**Electronic Supporting Information**

# Rod-like mesoporous silica nanoparticles with rough surfaces for enhanced cellular delivery

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## Materials and Methods.

**1. Synthesis of R-MCM-41.** The synthesis of rod-like MSN followed our previous method.[1] Typically, 0.2 g of cetyltrimethylammonium bromide (CTAB, Sigma) was dissolved in 96 g of deionized water with stirring at room temperature, and then 0.7 mL NaOH solution (2M) was added and stirred for about 20 min until CTAB powder was dissolved. The above solution was heated to 80 °C, and 0.01 g perfluorooctanoic acid (PFOA, Sigma) was added to the mixture and stirred for 2 h. Then 1.43 mL tetraethyl orthosilicate (TEOS, Sigma) was added to the above solution and the mixture was stirred for another 2 h at 80 °C. Products were collected by filtration, washed with ethanol for 6 times and dried at room temperature overnight.

**2. Amino group modification of R-MCM-41.** Amine silane was grafted onto the surface of R-MCM-41 to create positively charged particles. 0.2 g of R-MCM-41 was suspended in 30 ml of toluene, and then 0.19 ml of 3-aminopropyltriethoxysilane (APTES, Sigma-Aldrich) was added and the mixture was refluxed at 110 °C for 20 h. The products were collected by filtration, washed with ethanol 6 times and dried at room temperature overnight.

**3. Synthesis of small Stöber particles.** Silica nanoparticles with a small diameter were prepared using a modified Stöber method.[2] 87 mg L-arginine (Sigma) was dissolved in the solution of 69.5 mL deionized water and 5.23 mL octanes (Sigma), stirred overnight at 60 °C. Then 0.5 mL TEOS was added to the mixture and further stirred for 5 h. The as-synthesis small particles solution was used directly in following step.

**4. Synthesis of RR-MCM-41.** The amino group modified R-MCM-41 (100 mg) was dispersed in deionized water and followed with 10 mins' ultrasonic treatment, then the solution was heated to 60 °C. The as-synthesized small size Stöber particles solution (5.4mL) was added and the mixture was stirred at 60 °C for 24 h. The products were collected by filtration, washed with ethanol for 6 times and dried at room temperature. The dried R-MCM-41 and RR-MCM-41 were subjected to calcination at 550 °C for 5 h.

**5. Grafting of Rhodamine B isothiocyanate (RITC) and fluorescein 5(6)-isothiocyanate dye (FITC).** First R-MCM-41 and RR-MCM-41 were modified with amino group using the method mentioned above, and then 50 mg of each amino group modified particles were suspended in 20 ml ethanol. Afterwards, 5 mg of FITC or 6.2 mg of RITC was added and the reaction mixture was stirred for 20 h in dark. The products were separated by centrifugation and thoroughly washed with ethanol to remove free dyes. After re-collection and drying, the final products were obtained. To determine the grafted amount of FITC, 1 mg of particles was dissolved in 1 ml of 1 mol/L aqueous NaOH solution and the absorbance at 490 nm was recorded by using a Synergy HT microplate reader (Biotek).

**6. Curcumin Loading.** Loading curcumin into R-MCM-41 and RR-MCM-41 was performed using rotavapor technique. 30 mg of silica material, 10 mg of curcumin was added to rotavapor flask, followed by addition of 5 ml of methanol. This suspension was sonicated for 2 min in ultrasonic bath and the flask was affixed to the rotavapor assembly and the mixture was slowly evaporated at 50 °C. The solvent evaporation procedure was continued for about 30 mins until completely solvent removal.

**7. Cell culture.** Cell culture reagents were purchased from GIBCO Invitrogen Corporation/Life Technologies Life Sciences unless otherwise specified. Osteosarcoma cell line KHOS were purchased from ATCC (American Type Culture Collection). Cells were maintained as monolayer cultures in Dulbecco's Modified Eagle's Medium (DMEM) supplemented with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin at 37 °C and 5% $CO_2$. Fetal bovine serum, paraformaldehyde, antifade fluorescent mounting medium with 4'-6-diamidino-2-phenylindole (DAPI) and 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT) were purchased from Sigma-Aldrich. Alexa Fluor® 488 Phalloidin was purchased from Cell Signaling Technology.

**9. Confocal microscopy.** KHOS cells were seeded at $2\times10^5$ cells per well in 6 well-plates the day before the assay. The RITC and FITC-labelled R-MCM-41 and RR-MCM-41

were added into each well containing serum free medium at 37 °C for 4 h. Afterwards, the cells were washed twice with phosphate buffered saline (PBS) and fixed with 1 ml of 4% paraformaldehyde in PBS (PFA/PBS) for 30 minutes. Subsequently, the cells were washed again with PBS and the liquid was drained. For RITC-labelled nanoparticles group, the cells were pre-incubated with PBS containing 1% bovine serum albumin (BSA) for 20-30 mins. Then staining solution (200 μl) containing 1% (w/v) BSA and Alexa Fluor® 488 Phalloidin (3 μl) in PBS was added for 2 h at 37 °C. Then, the coverslips were washed three times with PBS and mounted onto glass slides by fluoroshield with DAPI. For the FITC-labelled nanoparticles group, the fixed cells were directly stained with DAPI. The slides were viewed using a confocal microscopy (LSM ZEISS 710).

**9. Cellular uptake assay.** KHOS cells were seeded in 6 well-plates at $2\times10^5$ cells per well the day before the assay. The FITC-labelled nanoparticles were diluted in PBS at a concentration of 5 mg/ml, respectively, and 100 μg of nanoparticles was added into each well with serum free medium for 4 hours at 37 °C, respectively. After washing with PBS 3 times, 1 ml of 0.4% (w/v) trypan blue was used to quench extracellular FITC-fluorescences, the non-quenched fraction thus representing internalized nanoparticles. 10 minutes later, the cells were washed 3 times with PBS and harvested by trypsination. After washing twice with PBS, the cells were re-suspended in 2% PFA/PBS for FACS analysis. The values of median influorescent intensity (MFI) were normalized by the FITC amount between FITC-conjugation R-MCM-41 and RR-MCM-41.

**10. MTT assay.** KHOS cells were used for cytotoxicity test. The cells were seeded in 96-well plates at a density of 5000 cells/well the day before the assay. Culture medium with different concentrations of free CUR, R-MCM-41 CUR and RR-MCM-41 CUR was prepared, culture medium with according concentrations of R-MCM-41 and RR-MCM-41 was also prepared. The cell culture medium was replaced with 100 μl of culture medium containing free CUR, blank R-MCM-41/RR-MCM-41 and R-MCM-41 CUR/RR MCM-41 CUR. After 24 h of incubation at 37 °C, the culture medium was replaced with MTT working solution (final concentration of 0.5 mg/mL) at 37 °C for 4 h. The dark blue formazan crystals formed were dissolved with DMSO. Cell viability was measured by reading the absorbance at 570 nm using a Synergy HT microplate reader. Samples were normalized to non-treated cells and all drug concentrations were tested in six replicates.

## Characterization

TEM images were taken using a JEOL 1010 microscope operated at 100Kv. The TEM specimens were dispersed in ethanol, and then transferred to a copper grid. ζ potential measurements were carried out at 25 °C using a Zetasizer Nano-ZS from Malvern Instruments. X-ray diffraction (XRD) patterns were recorded on a German Bruker D8 X-ray diffractometer with Ni-filtered Cu Kα radiation (λ = 1.5406 Å). The XRD patterns were obtained at 40 kV and 30 mA with a step size of 0.01°. Nitrogen adsorption-desorption isotherms were measured at -196°C by using a Micromeritics Tristar II 3020 system. The samples were degassed at 200°C overnight on a vacuum line. The Brunauer–Emmett–Teller (BET) method was utilized to calculate the specific surface areas. The pore size distribution curves were derived from the adsorption branches of the isotherms using the Barrett–Joyner–Halanda (BJH) method after gaussian fitting. The total pore volume was calculated from the amount adsorbed at a maximum relative pressure ($P/P_0$). Thermogravimetric analysis (TGA) and Differential Scanning Calorimetry (DSC) measurements were performed by a TGA/DSC 1 Thermogravimetric Analyzer (Mettler-Toledo Inc) with a heating rate of 5 °C/min in air flow.

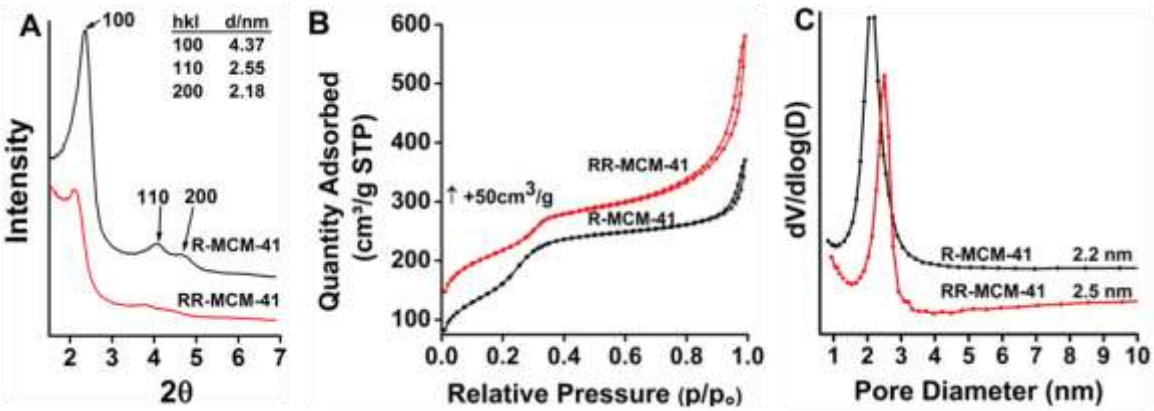


**Figure S1. Small-angle XRD patterns (A), nitrogen adsorption-desorption isotherms (B) and pore size distribution plot of R-MCM-41and RR-MCM-41.**

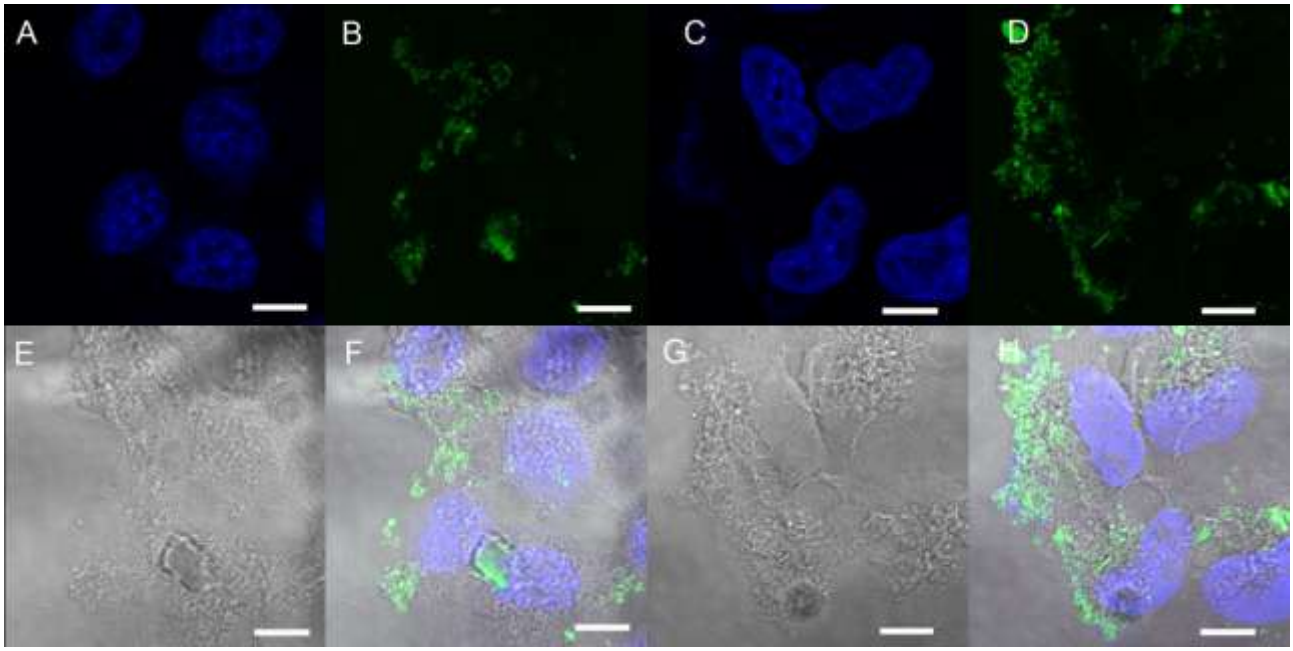


**Figure S2. Cellular uptake of R-MCM-41 and RR-MCM-41.** Confocal microscopy images of KHOS cells cultivated with R-MCM-41-FITC (A, B, E, F) and RR-MCM-41-FITC (C, D, G, H) for 4 h. A, C: cell nuclei stained in blue; B, D: green influorescence from FITC; E, G: bright-field images shows cell margin; F, H: merged fluorescence and bright-field images. Scale bar: 10μm.

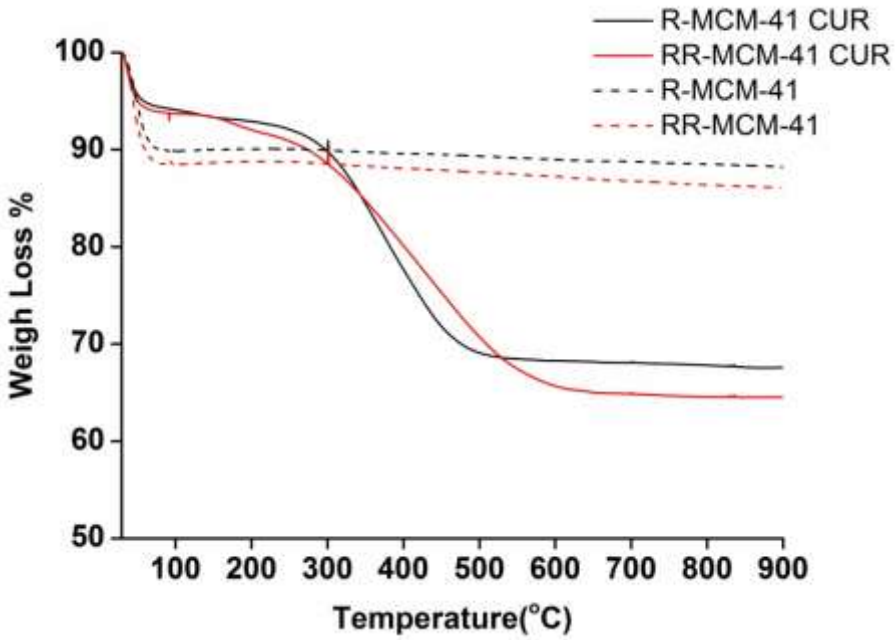


**Figure S3. Thermogravimetric analysis of R-MCM-41 CUR, RR-MCM-41 CUR, R-MCM-41 and RR-MCM-41.**

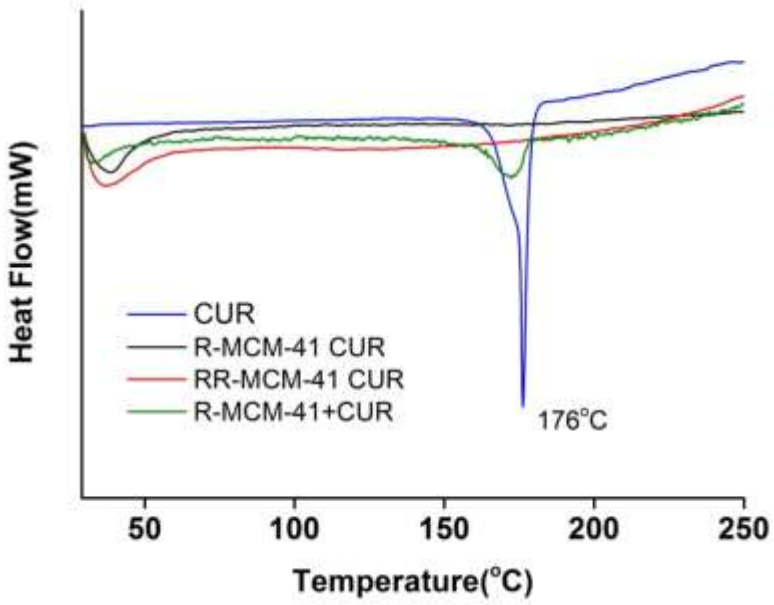


**Figure S4. Differential scanning calorimetry (DSC) of CUR, R-MCM-41 CUR, RR-MCM-41 CUR and R-MCM-41+CUR.**

**Table S1 Physical characterization of silica particles.**

| Sample | ζ potential (mV) | Pore Size (nm) | Total pore volume ($cm^3/g^{-1}$) | BET surface area ($m^2/g^{-1}$) |
|---|---|---|---|---|
| R-MCM-41 | -18.7 | 2.2 | 0.59 | 703 |
| RR-MCM-41 | -16.3 | 2.5 | 0.85 | 613 |

**Table S2 FITC-conjugation and MFI of internalized FITC**

| | $OD_{490nm}$ | Original MFI ($x10^4$) | Normalized MFI($x10^4$) |
|---|---|---|---|
| R-MCM-41-FITC | 2.807 | 1.21 | 1.21 |
| RR-MCM-41-FITC | 2.887 | 1.66 | 1.61 |

# References:

1. S. Yang, L. Zhao, C. Yu, X. Zhou, J. Tang, P. Yuan, D. Chen and D. Zhao, *J. Am. Chem. Soc.*, 2006, **128**, 10460-10466.

2. T. Yokoi, Y. Sakamoto, O. Terasaki, Y. Kubota, T. Okubo and T. Tatsumi, *J. Am. Chem. Soc.*, 2006, **128**, 13664-13665.