Electronic Supplementary Information (ESI)

Co-encapsulation of paclitaxel and 5-fluorouracil in folic acid-modified lipid-encapsulated hollow mesoporous silica nanoparticles for synergistic breast cancer treatment

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Preparation of 5-FU@FA-HMSNs

At first, HMSNs were prepared by reverse microemulsion method utilizing APS as the core template and CTAB as the pore-generating template and structure-directing surfactant, and utilizing the hydrolysis/condensation reactions of the crosslinker TEOS to synthesize SiO_2 shell. CTAB (15 mg) and Triton X-100 (17.88 g) were dissolved in a mixture of 72.6 mL of cyclohexane and 17.6 mL of n-octanol (oil phase), and 4.4 mL of deionized water containing 20 μL of APS (water phase) was added upon stirring to prepare water-in-oil (W/O) microemulsion. After 5 min, TEOS (800 μL) and ammonium hydroxide solution (800 μL) were added dropwise to trigger the polymerization reaction, and the mixture was stirred at room temperature for another 24 h. The reaction was stopped by adding 50 mL of acetone. The white precipitate was then obtained by centrifugation at 7,602 *g* for 10 min, and washed three times with ethanol and deionized water to form hollow nanoparticles, and extracted with a mixed solution of acetic acid and ethanol for 4 h to remove the pore-generating template CTAB and form hollow mesoporous nanoparticles. After centrifugation at 7,602 *g* for 10 min and washing three times with ethanol and deionized water, HMSNs were collected by vacuum drying at 60 ℃.

Secondly, FA was modified on HMSNs by sol-gel method. HMSNs (0.7 g) were dispersed in ethanol (12 mL) upon stirring in a round-bottomed flask, and 3-aminopropyltrimethoxysilane (1.5 mL) dissolved in ethanol was added. After stirring at room temperature for 48 h, the mixture was centrifuged at 7,602 *g* for 5 min, and the obtained precipitate was washed three times with ethanol and dried *in vacuo* at 60 ℃ to afford HMSNs-NH_2.

N-hydroxysuccinimide (0.26 g) and *N,N′*-dicyclohexylcarbodiimide (0.47 g) were added to a solution of FA (0.5 g) in 50 mL of anhydrous DMSO and 0.25 mL of triethylamine, and the mixture was stirred at room temperature in the dark for 12 h. After filtration,

HMSNs-NH_2 (0.6 g) were added to the filtrate and the resulting mixture was stirred at room temperature in the dark overnight. FA-HMSNs were collected by centrifugation, washed with ethanol and deionized water and freeze-dried at −50 ℃ for 24 h.

Finally, FA-HMSNs (100 mg) were dissolved in 100 mL of a 5-FU solution in ethanol (2 mg/mL) and stirred at room temperature for 24 h. After centrifugation at 7,602 *g* for 5 min, the mixture was washed with ethanol three times and 5-FU@FA-HMSNs were collected by vacuum drying at 30 ℃ for 24 h.

Preparation of PTX@p(NIPAAm-co-MAA)@LB

To prepare p(NIPAAm-co-MAA), NIPAM (125 mg), MAA (7.6 mL), and AIBN (1 mol%, in terms of total monomer moles) were dissolved in ethanol in a stoppered flask. After purging with nitrogen to remove residual oxygen, the mixture was stirred at 500 rpm and 60 ℃ for 12 h. After reaction completion, ethanol was removed *in vacuo*, and the residual mixture was redissolved in acetone and added dropwise to n-hexane upon stirring. The precipitated white crystals were filtered and dried *in vacuo* at 30 ℃ to obtain the temperature- and pH-responsive polymer p(NIPAAm-co-MAA). The molar ratio of NIPAM/MAA in the above preparation was 85:15, to optimize the ratio, more p(NIPAAm-co-MAA) polymers were prepared by the same method at the NIPAM/MAA molar ratios of 90:10 and 80:20, respectively.

1,2-Dipalmitoyl-*sn*-glycero-3-phosphocholine (36.702 mg), hydrogenated lecithin (19.04 mg), cholesterol (5.8 mg), and DSPE-PEG_{2000} (6 mg) were added to an eggplant-shaped flask at a molar ratio of 10:5:3:0.6. Then, a solution of p(NIPAAm-co-MAA) (60 mg) and PTX (30 mg) in 6 mL of chloroform was added, and the resulting mixture was probe-sonicated at 33 W (15 s on/off) for 5–20 min. The PTX@p(NIPAAm-co-MAA)@LB film was obtained after rotavaporation at 25 ℃ for 1 h, and stored in a chemical hood

overnight to remove the residual organic solvent.

Table S1. Physicochemical properties of hollow mesoporous silica nanoparticles prepared under various conditions.

Group	Conditions	Size (nm)	Polydispersity index	Zeta potential (mV)
1	APS:20 μL; CTAB:15 mg	62.40 ± 18.43	0.213	−20.97 ± 2.11
2	APS:15 μL; CTAB:15 mg	58.10 ± 21.60	0.361	−14.53 ± 0.85
3	APS:25 μL; CTAB:15 mg	155.00 ± 110.70	0.145	−15.13 ± 2.44
4	25 ℃, 24 h	72.40 ± 26.15	0.213	−20.73 ± 1.34
5	40 ℃, 24 h	141.80 ± 54.73	0.263	−17.87 ± 1.56
6	25 ℃, 12 h	120.20 ± 61.02	0.151	−17.43 ± 1.57
7	Low speed: 400 rpm	68.40 ± 26.15	0.521	−19.57 ± 4.77
8	Medium speed: 1300 rpm	90.10 ± 51.01	0.223	−19.57 ± 1.68
9	High speed: 2100 rpm	220.20 ± 55.56	0.401	−19.40 ± 2.65

Data are shown as mean ± SD (n = 3). APS, (3-aminopropyl)triethoxysilane; CTAB, cetyltrimethylammonium bromide.

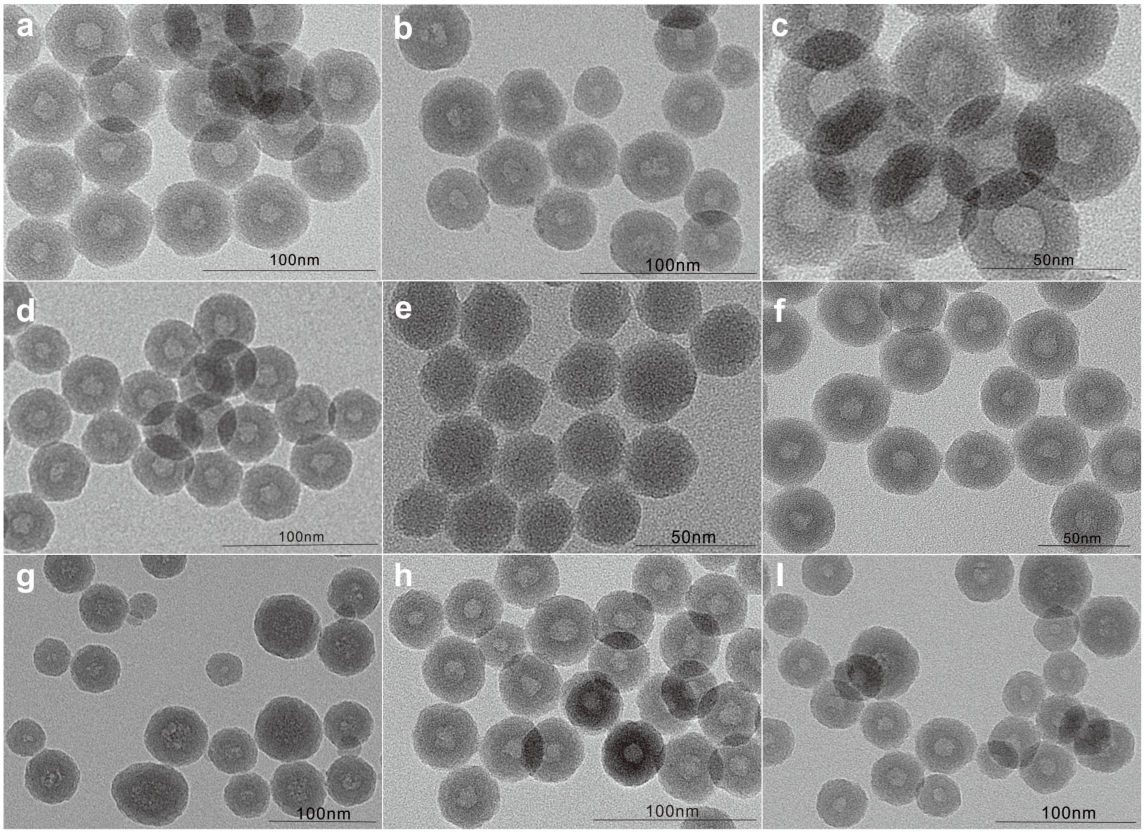

Fig. S1. Transmission electron micrographs of hollow mesoporous silica nanoparticles prepared with different formulations and processes. Pictures **a–i** correspond to groups 1–9 (Table S1).

The preparation of HMSNs was optimized by changing the feeding amount of the core template APS and the pore-generating template CTAB as well as the reaction conditions

(Table S1). Particle size gradually increased with increasing APS dosage, while the APS/CTAB ratios of groups 1 and 2 were considered as the most suitable, as the HMSNs were smaller than 100 nm. In addition, we found that high reaction temperatures (group 5) and a reaction time of 12 h (group 6) led to larger particles than those obtained at 25 ℃ for 24 h (group 4). Low and high stirring speeds resulted in uneven particle size distribution and large polydispersity indices, suggesting that medium stirring speed 1300 rpm is optimal for the preparation of HMSNs.

The TEM images of the nine groups (Fig. S1) showed that the hollow size could be adjusted by changing the APS-to-CTAB ratio, while the increase in the hollow size with increasing dosage of APS proved that APS was responsible for the formation of the hollow structure. TEM analysis also showed that a reaction temperature of 40 ℃ did not favor the formation of the cavity and afforded nanoparticles with irregular concavo-convex surfaces. The synthesis of HMSNs at low and high speeds was incomplete and afforded nanoparticles with uneven particle size distribution and irregular shape.

Taken together, the optimum conditions for preparation of HMSNs were chosen as the APS/CTAB ratio of 20 μL/15 mg, the reaction temperature of 25 ℃, the reaction time of 24 h, and the stirring speed of 1300 rpm.

Table S2. Drug loading (DL) and encapsulation efficiency (EE) of 5-fluorouracil (5-FU) onto hollow mesoporous silica nanoparticles prepared with different formulations and processes.

Nanoparticles	Carrier/drug ratio	Reaction time (h)	DL (%)	EE (%)
5-FU@HMSNs	1:1	24	26.62 ± 2.01	50.21 ± 2.17
5-FU@FA-HMSNs	1:1	24	5.30 ± 1.33	6.47 ± 2.68
5-FU@FA-HMSNs	1:1.5	24	10.58 ± 2.09	8.18 ± 1.05
5-FU@FA-HMSNs	1:2	24	28.49 ± 3.25	20.52 ± 3.31
5-FU@FA-HMSNs	1:2.5	24	25.48 ± 2.14	14.05 ± 3.54
5-FU@FA-HMSNs	1:1	48	11.04 ± 1.09	12.97 ± 1.02

Data are shown as mean ± SD (n = 3). FA, folic acid.

The synthesis and formulation of 5-FU@FA-HMSNs were optimized by changing the carrier/drug ratio and the reaction time (Table S2). 5-FU@HMSNs with a 1:1 carrier/drug ratio had high DL and EE values, which were considerably reduced after FA modification, probably because FA occupied part of the mesopore volume and pores. The optimum DL and EE values were obtained when the dosing ratio was increased to 1:2 and reaction time was 24 h. Interestingly, the DL of 5-FU@FA-HMSNs was not significantly different from that of 5-FU@HMSNs, suggesting that the pore volume of HMSNs is large enough to load a sufficient dose of drug and provide usable carrier space for drug delivery.

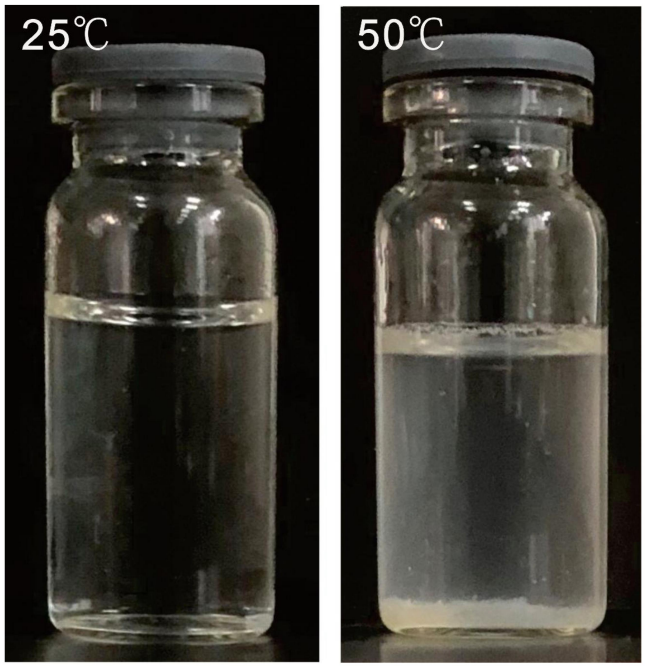

Fig. S2. Photographs of p(NIPAAm-co-MAA) solution in deionized water at 25 ℃ and 50 ℃.

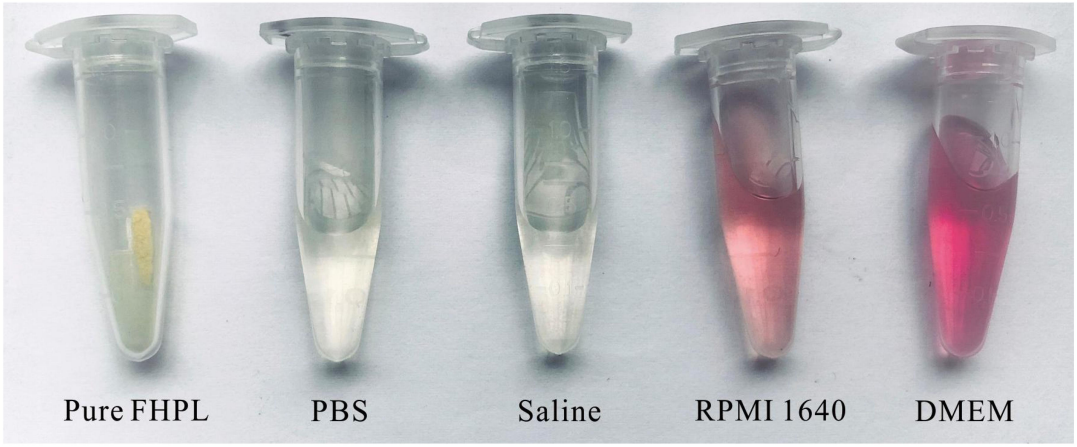

Fig. S3. Photographs of FA-HMSNs@p(NIPAAm-co-MAA)@LB (FHPL) nanoparticles after storage for 24 h in phosphate-buffered saline (PBS), saline, RPMI1640 medium, or Dulbecco's modified Eagle medium (DMEM).

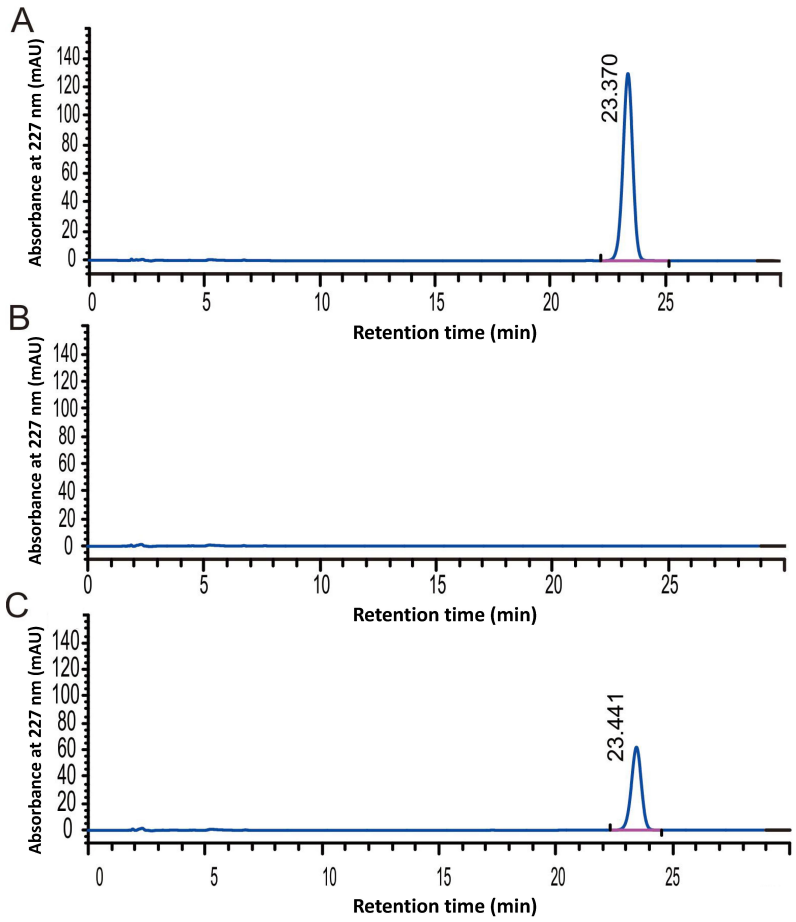

Fig. S4. High-performance liquid chromatograms of (**A**) paclitaxel, (**B**) FA-HMSNs@p(NIPAAm-co-MAA)@LB nanoparticles, and (**C**) their mixture.

Table S3. Fitting of drug release from (5-FU+PTX)@FHPL to six *in vitro* models of sustained drug release.

Drug	Model	Best-fit equation	R^2
5-Fluorouracil	Zero-order dynamics	$Q = 7.3751+2.0170t$	0.8628
	First-order dynamics	$Q = 96.9014[1-Exp(-0.0511t)]$	0.9733
	Weibull	$Q = 100\times[1-Exp(-t^{1.1847}/35.3179)]$	0.9774
	Higuchi	$Q = 14.7903t^{0.5}-8.8787$	0.9381
	Korsmeyer-Peppas	$Q = 8.6666t^{0.6206}$	0.9319
	Hixson-Crowell	$Q = 100\times [1-(1-0.0135t)^3]$	0.9678
Paclitaxel	Zero-order dynamics	$Q = 19.9887+1.9299t$	0.7262
	First-order dynamics	$Q = 89.0543[1-Exp(-0.1218t)]$	0.9951
	Weibull	$Q = 100\times[1-Exp(-t^{0.8606}/8.0727)]$	0.9821
	Higuchi	$Q = 15.1611t^{0.5}+1.2964$	0.9063
	Korsmeyer-Peppas	$Q = 20.0408t^{0.4202}$	0.9191
	Hixson-Crowell	$Q = 100\times[1-(1-0.0244t)^3]$	0.9582

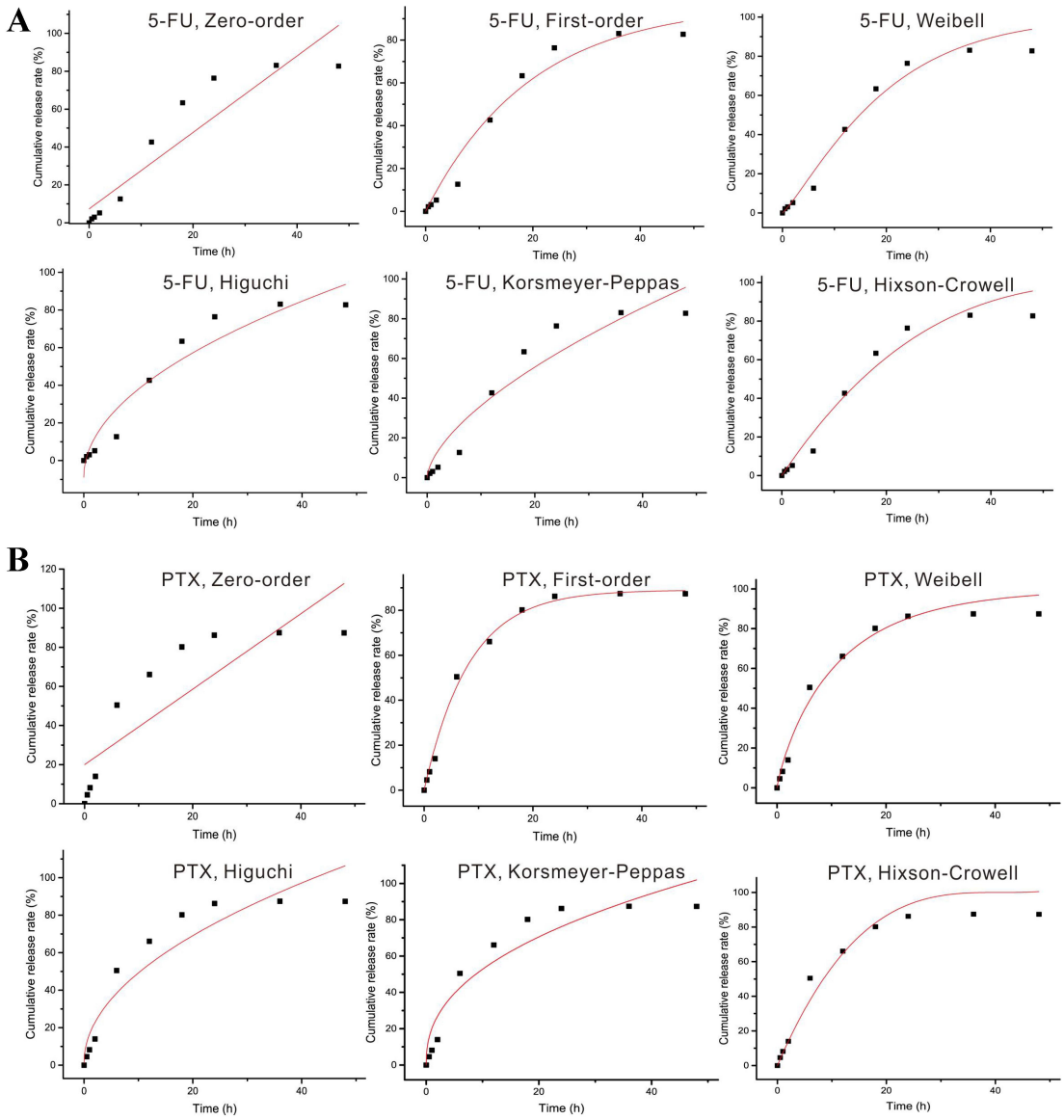

Fig. S5. Fitting of drug release from (5-FU+PTX)@FHPL to six *in vitro* models of sustained drug release. (**A**) 5-Fluorouracil (5-FU); (**B**) paclitaxel (PTX).

Fitting of the drug release curves of (5-FU+PTX)@FHPL at 41 ℃ and pH 5.0 to six commonly used *in vitro* models of sustained drug release revealed that the *in vitro* release of 5-FU and PTX fitted well all models except the zero-order dynamics model (Table S3 and Fig. S5). The Weibull model has no parameters associated with drug release and cannot adequately describe the kinetic properties of drug release. The Hixson-Crowell equation is often used to describe the erosion-mediated release of drug particles with reduced surface area and is especially suitable for multi-unit formulations. In contrast, the Korsmeyer-Peppas

model involves multiple release mechanisms. Parameter *n* in the equation $Q = k_{KP} \times t^n$ is a shape-related release parameter, which is explained as follows: $n \leq 0.45$, diffusion is dominant; $0.45 < n < 0.89$, drug release is mediated by both diffusion and erosion; $n \geq 0.89$, erosion is dominant. Here, the *n* value of 5-FU was 0.6206, indicating that its release involved both diffusion and erosion processes, whereas that of PTX was 0.4202, indicating that PTX was released mainly through diffusion.