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Electronic Supporting Information (ESI†)

Red fluorescent hybrid mesoporous organosilica for simultaneous cell imaging and anticancer drug delivery

**Madhappan Santha Moorthy,[a] Hyun-Jin Song,[a] Jae-Ho Bae,[b]
Sun-Hee Kim,[b] and Chang-Sik Ha[a]***

[a]Department of Polymer Science and Engineering, Pusan National University, Busan 609-735, Korea. E-mail: csha@pnu.edu

[b]Department of Biochemistry, School of Medicine, Pusan National University, Yangsan Hospital, Yangsan 626-870, Korea

Experimental

Materials and reagents

Cetyltrimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS), 3-(isocyanatopropyl) triethoxysilane (ICPTES), ammonia solution (28%), safranin and 5-Fluorouracil (5-FU) were purchased from Aldrich, USA. All the chemicals and reagents were used as received without further purification.

Synthesis of safranin-diurea bridged organosilane precursor (SDU)

0.5 g of safranin (1.42 mmol) was dissolved in 25 ml of tetrahydrofuran (THF). To this, 0.77 g (3.11 mmol) of 3-(isocyanatopropyl) triethoxysilane was added under vigorous stirring under inert condition at room temperature. Subsequently, the reaction mixture was kept for further stirring for 24 h under N_2 atmosphere. Upon completion, the obtained product mixture was concentrated under reduced pressure to yield dark pink oil as product. The crude product was purified using diethyl ether and hexane and the obtained product was dried under vacuum at room temperature (Yield: 82%). ^{1}H NMR (400 MHz, MeOH-d_4): δ 0.53 (t, 4H, $SiCH_2$), δ 1.12 (t, 18H, $CH_2CH_2CH_2$), δ 1.42 (t, 4H, $SiCH_2$-CH_2), δ 3.29 (t, 2H, NH), δ 7.5-8.7 (m, 18H, aromatic). ^{13}C NMR (400 MHz, MeOH-d_4): δ 1.85 ($SiCH_2$), δ 16.7 (CH_2), δ 25.8 (CH_2-CH_3), δ 36.2 (C-N), δ 152 (C=O), δ 57.6 (aromatic). FT-IR (KBr, ν cm^{-1}): 2872, 2928 cm^{-1} ($\nu_{CH2,\ CH3}$), 1622 ($\nu_{C=N}$), 1485 (ν_{N-H}), 1635 cm^{-1} ($\nu_{C=O}$), 1245 (νC-H, phenyl), 1068 cm^{-1} (ν_{Si-OEt}).

Synthesis of fluorescent hybrid mesoporous silica nanocarrier (SDU-HMS)

Fluorescent safranin-diurea bridged mesoporous organosilica hybrid (SDU-HMS) was synthesized by sol-gel method through structural directing surfactant template approach. Typically, 1.0 g of CTAB was dissolved in 47 ml of distilled water and 13 g (28%) of ammonia solution was added to CTAB solution followed by vigorous stirring at 35 ºC for 30 min to form a clear solution. A premixed solution of SDU precursor and TEOS, to afford a molar percentage of SDU/TEOS + SDU of 25 mol %, was then introduced slowly with the solution for dispersion under stirring for 6 h. The mixture was allowed to stir for 24 h at 35 ºC and another 24 h at 90 ºC. The obtained solid product was filtered, washed with

deionized water and dried at 60 ºC. To remove the occluded surfactant template, the sample was refluxed with the alcoholic solution of ammonium nitrate (120 ml/g) followed by extensive washing with deionized water and ethanol and dried 50 ºC, overnight. The obtained product was labelled as SDU-HMS.

Drug loading into the SDU-HMS nanocarriers

For drug loading and release experiment, 5-FU was chosen as model drug. Typically, 100 mg of synthesized SDU-HMS nanocarriers were dispersed into 10 ml of 5-FU in water (10 ml: 25 mg mL^{-1}), and the resulting suspension was kept for stirring at room temperature for 24 h. The drug encapsulated samples were collected by centrifugation, washed with minimum amount of water and dried in an oven at 50 ºC. The amount of encapsulated drug was determined according to the change in concentration before and after adsorption using UV-vis spectrometer (HITACHI U-2010) at a wavelength of 265 nm. The amount of encapsulated 5-FU into the samples was calculated to be 134 mg/g.

***In vitro* drug release test**

The *in vitro* drug release experiment was performed as follows. Typically, 50 mg of 5-FU drug encapsulated SDU-HMS nanocarriers were placed in a dialysis membrane bag (Molecular cut-off 5000 kDa) and immersed it into phosphate buffer solution (PBS) (50 ml) at various pHs (pH 7.4, 5.6 and 4.0, respectively) at 37 ºC with constant stirring at 100 rpm. The pH of the release medium was adjusted using 0.1 M HCl or 0.1 M NaOH solution. 2 ml of the released medium was taken at given time intervals. The amount of drug released in the medium solution was measured by UV-vis spectrophotometry at set times.

MTT assay analysis

Human breast cancer (MCF-7) cells were maintained in Dulbecco's modified Eagle's medium (DMEM) containing 10% fetal bovin serum (FBS), penicillin (100 units mL^{-1}), and streptomycin (100 mg mL^{-1}). The cells were then seeded in a 96-well plate at a density of $1x10^4$ cells/well for 24 h at 37 ºC in 5% CO_2. The cells were then treated with the blank and

5-FU drug loaded SDU-HMS carriers at the desired concentration. The pH of some wells was adjusted to 6.0 using 1.0 M HCl solution. After incubation for 24 h, the cell viability was determined using standard 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. The MTT solution (20 µL, 5 mg mL^{-1}) was added to each well and incubated for further 4 h. Subsequently, the medium was removed and dimethyl sulfoxide (DMSO; 200 µL) was added to each well. The optical density (OD) was measured at 570 nm using an ELISA reader (model MR 7000; Dynatech). The cell viability was calculated as follows.

$$\text{Viable rate} = (OD_{treated}/OD_{control}) \text{ X } 100$$

where $OD_{treated}$ was obtained for the cells treated by the 5-FU loaded SDU-HMS samples for 24 h. The $OD_{control}$ was obtained for the dells treated by the SDU-HMS samples without the drug loading, and the other cultured conditions were the same.

Intracellular uptake process

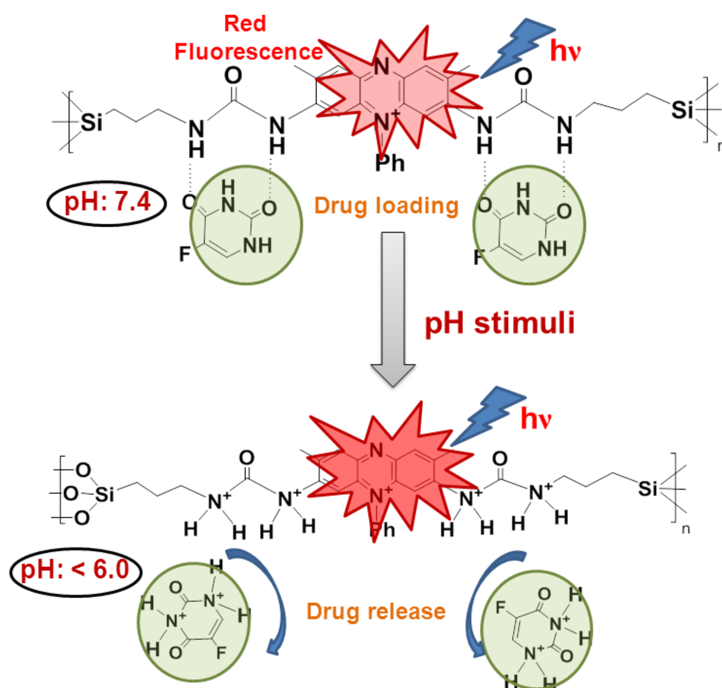
Even though the same pathway(s) are involved in the internalisation of nanoparticles in to the cells in the presence and absence of serum, the total amount of nanoparticles able to uptake by cells is lower in the presence of serum as compared to serum-free medium [S1-S5]. In this experiment, we performed the cellular uptake process in serum-free medium as following reported procedures.

To observe the cellular internalization of the prepared drug carriers, the MCF-7 cells were placed in a cell culture dish (7.5 x 10^4 cells) by using Dulbecco's modified Eagle's medium containing 10% fetal bovin serum supplemented with 100 U mL^{-1} penicillin G and 100 µg mL^{-1} streptomycin in 6-well plates and incubated at 37 ºC in an atmosphere containing 5% CO_2/95% air. After 24 h, the medium was removed and 100 µL of serum free medium was added to each well. 25 µL of freshly prepared sample solutions containing SDU-HMS nanocarriers and fluorescein (FITC) labelled 5-FU molecules loaded samples were added. After 4 h, the medium was exchanged to standard medium. After 20 h incubation, the cells were washed three times with PBS (pH 7.4, 0.1 M) and fixed with 4 %

paraformaldehyde for 15 min [S1,S5]. Fluorescence images of the sample treated cells were obtained by confocal microscopy (Leica,TCS-SP2). To further demonstrate their cellular uptake ability, the amount of internalised particles in MCF-7 cells was evaluated by the mean fluorescent intensity of the SDU-HMS particles that was internalised by MCF-7 cells by fluorescein-activated cell sorting (FACS) method [S6].

Characterization

Powder X-ray diffraction (XRD, Bruker, AXN) was performed using Cu-Kα radiation. The XRD data was collected in the range from 1.2-10º 2θ. Scanning electron microscopy (SEM) images were collected using a JEOL 6400 microscope. Transmission electron microscopy (TEM, JEOL 2010) was performed at an accelerating voltage of 200 kV. Fourier-transform infrared (FTIR, Jasco FTIR 4100) spectra were measured from KBr pellets. Particle size distributions in suspension were measured by using dynamic light scattering (DLS) on a Malvern Zetasizer Nano-ZS (Malvern Instrument). The Brunauer–Emmet–Teller (BET) method was used to calculate the specific surface area. The pore size distribution curve was obtained from an analysis of the adsorption branch using the Barrett–Joyner– Halenda (BJH) method. Thermogravimetric analysis (TGA, PerkinElmer Pyris Diamond) was carried out at a heating rate of 10 ºC min^{-1} in air.



Scheme S1. Drug binding and pH responsive release mechanisms of 5-FU from the SDU-HMS nanocarrier.

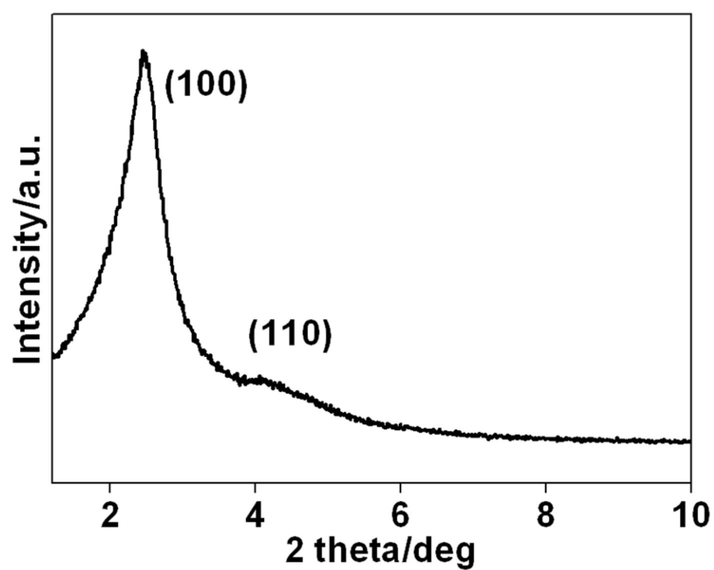


Fig. S1 Powder XRD pattern of the SDU-HMS material.

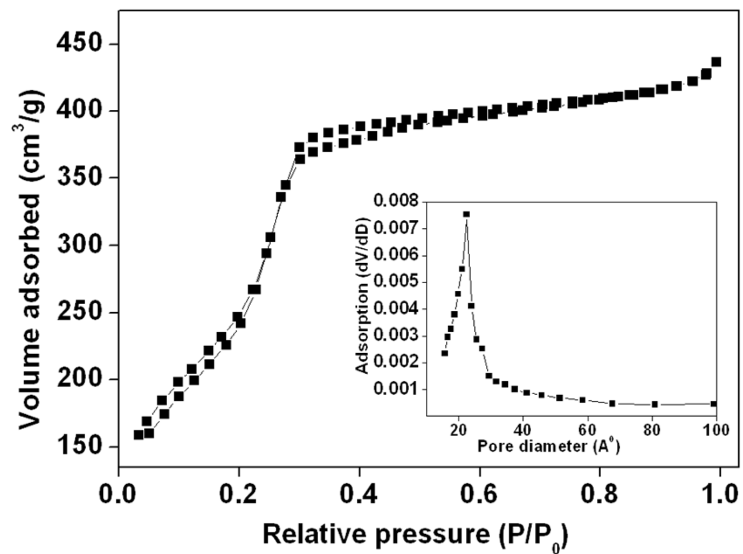


Fig. S2 Nitrogen adsorption-desorption isotherm and pore size distribution curve (inset) of the SDU-HMS material.

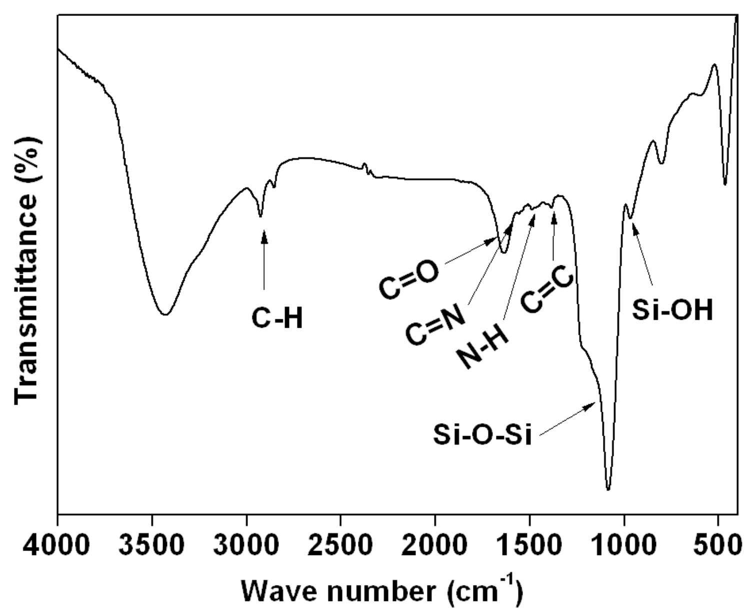


Fig. S3 FT-IR spectrum of the SDU-HMS material.

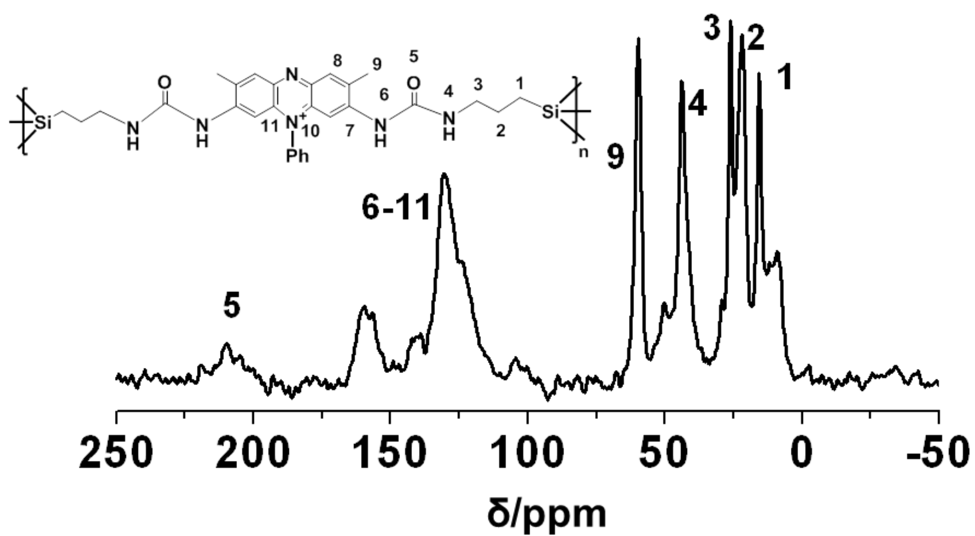


Fig. S4 Solid-state ^{13}C CP MAS NMR spectrum of the SDU-HMS material.

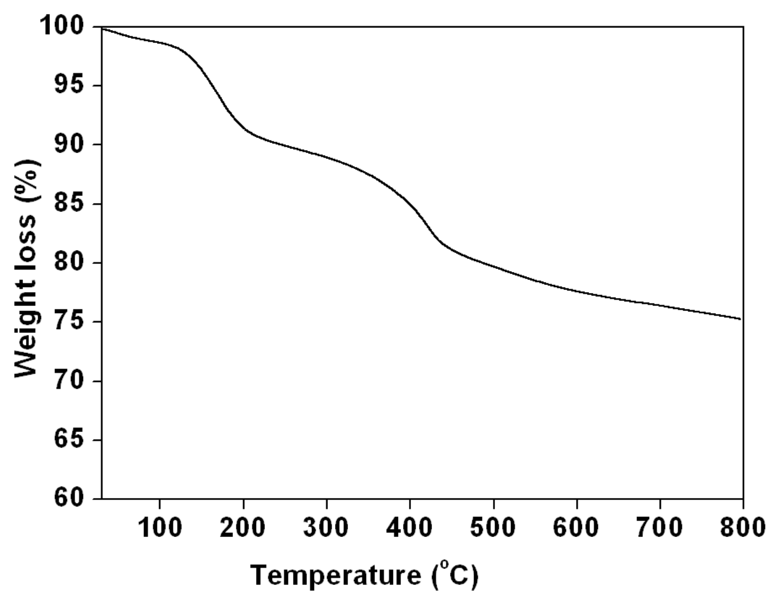


Fig. S5 TGA curve of the SDU-HMS material.

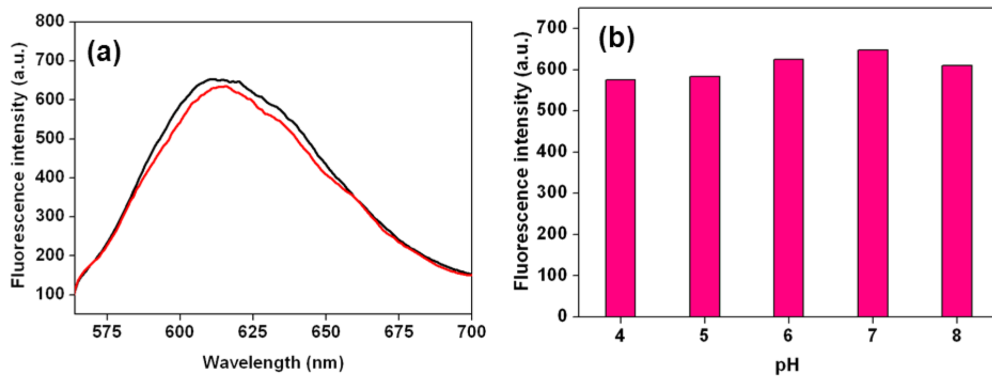


Fig. S6 The fluorescence intensities of the SDU-HMS nanoparticles remained almost constant when (a) incubation was carried out in PBS buffer (pH 7.4), 48 h and (b) at different pHs (4 to 8).

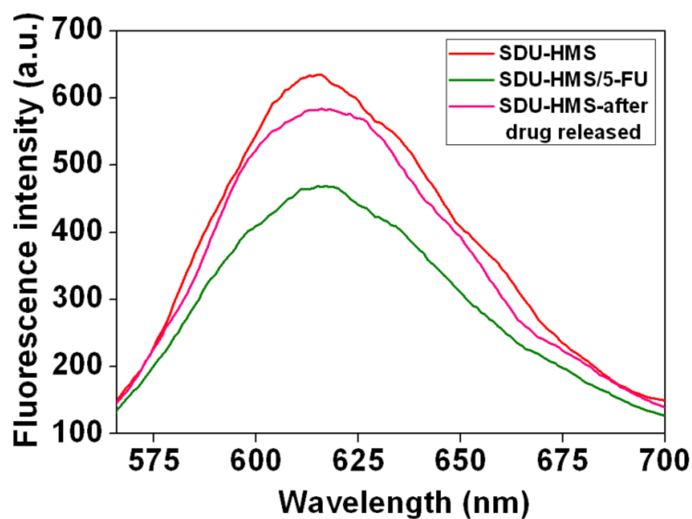


Fig. S7 Fluorescence intensity of the SDU-HMS, SDU-HMS/5-FU and the recovered sample of SDU-HMS-after drug release experiment.

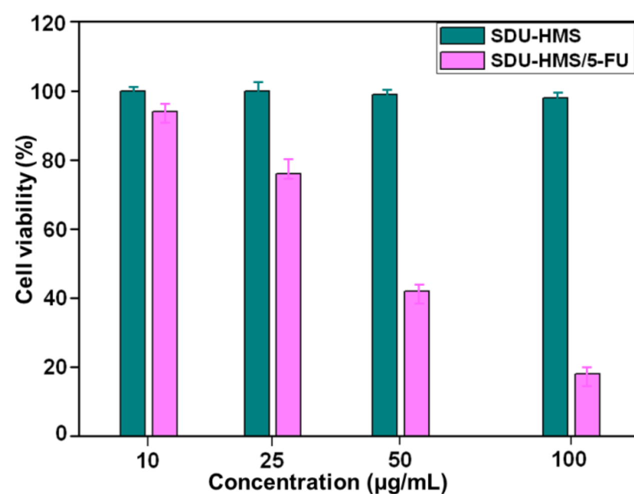


Fig. S8 *In vitro* cytotoxicity of the SDU-HMS carrier in various concentrations against MCF-7 cells. Cytotoxicity profiles 5-FU-loaded SDU-HMS nanoparticles (pH 6.0), at concentration of 10, 25, 50 and 100 μg mL^{-1}. Clear pH sensitivity of SDU-HMS carriers to antitumor activity can be observed.

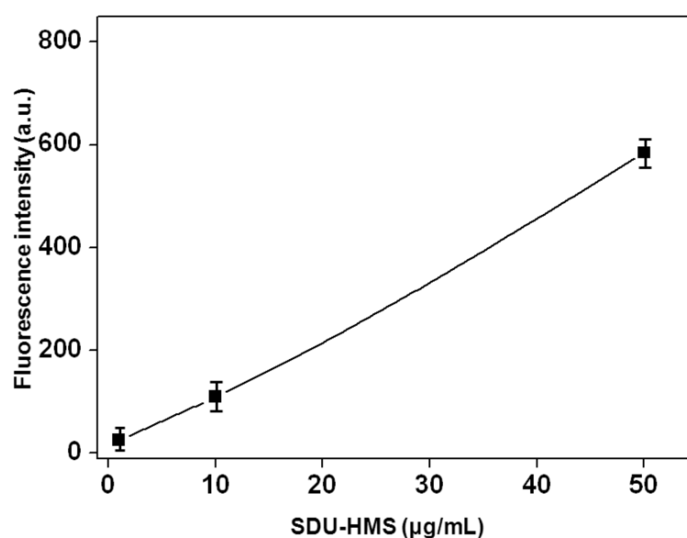


Fig. S9 Fluorescence intensity of the SDU-HMS nanoparticles in MCF-7 cells analysed by fluorescene-activated cell sorting (FACS) method.

Table S1 Structural properties of the SDU-HMS material.

Sample	d_{100} /nm[a]	S_{BET} /m^2/g	Pore volume/ cm^3/g[b]	Pore diameter/ nm[c]	Functional groups content (mol/g)[d]	5-FU loading/ mg/g
SDU-HMS	3.6	685	0.24	3.2	0.023	134

[a] Calculated from XRD analysis. [b]Calculated from the volume adsorbed with P/P_0 at 0.5. [c] Calculated from the adsorption branch of a nitrogen isotherm using the BJH model. [d] Calculated from elemental analysis.

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