Supplementary Information

Facile fabrication of pH-sensitive peptide-inorganic hollow spheres using a template-free method

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Experimental Section

Materials and reagents:

Phosphotungstic acid ($H_3PW_{12}O_{40,}$ PTA), 1,1,1,3,3,3-hexafluoro-2-propanol (HFIP) and doxorubicin (DOX) were purchased from Sigma-Aldrich. All reagents above were used without further purification. Deionized water purified by a Milli-Q system (Millipore, Milford, MA) was used in all experiments.

Preparation:

Fmoc-HP synthesis: The peptide fragment was synthesized by GL Biochem (Shanghai) Ltd. using Fmoc/t-Butyl solid-phase strategy on Wang Resin. The procedure is illustrated in Fig. S1.

Preparation of Fmoc-HP/PTA hollow spheres: Fmoc-HP was dissolved in HFIP using an ultrasonic bath until the solution was clear and transparent, yielding Fmoc-HP solution at a concentration of 100 mg/mL. The PTA aqueous solution was prepared at a concentration of 0.6 mg/mL. The Fmoc-HP solution was then diluted to a final concentration of 1.0 mg/mL by adding PTA aqueous solution. The mixture solutions were shaken in miniature oscillators for 10 s, and then aged at room temperature without disturbance.

DOX@Fmoc-HP/PTA hybrid spheres: DOX was dissolved into the PTA aqueous solution at a concentration of 0.53 mg/mL before injecting into to the Fmoc-HP solution (Fmoc-HP final concentration is 1.0 mg/mL). The mixture solution was shaken in miniature oscillators for 10 s, and then aged at room temperature in dark conditions. Subsequently, the obtained mixture was centrifuged (10000 rpm for 5 min) and washed with distilled water (1.0 mL × 3 times). The concentration of DOX in the supernatants was measured at 481 nm by using an ultraviolet-visible (UV-vis) spectrometer (Lambda 2, Perkin-Elmer) for indirect calculation of non-encapsulated drug. The non-encapsulated drug was estimated by measuring UV absorbance at 481 nm on a UV-vis

spectrophotometer. The drug-loading content (DLC%) was calculated by the following Eq. (1):

$$\text{DLC\%}=\frac{m_0 - cV}{m + m_0 - cV}\times 100 \qquad (1)$$

Where m_0 is drug dosage; m is the weight of hollow microspheres; c is the concentration of DOX in supernatants; V is the volume of supernatants.

Characterization:

The surface appearance of the Fmoc-HP/PTA hollow spheres was examined by scanning electron microscopy (SEM, JSM-5600LV, Japan). A drop of the mixture solution was carefully applied to the silicon substrate. Subsequently, it was allowed to dry at room temperature, then were affixed with conductive polymer on aluminum stubs, followed by sputtering a thin layer of gold. For examination of structural morphology, a transmission electron microscope (TEM, JEM-2000, Japan) was employed. A 400-mesh copper grid was immersed into the mixture solutions for several times and then it was allowed to dry at room temperature. EDX analysis was performed with an EDAX detector on the SEM. The FTIR spectra were recorded on a Nicolet-560 FTIR spectrometer (Nicolet, Madison) with 20 scans in the range of 1900-750cm^{-1}. Samples were prepared by mixing sample powder with KBr and scanned at 4 cm^{-1} resolution. XRD measurements were performed on a Bruker D&Advance X-ray powder diffractometer with a graphite monochromatized Cu Kα (k=0.15406 nm). A scanning rate of 0.05°/s was applied to record the pattern in the 2θ range of 5~50°. The turbidity was measured using a UV-1800 UV-vis spectrometer with UV-vis Chem Station software. The measurements at different pH and temperatures were carried out at a wave length of 500 nm where the absorption of the aggregates was minimized. Fluorescent microscopy images were acquired by NIKON ECLPSE 80i fluorescence microscope.

In vitro drug release studies:

The release behavior of DOX from DOX@Fmoc-HP/PTA hybrid spheres was evaluated by dialysis. DOX@Fmoc-HP/PTA (1.0 mg) dissolved in 2 mL PBS at the pH value of either 2.0 or 7.4 was placed in a dialysis bag with MWCO of 14,000, and then was immersed in 10 mL of corresponding release medium at pH of either 2.0 or 7.4 in PBS. The samples were incubated in a shaking incubator at 37 °C and were protected from light throughout the whole experimental period. At each predetermined time interval, 1.0 mL of the outside medium was taken. The DOX concentration in the dialysates was analyzed by UV-vis spectrometry in order to detect the rate of drug release. The volume of the outer phase buffer medium was kept constant by replenishing the corresponding buffer solution (1.0 mL).The cumulative drug release of hollow microspheres was calculated by the following Eq. (2):

$$\text{The cumulative release (\%)} = \frac{10C_n + \times 1}{m_0 - cV} \sum C_{n-1} \times 100 \qquad (2)$$

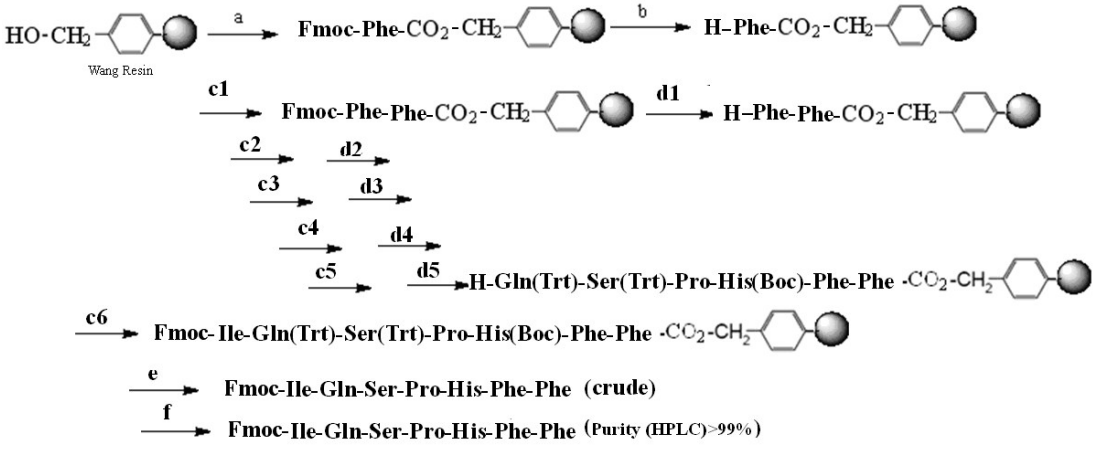
where C_n and C_{n-1} are the concentration of DOX released from the drug delivery system at n and n-1 times, respectively; n is the time of drawing out the buffer solution($n > 0$); Other symbols are as the same above mentioned.

MTT cell viability measurements:

L-929 mouse fibroblasts cells were purchased from the Institute of Cell Biology, Chinese Academy of Sciences (Shanghai, P.R. China). The cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) with DMEM-H30243.01B (Thermo HyClone) supplemented with 10% bovine serum albumin and 1% l-glutamine/penicillin and incubated at 37 °C in a humid atmosphere containing 5% CO_2.

The cell culture medium was changed every two days.

Cell viability was measured by MTT assays. L-929 mouse fibroblast cell suspension were seeded in a 96-well plate at a density of 2×10^4 cells/well and grown for 24 h. Then cells were incubated with different concentrations of Fmoc-HP/PTA hollow spheres for 24 h. Cells were cultured at 37ºC in a humidified atmosphere with 5% CO_2 for 24 h. For MTT assays, After washing the cells with PBS buffer, fresh DMEM medium (100 μL) and 20 μL of a 5 mg mL^{-1} MTT solution in PBS was added and the cells were incubated for 4 h. 150 μL of DMSO was then added to dissolve the precipitate, with 5 min gentle shaking. Absorbance was then measured with a microplate reader (MULTSIKANMK3, Thermo USA) at a wavelength of 570 nm. The cell viability was calculated by normalizing the absorbance of the sample well against that of the control well and expressed as a percentage, assigning the viability of non-treated cells as 100%.



a. DIC、HOBT、DMAP b. 20% PIP/DMF c1. Fmoc-Phe-OH、 HOBT、 HBTU、 DIEA

d1. 20% PIP/DMF c2. Fmoc-His(Boc)-OH、HOBT、 HBTU、 DIEA d2. 20% PIP/DMF

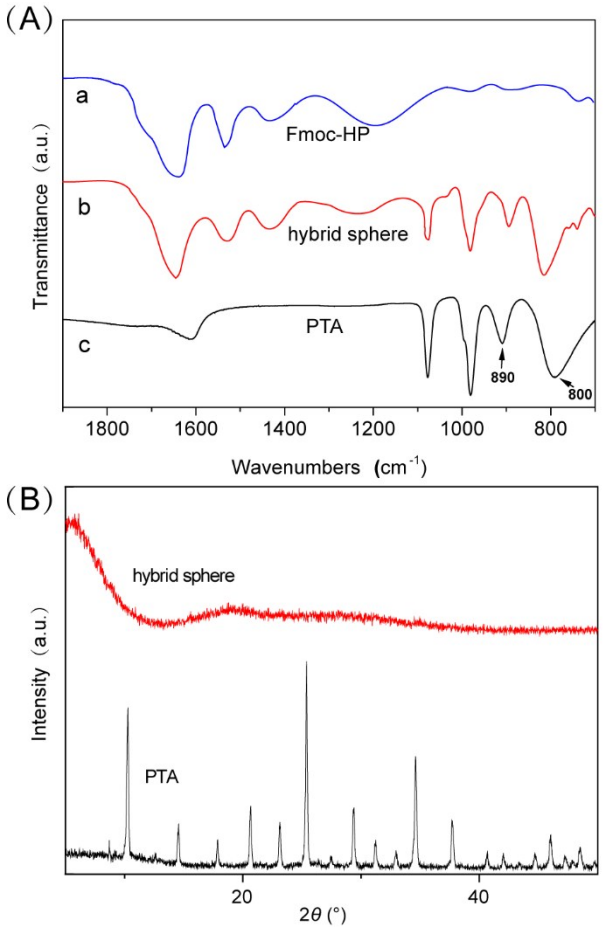
c3. Fmoc-Pro-OH、HOBT、 HBTU、 DIEA d3. 20% PIP/DMF

c4. Fmoc-Ser(Trt)-OH、HOBT、 HBTU、 DIEA d4. 20% PIP/DMF

c5. Fmoc-Gln(Trt)-OH、HOBT、 HBTU、 DIEA d5. 20% PIP/DMF

c6. Fmoc-Ile-OH、HOBT、 HBTU、 DIEA e. TIS、TFA、H2O f. HPLC Purifation

Fig. S1 The sketch of Fmoc-Ile-Gln-Ser-Pro-His-Phe-Phe synthesis.



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143 **Fig. S2** (A) FTIR spectra of (a) Fmoc-HP, (b) hybrid sphere and (c) PTA. (B) XRD

144 patterns of the as-prepared hollow sphere and PTA.