Supplementary Information

Outside-in stepwise functionalization of mesoporous silica nanocarriers for the matrix type sustained release of fluoroquinolone drugs

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1. Kinetic Equation Models for Drug Release:

To determine the release mechanism in *vitro* test, three kinds of release kinetic statistic models were chosen to evaluate and describe the unique release profiles of Enro-MCM-41 delivery system.

Zero-order kinetics was dedicated to the controlled release system with a constant releasing rate which was independent of the drug concentration in the solvent. The equation for zero order release can be expressed as:

$$Mt / M_{\infty} = kt \quad (1)$$

With a reaction rate only depending on the concentration of drug molecules, first order kinetics was applied to describe the typical sustained-release system, the release mechanism of which follows Fick's law. The equation for first order release can be described as:

$$\ln(1-Mt / M_{\infty}) = -kt \quad (2)$$

The release kinetics of drugs delivered from insoluble porous materials was commonly described by an empirical Higuchi model which was well appropriate for matrix systems. The model was based on a square root of a time which was still dependent on the process of Fickian diffusion.

$$Mt / M_{\infty} = kt^{1/2} \quad (3)$$

In above equations, Mt and M∞ represented the amount of drug release at time t and the final maximum cumulant, respectively. Meanwhile, k is the corresponding release rate constant.

2. Cell Culture

Human embryonic kidney (HEK) 293T cells and rat pheochromocytoma PC12 cells were cultured and maintained in Dulbecco's modified Eagle's medium (DMEM) (GIBCO, Invitrogen, USA) with 10% fetal bovine serum (FBS), 1% penicillin-streptomycin at 37 °C in humidified atmosphere containing 5% CO_2. Medium was

replaced every 2 days. When grown to 80% ~ 90% confluence, cells were passaged by repeated trypsinization (0.25% trypsin/0.02% EDTA for 2 ~ 3 min) and replanting. Cell numbers were determined with an electronic cell counter device (CASY1, Schärfe Systems, Reutlingen, Germany).

3. Cell Viability Study

The cytotoxicity of the modified MCM-41 nanospheres against different cell lines, including 293T and PC12 cells was evaluated in vitro using the Cell Counting Kit-8 (CCK-8) (Dojindo, Kumamoto, Japan), according to the manufacturer's manual. In brief, 293T and PC12 cells were seeded in a 96-well plate at a density of 2.5×10^3 cells per well. DMEM (100 μL) containing 10% FBS was added to each well, and incubated for 24 h (37 °C, 5% CO_2). The medium was aspirated off, and then each well was washed three times with 1× PBS. Fresh medium (100 μL) containing modified MCM-41 in PBS at different concentrations (1000 μg/mL, 500 μg/mL, 250 μg/mL, 125 μg/mL, 62.5 μg/mL, 31.25 μg/mL) was added in triplicate. The liquid was aspirated off after a 48h incubation and replaced with 100 μL of fresh medium and 10 μL of CCK-8 reagent. The cells were further incubated for 2h, and then the absorbance at 570 nm was measured in a microplate reader (Bio Tek Instruments, Inc.). Negative controls were 293T and PC12 cells incubated without any treatment of nanospheres. The cytotoxicity was expressed as the percentage of cell viability compared to untreated controls.

4. Hemolysis Assay

The blood samples from healthy adult rats were used to evaluate the blood

compatibility of modified MCM-41 nanospheres. Rat red blood cells (RBCs) were collected by removing the serum from the blood after centrifugation and suction. RBCs were purified by washing with PBS five times, and then diluted to 1/10 of their initial volume with PBS solutions. A 0.5 mL suspension of diluted RBCs suspension was then mixed with 1.0 mL 0.9% NaCl as a negative control; 1.0 mL 10% Triton-100 as a positive control; or 1.0 mL of MCM-41 nanospheres suspension (0.9% NaCl) at concentrations ranging from 31.25 to 1000 μg/mL. The mixtures were shaken slightly and then kept still for 2 h at room temperature. The samples were centrifuged, photographed, and the absorbance of the supernatants at 570 nm was measured in a microplate reader (Bio Tek Instruments, Inc.). All experimental protocols and animal handling procedures were approved by the Institutional Animal Care and Use Committee of Sun Yat-Sen University and in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

5. Cell Apoptosis Study

Microscopic fluorescence images of the 293T and PC12 cells were obtained using an upright fluorescence microscope (Olympus, BX51, Tokyo, Japan). The 293T and PC12 cells were cultured and maintained in modified MCM-41 nanospheres for 72 h at concentrations ranging from 31.25 to 2000 μg/mL and normal DMEM for 36 h, and then it was stained with Hoechst33342 for nucleus and analyzed using ImagePro Plus 6.0 software (Media Cybernetics, Bethesda, MD, USA).

Table. S1 Loading capacity and encapsulation efficiency of amino and stepwise functionalized samples

Sample	Pure	$MCMN_2$	$MCMN_4$	$MCMN_6$	$MCMN_8$	$MCMN_{10}$	$MCMSN_6$	$MCMSN_8$	$MCMSN_{10}$
LC	3.89	20.61	61.55	67.00	74.38	79.18	45.43	58.64	75.27
(%)	± 0.57	± 2.15	± 3.76	± 3.85	± 2.18	± 3.29	± 3.09	± 2.85	± 3.97
EE	1.01	6.51	40.65	51.80	73.29	98.15	20.96	35.74	78.77
(%)	± 0.16	± 0.86	± 6.42	± 8.96	± 8.37	± 19.46	± 2.6	± 4.19	± 16.66

Table S2. Fitted Kinetic Release Parameters for modified MCM-41 nanosystems.

	$Mt / M\infty = kt + K$ (Zero-order model)			$\ln(1-Mt / M\infty) = -kt + K$ (First-order model)			$Mt / M\infty = kt^{1/2} + K$ (Higuchi model)		
Sample	Slope(k)	Intercept(K)	R^2	Slope(k)	Intercept(K)	R^2	Slope(k)	Intercept(K)	R^2
$MCMN_2$	0.01312	0.53119	0.33696	0.18884	-0.38889	0.98588	0.11948	0.36732	0.64413
$MCMN_4$	0.01076	0.39939	0.56585	0.35138	-0.07984	0.97529	0.10958	0.23507	0.82295
$MCMN_6$	0.01154	0.30744	0.74608	0.06677	-0.11797	0.95217	0.11113	0.15249	0.94008
$MCMN_8$	0.01125	0.34634	0.63575	0.06354	-0.27244	0.988	0.11196	0.1832	0.8706
$MCMN_{10}$	0.03327	0.34435	0.60329	0.32977	0.22329	0.90856	0.19845	0.15729	0.8793
$MCMSN_6$	0.00839	0.36871	0.65078	0.07259	-0.07393	0.91253	0.09518	0.21173	0.87184
$MCMSN_8$	0.00763	0.28201	0.73640	0.04179	-0.14364	0.98981	0.09384	0.1158	0.92401
$MCMSN_{10}$	0.01059	0.42169	0.51184	0.10811	-0.2226	0.95446	0.10999	0.25298	0.78408

Fig. S1 Fitted dissolution-diffusion kinetic models of Zero-order(a-b), First-order(c-d) and Higuchi(e-f)