to completely remove Na\(_2\)CO\(_3\) and dried under vacuum at room temperature overnight. Finally, the obtained samples were calcined at 550 °C for 6 h to remove the organic templates. The as-synthesized MHS (200 mg) were added to a 100 ml round bottom flasks containing 20 ml toluene and the mixture were stirred for 30 min to disperse the particles in the solvent before adding 0.19 ml 3-aminopropyltriethoxysilane (APTES). After refluxing at 110 °C for 20 h, the APTES modified particles (denoted as APTES-HS) were extensively washed with ethanol and water, and dried in a vacuum oven at 40 °C overnight. Separately, the small silica (SSNPs) was prepared by a modified Stöber method. Typically, absolute ethanol (50 ml) was mixed with deionized water (3.8 ml) and ammonium hydroxide solution (1.4 ml, 28%) at 70 °C. Then, TEOS (3.0 ml) was added to the solution under vigorous stirring and continuously stirred for 1 hr. 200 mg APTES-HS was added into the as-synthesized Stöber suspension and was allowed to stir for 30 min to obtain homogenously disperse particles with rough morphology. Products were isolated by filtration, dried in a vacuum oven at 40 °C overnight and further calcined at 550 °C for 6 h in air to remove aminopropyl groups. Finally stable rough mesoporous hollow silica (RMHS) was obtained with pure silica in composition. The same method was repeated to prepare the RSS using the monodipersed Stöber silica particles as the core.

Sample Characterization

HRTEM images were obtained with JEOL 2100 operated at 200 kV, respectively. For HRTEM measurements, the samples were prepared by dispersing the powder samples in
ethanol by sonication and were then dropped on carbon film on a Cu grid and air dried. The morphologies of the samples were also observed using a JEOL 7001 SEM operated at 15 kV. For SEM measurements, the samples were prepared by adding the nanoparticles on the carbon tape. Nitrogen (N\textsubscript{2}) adsorption-desorption isotherms were measured at -190 °C by using a Micromeritics Tristar II system. Before measuring, all samples were degassed at 180 °C under vacuum overnight. The pore size distributions were calculated by the Barrett–Joyner–Halenda (BJH) method while the total pore volume and surface area were calculated by using typical Brunauer–Emmett–Teller (BET) method. Fourier transform infrared spectroscopy (FTIR) spectra were performed on ThermoNicolet Nexus 6700 infrared spectrometer equipped with a Diamond ATR (attenuated total reflection) crystal. For each spectrum, 128 scans were collected at resolution of 4 cm\textsuperscript{-1} over the range 400-4000 cm\textsuperscript{-1}. TGA analysis was conducted using a TGA/DSC 1 Thermogravimetric Analyzer (Mettler Toledo Inc) at a heating rate of 2 °C min\textsuperscript{-1} from 25 to 800 °C under nitrogen. The contact angles of MHS and RMHS were measured by gel trapping technique (GTT).\textsuperscript{2} A detailed GTT protocol is provided in Figure S6. HRSEM images from the GTT were observed using JEOL 7800 field operated at 15 kV. The deposition of about 10 nm of a thin carbon layer was performed over the particle monolayer on the polydimethylsiloxane (PDMS) surface prior to SEM imaging. DLS and \(\zeta\)-potential measurements were carried out at room temperature using a Zetasizer Nano ZS from Malvern Instruments. The samples were dispersed in deionized water by ultrasonication before analysis.

**Organic Solvent-Water Interfaces**

In a typical experiment, 5 ml of deionized water was first added into a 20 ml glass vial followed by 5 ml of diethyl ether. Then, 5 mg of the samples were added into the water-solvent layer and a gentle shaking was applied for 5 min. The digital images of the samples were taken when the particles come to rest.

**Loading of Hydrophobic Dye and Hydrophobic Drug**

Disperse red 1 (DR1), a hydrophobic dye was loaded into the nanoparticles to evaluate the difference in hydrophobicity between the MHS and RMHS. Nanoparticles (1.0 mg) were dispersed in 1.0 ml DR1 in tetrahydrofuran (THF) (1 mg ml\textsuperscript{-1}) and the solution was rotated for 24 h at room temperature. The dye loaded nanoparticles were collected by centrifugation (13, 300 rpm, 5 min) and were washed twice with water. To measure the amount of DR1 loaded on the nanoparticle, THF (0.2 ml) was added into the DR1 loaded nanoparticles, vortexed for 1
min and supernatant was collected and measured by (UV-Vis spectrophotometer, Shimadzu) at 480 nm. For DR1 uptake rate study, 10 ml of the dye in THF (200 μg ml⁻¹) was added to 10 mg nanoparticles. The mixture was allowed to rotate at room temperature and the supernatant was collected at different time point and was measured by UV-Vis spectrophotometer. Antifungal drug, griseofulvin (GRIS) in methanol (1 mg ml⁻¹, 1.0 ml) was added to 1.0 mg of the nanoparticles. The solution was rotated for 24 h and nanoparticles were separated from the solution by centrifugation. The absorbance of the supernatant was measured by UV-Vis spectrophotometer at 296 nm. The loading amount of the GRIS can be calculated based on the original and residual drugs concentrations and volumes. All experiments were performed in triplicates.

**Loading and Release of Vancomycin**

The vancomycin (VAN) loading was carried out by dissolving the drug in a PBS buffer (pH 7.4). Nanoparticles (10 mg each) were soaked in 10 ml of VAN-PBS solution (5 mg ml⁻¹) and were rotated for 24 h. The mixtures were then centrifuged at 13, 300 rpm for 5 min. To evaluate the loading amount, the supernatant were collected and the residual drug content was measured by using UV-Vis spectrophotometer (Shimadzu) at 289 nm. To obtain the drug release profiles, precipitates of VAN loaded nanoparticles were soaked in PBS and rotated at 37 °C. At a selected time point, samples were centrifuged and the supernatant was collected for UV-Vis measurement. The points in each release profile are the average values collected from two independent experiments.

**Protein Loading and Release**

Ribonuclease A (RNase A), insulin (INS) and lysozyme (LYS) proteins have been selected as a model protein. Typically, nanoparticles (1.0 mg) were dispersed in 1.0 ml protein solution in PBS (1 mg ml⁻¹) and the solution was rotated for 24 h at room temperature. After rotating at 25 °C for 24 h, the mixtures were centrifuged at 13,300 rpm for 5 min. To evaluate the loading amount, the supernatants were collected and the residual protein content was measured by using a UV-2450 (UV-Vis spectrophotometer, Shimadzu) at a wavelength of 277.5 nm, 284.5 nm and 285 nm for RNase A, INS and LYS respectively. The loading amount of the protein can be calculated based on the original and residual protein concentrations and volumes. All experiments were performed in triplicates. For protein release study, the dried precipitates (LYS loaded nanoparticles) were mixed with PBS and allowed to rotate at room
temperature for 24 h. The supernatants were collected at different time point and the released LYS content was measured and evaluated by using a UV-Vis spectrophotometer.

**Antibacterial Study**

Bacterial suspension (100 μL of $1 \times 10^7$ CFU ml$^{-1}$) and EDTA (100 μL, final concentration was 1 mM) was added into luria broth (LB) medium (700 μl) for each 1.5 ml centrifuge tubes. Then 100 μl of the samples diluted in PBS (VAN, MHS200-VAN, and RMHS200-VAN) at different concentration and PBS only as the blank control were separately added and shaken at 37 °C on a shaker bed at 220 rpm for 18 h. The bacterial viability was determined by OD at 600 nm using a multifunctional microplate reader (Tecan infinite M200). Sample was shaken for 20 sec prior to the OD measurement to obtain homogenous distribution of nanoparticles. The morphological changes of *E. coli* treated at 18 h with PBS (control), VAN, MHS200-VAN, and RMHS200-VAN (all 25 μg ml$^{-1}$ calculated based on VAN) were observed by JEOL 1010 microscope operated at 100 kV. The TEM samples were prepared by drop-coating the treated *E. coli* cells onto copper grids for 45 s, the excess amount of droplets were removed with filter papers. The *E. coli* cells on the copper grids were stained with uranyl acetate (2%) for 30 s prior to imaging. In a separate experiment, OD measurements at 600 nm were taken at 4 h, 8 h, 18 h, and 24 h to study the antibacterial performance in a timely dependent manner. Each concentration was prepared and measured in triplicate, and all experiments were repeated at least twice in parallel.

**Rose Bengal Partitioning**

Hydrophobicity of the nanoparticles was studied using the rose bengal partitioning method as previously described. A solution of rose bengal (RB) reagent in 0.1 M phosphate buffer (pH 7.4) was diluted to 20 μg ml$^{-1}$. Various concentrations of nanoparticles were added to the solution to create a wide array of RB-nanoparticle suspensions. Each sample was incubated at 25 °C for 3 hr. Suspensions were subsequently centrifuged at 13,300 rpm for 5 min. The supernatant was collected and absorbance was read using a UV/Vis spectrophotometer at 543 nm (UV-Vis spectrophotometer, Shimadzu). PQ was calculated as the ratio of bound RB and free RB in solution.

**MTT Assay**

The cytotoxicity of nanoparticles was tested with a human dermal fibroblast (HDF) cell line. One day before the test, 5.0 x 10$^3$ cells per well were seeded in 96 well plates. Nanoparticles
were dispersed in culture medium supplemented with 10% FBS and 1% penicillin-streptomycin. After 24 h incubation at 37 °C, the liquid was replaced with fresh medium containing nanoparticles and the cells were cultured for subsequent 24 h. After 24 h incubation, the culture medium was replaced with MTT working solution (final concentration of 0.5 mg ml⁻¹) and further incubated at 37 °C for 4 h. The dark blue formazan crystals formed were dissolved with DMSO and the absorbance at 570 nm was measured using a Synergy HT Microplate Reader. Results were normalized by cells not exposed to nanoparticles as a control.

**Supporting Data**

![Figure S1](image)

**Figure S1** The morphology of the nanoparticles. SEM images of MHS showing relatively smooth surface (a) and hollow core (b), TEM image of MHS (c) and RMHS (d) showing homogenous structure with uniform size and morphology.
**Figure S2** Particles size distribution of nanoparticles measured by DLS.
Figure S3 a) Nitrogen adsorption and desorption isotherms and b) pore size distribution curves of nanoparticles.
Figure S4 FTIR spectra of MHS (a) and RMHS (b).
Figure S5 a) TEM images of RSS, b) dispersion behaviour of RSS in water/diethyl layer before and after shaking and c) loading of LYS on RSS.

Figure S6 GTT preparation protocol for the contact angle measurement of the nanoparticles.
Figure S7 a) LYS uptake and b) the release profile as a function of time of LYS from MHS and RMHS.
**Figure S8** TEM images of MHS200 (a) and RMHS200 (b) and SEM images of MHS200 (c) and RMHS200 (d).
Figure S9 Cumulative release of vancomycin from MHS200 and RMHS200 over 24 h. Each point represent the average values collected from two independent experiments.
Figure S10  a) TEM images of *E.coli* treated in PBS, b) *E.coli* treated in VAN, c) *E.coli* treated in MHS200-VAN and d) *E.coli* treated in RMHS200-VAN at the dosage of 25 µg ml⁻¹ for 18 h. Scale bar = 500 nm.
Figure S11 MTT assay of nanoparticles in HDF cell line cultured at 24 h.
Figure S12 The TEM image of RMHS after shaking (a) and after vigorous stirring (b) in PBS for 36 h
Table S1 Physiochemical properties of silica nanoparticles.

| Sample       | Surface Area ($\text{m}^2/\text{g}$) | Pore diameter (nm) | $V_t$ ($\text{cm}^3/\text{g}$) | $V_{\text{meso}}$ ($\text{cm}^3/\text{g}$) | Surface charge (mV) | Size (nm) | Hydrophobicity ($10^9$ ml/µm$^2$) |
|--------------|--------------------------------------|--------------------|---------------------------------|-------------------------------------------|---------------------|----------|-----------------------------------|
| MHS          | 427                                  | 3.5                | 0.38                            | 0.34                                      | -34.9$^p$           | 396      | 1.803e-5 ± 6.817e-5                |
|              |                                      |                    |                                 |                                           | -23.3$^p$           |          |                                   |
| RMHS         | 342                                  | 3.5                | 0.46                            | 0.35                                      | -33.6$^w$           | 459      | 0.001058 ± 7.106e-5                |
|              |                                      |                    |                                 |                                           | -24.5$^p$           |          |                                   |
| RSS          | 14                                   | -                  | 0.05                            | N/A                                       | -38.4               | 443      | 0.000675 ± 0.0004                  |
| MHS200       | 310                                  | 3.4                | 0.38                            | 0.24                                      | -30.8               | 164      | -                                 |
| RMHS200      | 371                                  | 3.4                | 0.62                            | 0.29                                      | -31.4               | 190      | -                                 |

$^a$Vt: The total pore volume calculated at P/Po=0.99; $^b$Vmeso: The mesopore volume calculated in the range of 1 to 6 nm by the BJH method; w: water; p: PBS (pH 7.4)

Table S2 Loading capacity of nanoparticles towards different molecules

| Sample  | Loading capacity (mg/g) | Protein | Drugs | Dye |
|---------|------------------------|---------|-------|-----|
|         |                        | INS     | LYS   | RNASE | GRIS | VAN | DR1 |
| MHS     | 156.8                  | 161.5   | 150.2 | 23.3  | 231.8 | 7.2 |
| RMHS    | 287.8                  | 263.1   | 303.2 | 53.8  | 237.5 | 21.6 |

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