## **Electronic Supplementary Information (ESI)**

## Hierarchical Mesoporous Bio-Polymer/Silica Composites Co-Templated by Trimethyl Chitosan and a Surfactant for Controlled Drug Delivery

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## Experimental

**Materials:** The materials that were used for this work, including chitosan (degree of deacetylation 85 %; number-average molecular weight; 190 KDa), tetraethoxy silane (TEOS), sodium dodecyl sulfate (SDS), iodomethane, sodium iodide, Ibuprofen (IBU, 99 %) and 5-fluorouracil (5-FU, 99 %) were supplied by Sigma-Aldrich Chemical Co. All of the chemicals were used as received without any further purification.

**Methylation of Chitosan (N,N,N,-trimethyl chitosan):** N,N,N-trymethyl chitosan (TMCs) was synthesized using the Campana-filho's method [**S1**. E. Curti, D. de Britto, S. P. Campana-Filho, *Macromol. Biosci.* **2003**, *3*, 571.] ]. Briefly, 1 g of chitosan was suspended in 40 mL of N-methyl-2-pyrrolidone and 5.5 mL aqueous NaOH (15 wt%) with 5.7 mL of iodomethane, in the presence of 2.4 g of sodium iodide. The dispersion was constantly, magnetically stirred, and the reaction proceeded at room temperature for a desired amount of time. At the end of the reaction, the pH of the suspension was adjusted to 7.5, and the suspension was dialyzed, filtered and finally freeze-dried. (Scheme S1)

Synthesis of Mesoporous Bio-Polymer/Silica Composites (TMCs-Silica): In the typical synthesis method, a solution containing 0.35 g of SDS, 15 mL of 0.1 M NaOH, and 22.5 mL of  $H_2O$  was stirred at room temperature. After SDS was completely dissolved, 25.35 g of an aqueous TMCs solution (1.4 wt%) was added to the system. After about 4 h of stirring, 7.5 mL of TEOS was also added, and the mixture was continuously stirred for 24 h. The resulting samples were collected through centrifugation and washed with water. Finally, the product was washed with ethanol three times and then dried at 60 °C to 12 h. For comparision purpose, mesoporous silica was obtained through calcination at 550 °C for 5 h The mesoporous biopolymer/silica (TMCs-silica) composites were obtained through extraction with an ethanol solution containing HCl as the ion-exchanger. (Scheme S1)

**Drug Loading and Release:** In this study, 100 mg of the TMCs-Silica composites was dispersed in 4 mL/20 mg hexane/IBU or aqueous 5-Flurouracil solutions. The mixtures were incubated for 48 h in order to the fabricate drugs-loaded TMCs-Silica composites. The solutions were covered with a polyethylene (PE) film to prevent solvent evaporation.

The drug-loaded samples were separated from the solution through vacuum filtration, washed with the same solvent at least three times, and dried at room temperature. After the filtrates were properly diluted, a UV-vis spectrophotometer was used to determine the drug-loading amount. Then 20 mg of the drug loaded sample was dispersed into 3 mL of a phosphate buffer solution that simulated intestinal fluid (SIF; pH 7.5) and simulated stomach fluid (SSF; pH 1.4). The sample was placed into a dialysis membrane bag (molecular weight cutoff 5000 kDa), and then immersed into 27 mL of both the SIF and SSF solutions at 37 °C. At periodic intervals (up to 120 h), the release media were withdrawn and 1 mL of the fresh buffer solutions were added. The

amounts of IBU and 5-FU were determined using a UV-vis spectrophometer at 223 nm and 265 nm, respectively.

**Cell Proliferation Assays:** The cell proliferation was measured using the 3-(4,5dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT; Sigma-Aldrich Co., St. Louis, MO) assay and the colorimetric dye-reduction method. The exponentially growing cells (5 x  $10^3$  cells/well) were plated into 96 wells and incubated in a growth medium containing all of the samples at 37°C. After five days, the medium was aspirated after centrifugation, and the MTTformazan crystals were solubilized in 100 µl dimethylsulfoxide (DMSO). The optical density of each sample was measured at 570 nm using an ELISA reader (Bio-Tec Instruments, VT, USA). The optical density of the media was proportional to the number of viable cells. The inhibition of the proliferation was evaluated as a percentage of the control growth (no drug in the sample). All of the experiments were repeated at least twice.

**Characterization:** The adsorption/desorption isotherms of nitrogen were measured at -196°C using a Micromeritics ASAP 2010 surface area and pore size analyzer. All of the samples were outgassed at 100 °C for 12 h under vacuum. The specific surface area was determined by the BET (Brunnauer-Emmett-Teller) method and the pore size distribution by BJH (Barrett-Joyner-Halenda) method using the adsorption branch of the isotherm plot. The scanning electron microscopy (SEM) images and the energy-dispersive X-ray (EDX) analysis were obtained using a Model 952888(8) microscope from Hitachi Ltd, with an acceleration voltage of 20 kV. The transmission electron microscopy (TEM) images were obtained using a JEOL 2021F microscope that was operated at 200 kV. The samples were characterized using spectrum-GX FT-IR spectroscopy with KBr pellets in an ambient atmosphere. The <sup>29</sup>Si and <sup>13</sup>C cross-polarization (CP) MAS NMR spectra were obtained using a Unity-Inova 400 NMR 400 MHz spectrometer at

room temperature with a 4 mm zirconia rotor that was spinning at 6 kHz. The UV absorption spectra were obtained using a UV-visible spectrophotometer U-2010, HITACHI Co. The thermogravimetric analysis was conducted under nitrogen using a TA instruments Q50 at heating rate of 10 °C/min. The zeta potential was measured using an electrophoretic light scattering spectrophotometer (ELS-8000). The samples were made up in deionized water at a concentration of 1 mg/50 ml.

Sample	$\frac{S_{BET}}{(m^2g^{-1})}$	Small Pore (nm)	Large pore (nm)	Vol. small pore (m <sup>3</sup> g <sup>-1</sup> )	Vol. large pore (m <sup>3</sup> g <sup>-1</sup> )	Drug (wt%)
TMCs-Silica	351	7.1	35.3	0.65	0.78	-
TMCs-Silica + IBU	190	4.9	-	0.27	-	41.9
TMCs-Silica + 5-FU	200	5.2	-	0.30	-	33.4
Silica	420	6.6	36.6	0.50	0.50	-
Silica + IBU	210	4.7	-	0.29	-	44.2
Silica+ 5-FU	222	5.1	-	0.33	-	29.9

Table S1. Physicochemical properties and drug loading amount of TMCs-Silica and pure silica.



Scheme S1. Schematic representation of the preparation of TMCs-Silica and the drug loading/release concept.(slightly modified scheme as that of the Scheme 1 in the text for more clear molecular structures of materials used in this work.)



**Figure S1.** Additional TEM micrographs of hierarchical mesoporous bio-polymer/silica composites.



Figure S2. <sup>29</sup>Si CP/MAS spectra of the TMCs-Silica and silica nanoparticles.



Figure S3. SEM images of TMCs-Silica nanoparticles.



**Figure S4.** (a) FT-IR spectra, (b) TG weight change curves, and (c) DTG curves corresponding to the TG curves shown in S2 (b) of TMCs-Silica and pure silica nanoparticles, where TMCS-M denotes as-made TMCS before preparing composites with silica.



Figure S5. X-ray energy dispersion spectrum of (a- b) TMCs-Silica, and (c-d) pure silica.



**Figure S6.** <sup>13</sup>C CPMAS NMR spectrum of TMCs-Silica to confirm the existence of chitosan in the silica framework.



**Figure S7.** TG curves of **(a)** TMCs-Silica and TMCs-Silica+ IBU, **(b)** TMCs-Silica and TMCs-Silica+ 5-FU, **(c)** Silica and Silica+ IBU, and **(d)** Silica and Silica+ 5-FU.