Supporting Information

for

Mesoporous Silica Nanoparticles Coated by Layer-by-Layer Self-Assembly Using Cucurbit[7]uril for in Vitro and in Vivo Anticancer Drug Release

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1. Materials and Methods

Tetraethoxysilane (TEOS), 1,3,5-trimethylbenzene, 3-isocyanatopropyl triethoxysilane (IPTES),
cetyltrimethylammonium bromide (CTAB), adamantaneamine hydrochloride (AH),
2-bromoiso-butryl bromide, glycidyl methacrylate (GMA), ethanediamine (EDA),
1,4-butanediamine (BDA), 1,6-hexamethylenediamine (HDA) and 2,2′-bipyridine were
purchased from the Aladdin Reagent Co. Ltd. DOX was purchased from Beijing Huafeng United
Technology Co. Ltd. CuBr was purchased from J&K Co. Ltd. Cell Counting Kit-8 (CCK-8) was
obtained from Dojindo (Beijing, China). Propidium iodide (PI) was purchased from
Sigma-Aldrich. All the starting materials and reagents were used as received. A series of
phosphate buffers (PBS buffers) were prepared according to the Appendix XV of the Chinese
Pharmacopeia (Second Part, 2010 Edition). CB[7] was synthesized according to a procedure
reported by Day. Unless otherwise noted, all reactions were performed under nitrogen
atmosphere and in dry solvents. Scanning electron microscope (SEM) images were collected on
a JEOL JSM 6700F instrument. Au coating of nanoparticles used for imaging was carried out by
sputtering for 90 sec. Transmission electron microscopy (TEM) images were collected on a
JEM-2100F instrument, employing an accelerating voltage of 200 kV. Fourier transform infrared
(FT-IR) spectra were recorded on a Bruker Vertex 80V spectrometer. Thermogravimetric
analysis (TGA) was carried on a TA Q500 instrument with a heating program consisting of a
heating rate of 10 °C/min from 308 to 1173 K. 1H NMR Spectra were recorded on a Bruker 500
MHz NMR spectrometer. Powder X-ray diffraction (XRD) measurements were carried out using
a Rigaku SmartLab III powder diffractometer. The radiation source was copper (Kα = 1.39225
Å). N2 adsorption and desorption isotherms were obtained using a Micromeritics Gemini
instrument. Specific surface areas were calculated from the adsorption data in the low-pressure
range using the Brunauer-Emmett-Teller (BET) model. Pore sizes were determined following the
Barrett-Joyner-Halenda (BJH) method. Controlled release profiles were obtained via UV-vis
spectroscopy using a Shimadzu UV-2550 spectrophotometer.
2. Syntheses and Characterizations

2.1. Synthesis and Gel Permeation Chromatography (GPC) of 5-Arm PGMA

5-Arm PGMA was synthesized according to the reported procedure by Gao, Leroux et al.\textsuperscript{S1} The synthetic route to 5-arm PGMA is shown in Scheme S1. The molecular weight of the synthesized polymer was determined by GPC using THF as the eluent at a flow rate of 1.0 mL/min at 35 °C. The molecular weight and PDI of the 5-arm PGMA is recorded in Figure S1. The molecular weight of the 5-arm PGMA is $7.92 \times 10^3$ with a polydispersity index (PDI) of 1.36, endowing the polymer with the appropriate properties for biomedical applications.

Scheme S1. Synthetic route to 5-arm PGMA

Figure S1. Gel permeation chromatography (GPC) of 5-arm PGMA.
2.2. Synthesis of 5-Arm BA-PGOHMA

The general synthetic route to the 5-arm BA-PGOHMAs is shown in Scheme S2. EDA-PGOHMA and BDA-PGOHMA were prepared according to a previous report.\textsuperscript{52} The synthesis of HDA-PGOHMA is best described as follows: 5-arm PGMA (1.0 g) was dissolved in MeCN at a concentration of 12.5 g/L. Then, excess of amine was added (amine/epoxy group 10:1 molar ratio) to ensure the completion of the reaction. The mixture was heated under reflux at 90 °C overnight in an atmosphere of argon. After refluxing at 90 °C overnight, distilled H₂O (ratio of H₂O / initial epoxy group in PGMA was 2:1) was added and the mixture was refluxed at 90 °C for another 12 h. The solution was cooled and the products were dialyzed (molecular weight = 7000) either against H₂O or against EtOH for 48 h, and then freeze-dried or rotary evaporated to obtain the pure products yields in the region of 90–95%.
2.3. $^1H$ NMR Spectra of 5-Arm HDA-PGOHMA

The $^1H$ NMR spectrum of HDA-PGOHMA is shown and compared with the $^1H$ NMR spectrum of 5-arm PGMA in Figure S2.

![Figure S2. $^1H$ NMR spectra (400 MHz, D$_2$O) of the 5-arm HDA-PGOHMA and 5-arm PGMA.](image)

2.4. SEM Images

The SEM images (Figure S3) of a) MSN-1, b) MSN-2, e) DOX-loaded LbL-MSN-1, f) DOX-loaded MSN-2 indicate the monodispersion and homogeneous spherical particle morphology of the nanoparticles. In addition, the average diameter of MSN-1 is ca. 190 nm. After polymer-CB[7] self-assembly, the diameter of DOX-loaded LbL-MSN-1 (or LbL-MSN-2) is larger than MSN-1 (or MSN-2). More importantly, both DOX-loaded MSN-1 and MSN-2 have appropriate sizes within the range that can be taken up by cells via endocytosis.
Figure S3. SEM images and corresponding histograms: a) SEM image of MSN-1; b) SEM image of MSN-2; c) corresponding histogram of MSN-1 diameter based on 200 particles, and the average diameter is ca. 189 nm; d) corresponding histogram of MSN-2 diameter based on 200 particles, and the average diameter is ca. 190 nm; e) SEM image of LbL-MSN-1; f) SEM image of LbL-MSN-2; g) corresponding histogram of LbL-MSN-1 based on 100 particles, the average diameter is ca. 199 nm; h) corresponding histogram of LbL-MSN-2 based on 100 particles, the average diameter is ca. 197 nm.
2.5. TGA Experiments

Based on the TGA curves (Figure 4), the final weight losses for all the samples are displayed in Table S1. After both loading DOX and self-assembly, the weight losses have increased. Results show that the LbL-MSNs have been successfully loaded and assembled.

Table S1. Final Weight Losses for all the TGA Curves in Figure 4

| Samples                     | a)     | b)     |
|-----------------------------|--------|--------|
|                             | MSN-1  | DOX-loaded | DOX-loaded | MSN-2  | DOX-loaded | DOX-loaded |
|                            | MSN-1  | LbL-MSN-1 | LbL-MSN-1 | MSN-2  | LbL-MSN-2 | LbL-MSN-2 |
| Final weight loss [ wt% ]   | 14.2   | 18.6    | 30.9      | 7.4    | 13.8      | 32.5      |

2.6. Determination of DOX Loading Efficiency

LbL-MSNs (5 mg) were dispersed in PBS (1 mL, pH = 2), and dialyzed against the corresponding buffer solutions (20 mL) in capped beakers under stirring at 37 °C. After a long enough period of time, the final amount of DOX released into the buffer solution was analyzed using a UV-vis spectrophotometer by monitoring the absorption at 498 nm.

2.7. ζ-Potential Measurements

In order to measure the ζ-potentials of MSN-1, MSN-2, DOX-loaded LbL-MSN-1 and DOX-loaded LbL-MSN-2, suspensions of each material in water were prepared and tested for 12 times at 25 °C. It turns out that these materials hold certain stability in water. (Table S2)

As shown in Table S2, a negatively charged surface of MSN-1 (with carboxyl groups) or MSN-2 turned into positively charged after loading positively charged DOX and being coated with polycationic PGOHMA. The final positively charged surface of LbL-MSN hybrids facilitates cellular uptake via electrostatic interaction with the negatively charged cell surfaces.

Table S2. ζ-potential of MSN-1, MSN-2, LbL-MSN-1 and LbL-MSN-2

| Samples                  | Zeta-potential [mV] |
|--------------------------|---------------------|
| MSN-1                    | -22.3               |
| MSN-2                    | -25.9               |
| DOX-loaded LbL-1         | 16.7                |
| DOX-loaded LbL-2         | 21                  |
3. Controlled Release Experiments

3.1. Spectroscopic Setup for Controlled Release Experiments

The spectroscopic setup for all the controlled release experiments is portrayed in Figure S4. The release samples were dispersed in PBS buffer and dialyzed against their corresponding buffer solution in capped beakers under stirring at 37 °C.

3.2. Comparison of DOX Release between DOX-Loaded LbL-MSN-1 and DOX-Loaded LbL-MSN-2

Both kinds of DOX-loaded LbL-MSNs were activated lowering the pH value to 2. The relative release curves are shown in Figure S5. MSN-2 reveals a lower release capacity because of the relatively lower specific internal area, even though it has larger pore size.

![Figure S4. Spectroscopic setup for controlled release experiments.](image-url)
3.3. Loading Efficiency of LbL-MSNs

The actual DOX loading efficiencies of LbL-MSNs were estimated by loading and release experiments. Per 1 mg of DOX-loaded LbL-MSN-1 contains ca. 100 µg of DOX, and that of DOX-loaded LbL-MSN-2 contains around 86 µg DOX.

4. Cell Study - Confocal Laser Scanning Microscopy (CLSM)

CLSM confirms the cell uptake of the PI-loaded LbL-MSNs. The Figure S6 shows a qualitative time-dependent uptake kinetic picture of the nanocarrier in the cell and the following release of fluorescent dye PI which is corroborated by the nuclear staining (Figure S7). The lambda scan experiment (Figure S8) records the emission spectrum of the sample, which agrees well with the manufacturer’s data (Invitrogen).
Figure S6. Confocal microscopy images of the nuclei staining of MDA-MB-231 cancer cells for the indicated time. LbL-MSNs were loaded with plasma-membrane-impermeable propidium iodide (PI) molecules and incubated with the cells. The cells were examined with confocal microscopy ($\lambda_{\text{ex}} = 488 \text{ nm}, \lambda_{\text{em}} = 617 \pm 15 \text{ nm}$). In the early time stage ($< 3 \text{ h}$), PI was retained in nanocarriers localized in perinuclear region. The gradual increase in nuclear staining indicates the release of PI dye from the nanocarrier to the nucleus.

Figure S7. Cellular uptake experiment in MDA-MB-231 cancer cells. Nuclear staining after the PI release from LbL-MSNs confirmed by orthogonal sectioning experiment. $\lambda_{\text{ex}} = 488 \text{ nm}, \lambda_{\text{em}} = 617 \pm 15 \text{ nm}$. 
Figure S8. Cellular uptake experiment in MDA-MB-231 cancer cells. Nuclear staining after the PI release from LbL-MSNs. Emission spectra of PI in the cell nucleus. $\lambda_{ex} = 488$ nm.

5. Body Weight of Nude Mice

The mice treated with DOX-loaded LbL-MSNs maintained their body weights, similar to those treated with DOX and the controls (Figure S9).

Figure S9. Body weight curves for BALB/c nude mice with HeLa cancer cells after treatment with DOX (red), DOX-loaded LbL-MSNs (blue), and blank control (black).
6. References

S1  Gao, H.; Jones, M.-C.; Tewari, P.; Ranger, M.; Leroux, J.-C. Star-Shaped Alkylated Poly(Glycerol Methacrylate) Reverse Micelles: Synthesis and Evaluation of Their Solubilizing Properties in Dichloromethane. *J. Polym. Sci. Pol. Chem.* **2007**, *45*, 2425–2435.

S2  Li, C.; Yang, Y.-W.; Liang, Z.; Wu, G.; Gao, H. Post-Modification of Poly(Glycidyl Methacrylate)s with Alkyl Amine and Isothiocyanate for Effective pDNA Delivery. *Polym. Chem.* **2013**, *4*, 4366–4374.