Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Synthesis of selenium nanoparticles with mesoporous silica drug-carrier shell for programmed responsive tumor targeted synergistic therapy

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Experiment section

Synthesis of SeNP

The selenium nanoparticles (SeNP) were synthesized according to previous procedures with modification[1]. As a typical procedure, polyvinylpyrrolidone (PVP, K30) aqueous solution was added into 1 mL of 125 mM H_2SeO_3 solution under magnetic stirring. Then 5 mL of 100 mM ascorbic acid solution was added into the above solution. The reaction solution was adjusted to final volume of 25 mL with Milli-Q water and the reaction lasted 24 h. After centrifuged at 10000 rpm for 30 min, the product was re-dispersed in 25 mL of water for subsequent use.

Synthesis of SeNP@nSiO$_2$

100 μL of (3-aminopropyl)-triethoxy-silan (APTES) was added slowly into the above SeNP product under agitated stirring. After 6 h, the SeNP@nSiO$_2$ was separated by centrifugation and washed by ethanol and water for three times.

Synthesis of SeNP@nSiO$_2$@mSiO$_2$

The SeNP@nSiO$_2$@mSiO$_2$ nanoparticles were prepared through a surfactant-templating sol-gel approach by using CTAB as a template [2]. In brief, the SeNP@nSiO$_2$ were added into the solution containing 25 mL of water, 15 mL of ethanol, 75 mg of CTAB and 0.25 mL of ammonia aqueous solution (28 wt %). It was followed by the addition of 200 μL of TEOS dropwise with continuous stirring within about 10 seconds. Then the reaction lasted 6 h. The particles were collected by centrifugation and washed with ethanol and water, respectively. The CTAB surfactant was removed by solvent extraction method using 60 mL of NH_4NO_3/ethanol solution (6 g/L) and refluxed at 60 °C for 1 h. This extraction process was repeated twice. The channel-opened SeNP@nSiO2@mSiO$_2$ was named meso-SeNP.

Synthesis of folate-functionalized chitosan (FFC)

The conjugate was synthesized as reported in our earlier work [1]. A solution of folic acid and EDC in 20 mL DMSO (molar ratio was 1:1) was prepared and stirred at room temperature until reaction agents were well-dissolved and mixed. The mixture was then added to 1% (w/v) chitosan in acetic acid aqueous solution and stirred at room temperature in dark for 24 h. Finally, the product was isolated by lyophilization and kept for follow-up study.

Modification of dextran with acid cleavable side chain (Dex-COOH)

modification of dextran with a side chain of an acid cleavable citraconic amide group was synthesized and characterized as reported in our earlier work [3].

Preparation of Dox-loaded nanoparticles (I)

In a typical procedure, 400 μL of different concentration of Dox·HCl (1.0, 0.5, 0.2 mg/mL) was mixed with 10 mg of meso-SeNP. After 24, the Dox-loaded nanoparticles were collected by centrifugation at 10000 rpm for 30 min and washed with 100 μL water twice. The supernatant was collected to monitor the drug loading amount and efficiency.

The amount of Dox loaded in nanocarriers was determined by referring to the fluorescent characteristic of Dox (Ex at 480 nm and Em at 590 nm) using a calibration curve constructed from

Dox solution. The equations were as follows:

Drug loading content (%) = $(\mathbf{W}_{\text{initial Dox}} - \mathbf{W}_{\text{Dox in supernatant}})/\mathbf{W}_{\text{nanocarrier}} \times \mathbf{100\%}$;

Drug loading efficiency (%) = $(\mathbf{W}_{\text{initial Dox}} - \mathbf{W}_{\text{Dox in supernatant}})/\mathbf{W}_{\text{initial Dox}} \times \mathbf{100\%}$.

Preparation of I decorated with FFC (II)

I was re-dispersed in 1 mL of FFC solution (contains 1% acetic acid) and continuous stirred for 24 h. Then this solution was washed twice with 1% acetic acid and once with water. **II** was lyophilized. FFC content was calculated to be 4.1 wt% gravimetrically.

Synthesis of II coated with Dex-COOH (III)

II was re-dispersed in 1 mL of Dex-COOH solution (2 mg/mL) and stirred for 6 h. Then this solution was washed twice with water. **III** was lyophilized. Dex-COOH was calculated to account for 9.3 wt% gravimetrically.

***In vitro* Dox release**

Two copies of 10 mg of **III** were respectively suspended in 10 mL PBS solution at pH 5.0, 6.8 and 7.4 with constantly shaking in dark tubes at 37 °C. At specific intervals, a certain volume of buffer was taken out from tubes and same volume of fresh buffer was replaced. The collected buffer was centrifuged and the Dox concentration was detected by fluorescence quantity analysis[3].

MTT assay

Cell viability was determined by measuring the ability of cells to transform MTT to a purple formazan dye [3].

Intracellular localization

Intracellular uptake of nanoparticle was studied in HeLa cells by confocal microscope analysis. HeLa cells were seeded in the culture dish with a coverslip at a density of ~2×10^5 cells per well and cultured for 24 h. The cells were then incubated with nanoparticle for 4 h or 24 h at 37 ℃. The final concentration of Dox was set at double value of IC_{50}. At predetermined time, the culture media were subsequently removed and the cells were washed thrice with PBS and fixed with 4 % (w/v) paraformaldehyde for 15 min at room temperature. The slides were then rinsed thrice with PBS. Finally, the cells were stained with Hoechst 33258 (5 mg/mL in PBS) at 37 ℃ for 10 min. After further rinsed with PBS thrice, the prepared slides were examined by CLSM (Nikon, TE2000, EZ-C1, Japan).

Characterization of materials

Fourier transform infrared (FTIR) spectra were recorded on a Nicolet 5700 spectrometer in the wavenumber range of 400–4000 cm^{-1}. The size, size distribution, and zeta potential were investigated by dynamic light scattering (DLS) and electrophoresis, respectively, using a Zetasizer ZEN3690 (Malvern) with a He–Ne laser beam at 633 nm at 25 °C. Morphology was observed under a transmission electron microscope (TEM) using Tecnai G2S-Twin at an accelerating voltage of 200 kV. The analysis of EDX and XRD were performed on Falcon device and Bruker D8 Advance device, respectively. **III** treated under mild acid (pH 6.8 PBS) for 12 h was examined by CLSM (Nikon, TE2000, EZ-C1, Japan) and TEM.

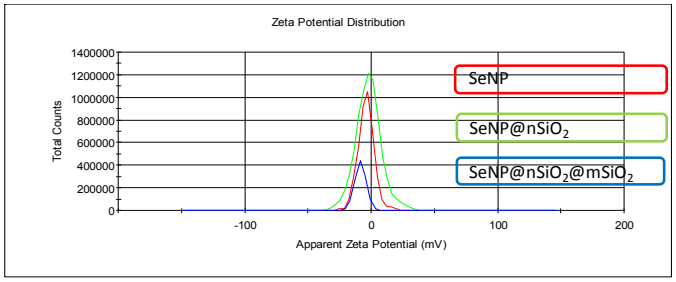

Fig. S1 Zeta potential analysis.

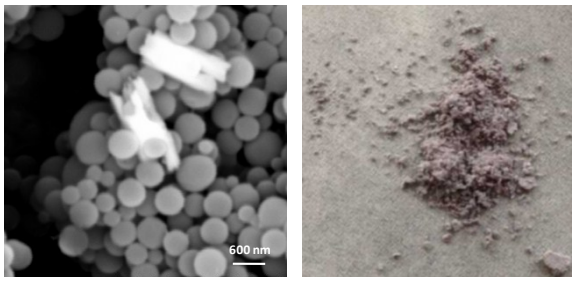

Fig. S2 SEM observation (left) and the picture of product (right) where the mixture solvent of ethanol and water in place of pure water was employed.

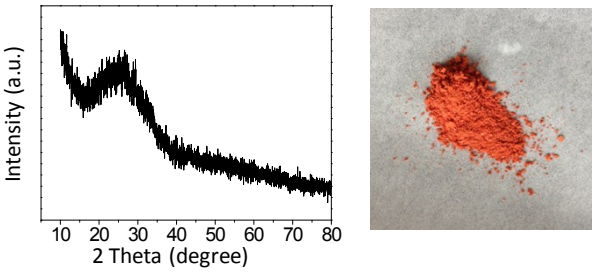

Fig. S3 XRD pattern (left) and the pictureof meso-SeNP (right).

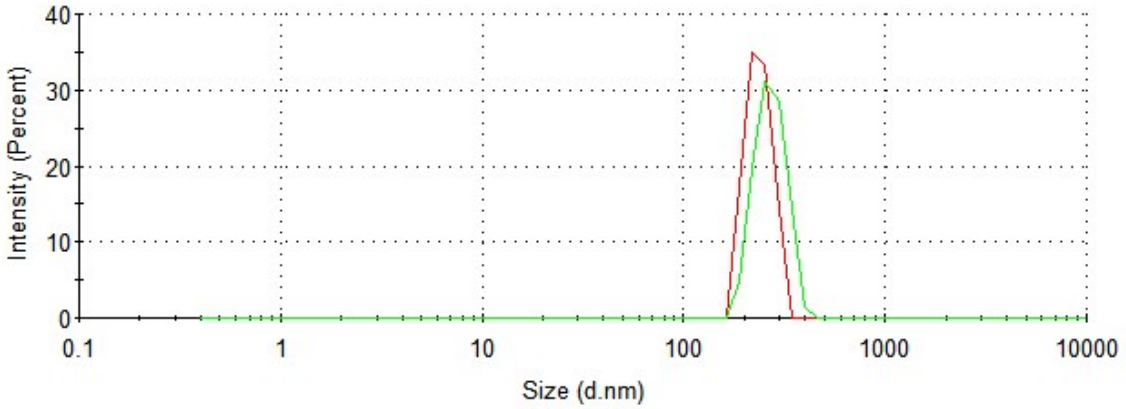

Fig. S4 Size distribution of nanoparticles analyzed by dynamic light scattering (Red line : II, Green line : III).

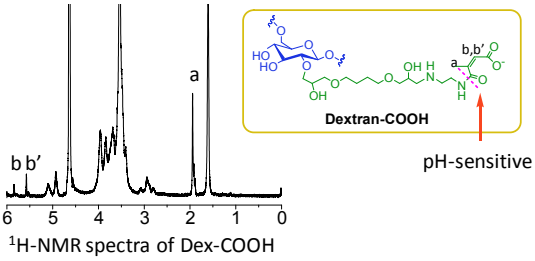

Fig. S5 H-NMR spectra of Dex-COOH.

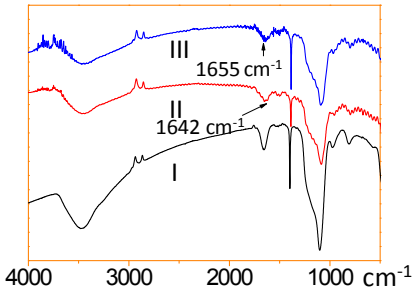

Fig. S6 FT-IR spectra of **I**, **II** and **III.** No Dox was loaded for clear comparison. The weak peak at 1655 cm^{-1} is assigned to the hydrogen bonding in dextran [3]. The characteristic peak of silica was consistent with the reported work [4]. The peak at 1642 cm^{-1} is attributed to the amide in FFC [5].

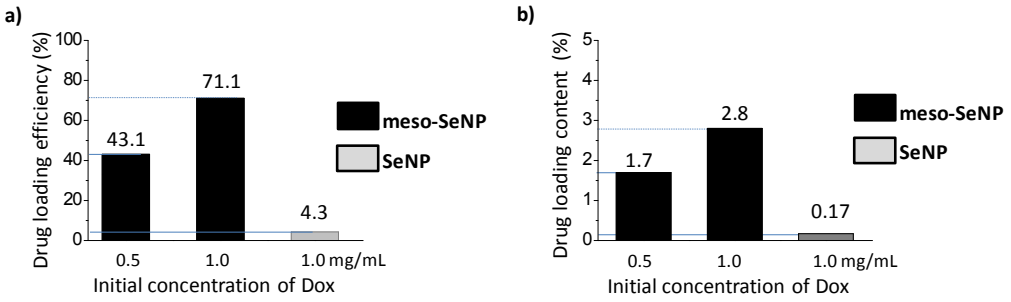

Fig. S7 The drug loading efficiency (a) and content (b) dependent on initial concentration of Dox while both of meso-SeNP and SeNP are kept at 10 mg.

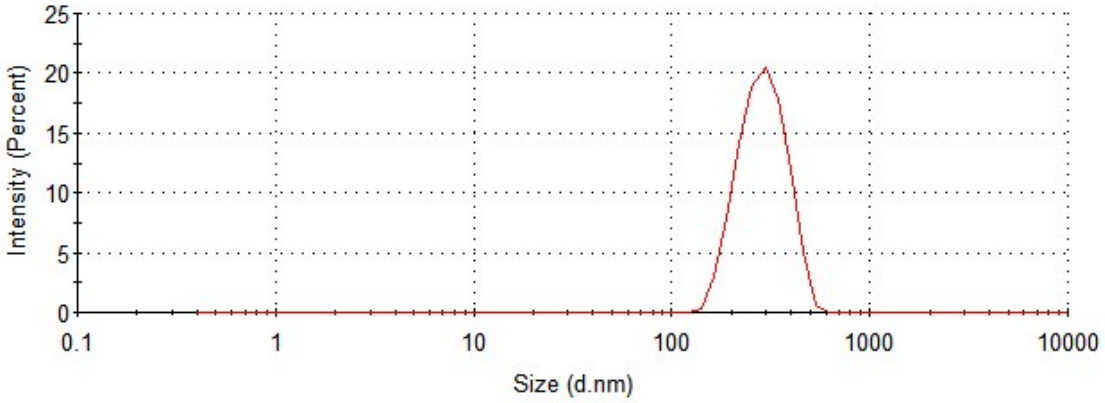

Fig. S8 Size distribution of nanoparticles after 12 h acid treatment (pH 6.8) .

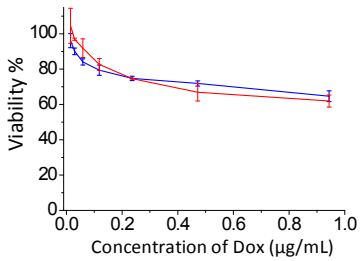

Fig. S9 Inhibition of HepG2 cells proliferation induced by **II** (red curve) and **III** (blue curve), incubation time: 24 h.

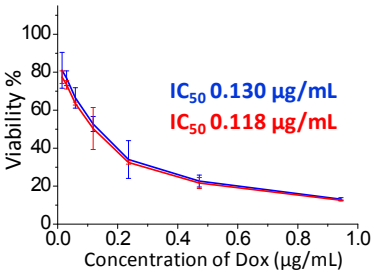

Fig. S10 Inhibition of HeLa cells proliferation induced by **II** (red curve) and **III** (blue curve) , incubation time: 72 h.

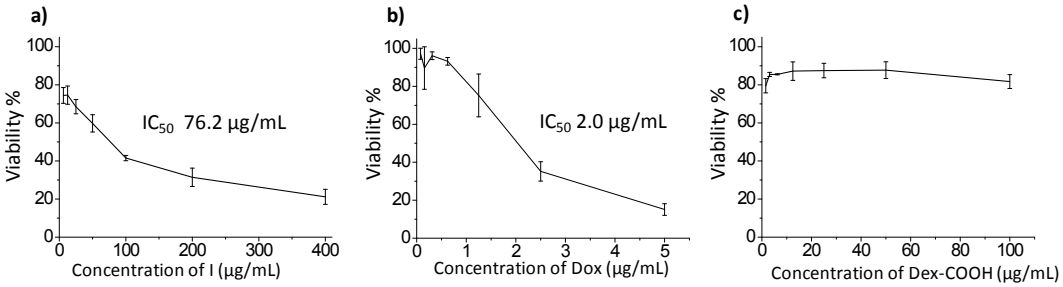

Fig. S11 Inhibition of HeLa cells proliferation induced by meso-SeNP (a), Dox (b) and Dex-COOH (c), incubation time: 72 h.

Reference

1. B. Yu, X. Li, W. Zheng, Y. Feng, Y.-S. Wong and T. Chen, *Journal of Materials Chemistry B*, 2014, **2**, 5409-5418.
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