Electronic Supplementary Information

A novel and facile synthesis of porous SiO_2-coated ultrasmall Se particles as drug delivery nanoplatform for efficient synergistic treatment of cancer cells

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1. Experimental Section

1.1. Chemicals and reagents.

All reagents were used without further purification. Copper (I) chloride (CuCl), polyvinyl pyrrolidone (PVP, K30), ammonium hydroxide (25%-28%) and anhydrous ethanol are analytically pure and were purchased from Sinopharm Chemical Reagent Co. (Shanghai, China), and tetraethylorthosilicate (TEOS, GR) , selenium powders, oleic acid, oleylamine (approximate C18 from 80-90%)were obtained from Aladdin, and doxorubicin hydrochloride (DOX) was got from Huafeng United Technology CO., Ltd. (Beijing, China).

1.2. Characterization.

Sizes and morphologies of the nanoparticles were determined by a transmission electron microscope (TEM) using JEM-2100F microscope. Powder X-ray diffraction (XRD) was measured by a D/max-2550 PCX-ray diffractometer (Rigaku, Japan). The surface area, pore size, and pore-size distribution were determined by a Micromeritics automated sorption analyzer (ASAP2020). An IRPRESTIGE-21 spectrometer (Shimadzu) was used for Fourier transform infrared (FTIR) characterization by ATR method. Optical absorbances of colloidal nanoparticles were measured using a Phoenix 1901 UV-visible-NIR Spectrophotometer. A Leeman Laboratories Prodigy high-dispersion inductively coupled plasma atomic emission spectroscopy (ICP-AES) was used to measure the content of Se in the solution.

1.3. Synthesis of $Cu_{2-x}Se$ nanocrystal.

$Cu_{2-x}Se$ nanocrystals were prepared by a thermal injection method based on a previous research.[1] Briefly, 39.5 mg of selenium powders was dispersed in 5 mL of oleic acid (OA) in a three-neck bottle flask and heated to 120 °C under a nitrogen atmosphere for 30 min with magnetic stirring. Subsequently, the mixture were heated to 280 ℃ and maintained for 30 min. After being cooled to 220 °C, Se-OA precursor was prepared for following use. In another three-neck bottle flask, the mixtures of 5 mL of oleylamine (OAM) ,5 mL of OA and 49.5 mg of CuCl were heated to 120 °C and maintained for 30 min with magnetic stirring under a nitrogen atmosphere. Subsequently, the

mixtures were heated to 220 °C and maintained for 5 min. The dark solution was formed when the above Se-OA precursor quickly injecting into the CuCl mixtures. And the resulting solution was aged at 220 °C for 5 min and then rapidly cooled to 60 °C. Then ethanol was added to the above solution and washed by centrifuging three times with ethanol. Finally, the $Cu_{2-x}Se$ nanocrystals were dispersed in 10 mL of normal hexane for later use.

1.4. Synthesis of Se@SiO_2 core-shell nanospheres.

Se @SiO_2 core-shell nanospheres was prepared by water-in-oil microemulsions methods. In a typical process, 30 mL of *n*-hexane, 3 mL of n-hexanol, 3 mL of Triton X-100, 0.9 mL of deionized water, 5 mL of above $Cu_{2-x}Se$ n-hexanol solution were added in a flask. Then 0.05mL TEOS and 0.1 mL of aqueous ammonia was dropwise added into the mixtures in sequence under rapid stirring. After the reaction mixtures were stirred at room temperature for 24 h, the Se@SiO_2 particles were isolated by adding ethanol. Then, the Se@SiO_2 core-shell nanospheres were collected by centrifuging and washing with ethanol three times.

1.5. Synthesis of porous Se@SiO_2 core-shell nanospheres.

The fresh obtained Se@SiO_2 core-shell nanospheres were dispersed in 20 mL of PVP (K30) solution (10g/L) under stirring. After stirring for 1h, the mixtures were heated to 95°C and maintained at the temperature for different time in standstill condition. Then porous Se@SiO_2 core-shell nanospheres were obtained by centrifuging and washing with ethanol three times.

1.6. In vitro drug loading and release of porous Se@SiO_2 core-shell nanospheres.

5 mg of porous Se@SiO_2 core-shell nanospheres (heat treatment for 2h) were mixed with 2.5 mL of 0.5mg/mL water solution with stirring for 48 h in darkness at room temperature. The DOX-loaded porous Se@SiO_2 nanospheres(Se@SiO_2/DOX) samples were obtained by centrifugation and washed with water twice. The supernatant solution was collected and measured by the 1901 UV–visible-NIR spectrophotometer at 490 nm to calculate the amount of DOX loading in the nanospheres. The DOX release was performed by transforming 5 mg of DOX-loaded porous Se@SiO_2 nanospheres to 2.5 mL of PBS at pH 7.4 and pH 5.0 in a shaker, respectively. At different time intervals, the supernatant solution was collected for analysis and replaced with an equal volume of fresh PBS buffer. The release percentage of DOX was calculated by the value of absorbance at 490 nm.

1.7. Release of Se from porous Se@SiO_2 core-shell nanospheres.

5 mg of porous Se@SiO_2 core-shell nanospheres (heat treatment for 2h), were dispersed in 5 mL of PBS at pH 7.4 and pH 5.0 by ultrasound, respectively. Then they were put in a shaker at 37 °C for Se release. At different time intervals, the supernatant solution was collected by centrifuging and replaced with an equal volume of fresh PBS buffer. The supernatant solution was filtered by 0.22 μm filter, then was added 2 mL aqua regia. Finally, the amount of Se in the solution was measured by ICP.

1.8. CLSM imaging.

The delivery DOX to cancer cells by porous Se@SiO_2 nanospheres was qualitatively detected through CLSM (Confocal Laser Scanning Microscopy). HeLa cells were cultured for 24 h in a 6-well plate for HeLa cells attaching onto the coverslips. Before CLSM imaging, the above coverslips

with HeLa cells were incubated with complete medium containing the $Se@SiO_2$/DOX nanocomposites (DOX concentration 5 $\mu g \cdot mL^{-1}$) for 2 h and 6 h, respectively. Then the cells were washed with PBS, fixed with glutaraldehyde, and counterstained with Hoechst 33342 using a standard procedure. Finally, the coverslips were imaged by CLSM (Leica TCS SP5, Germany).

1.9. Cell culture and viability measurements.

For cell viability measurements, HeLa cells and Sprague-Dawley rat cartilage cells were seeded in a 96-well plate and cultured in 5% CO_2 at 37 ^oC for 24 h. The culture medium was replaced and cells were incubated with complete medium containing porous $Se@SiO_2$ nanospheres, DOX and $Se@SiO_2$/DOX at a series of concentrations in 5% CO_2 and 95% air at 37 ^oC in a humidified incubator for further 24 h, respectively. Then cell viabilities were measured by Cell Counting Kit-8 system (CCK-8) according to manufacture's instructions. The IC50 Values were calculated by cell viabilities of different concentrations of porous $Se@SiO_2$ nanospheres, DOX and $Se@SiO_2$/DOX.

2. Supplementary Figures

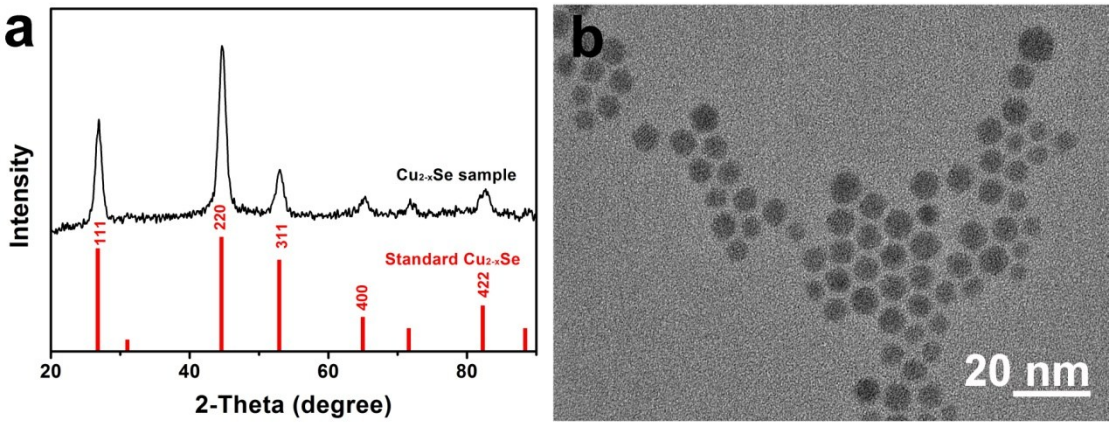

Fig.S1 (a) XRD pattern of the $Cu_{2-x}Se$ sample and standard berzelianite phase of selenium (JCPDS card no: 06-0680). (b) TEM images of $Cu_{2-x}Se$ nanocrystals.

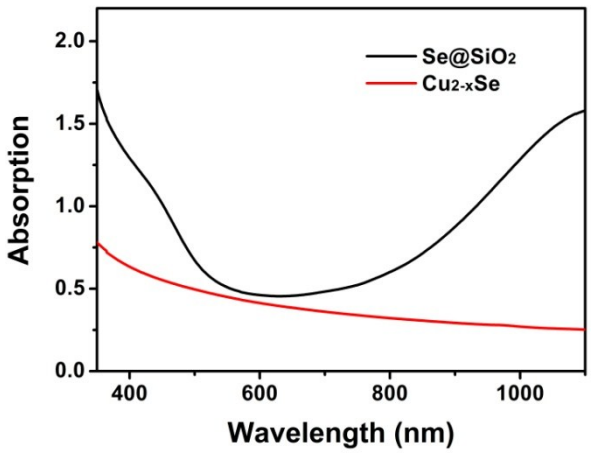

Fig.S2 UV-Vis-NIR absorption spectra of $Cu_{2-x}Se$ (reactant) and $Se@SiO_2$ (product)

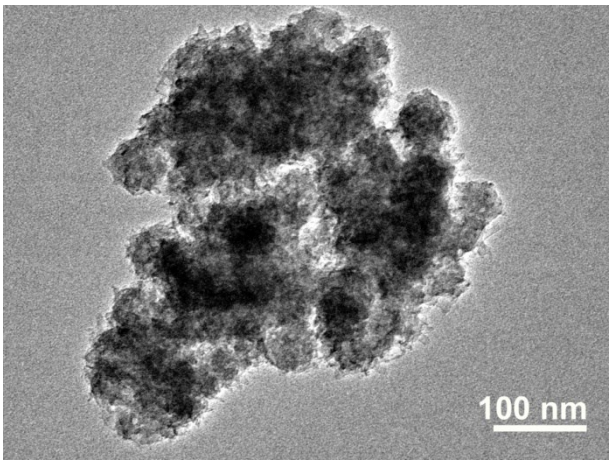

Fig.S3 TEM images of $Se@SiO_2$ nanospheres after heat treatment with water at 95 ^oC for 3h

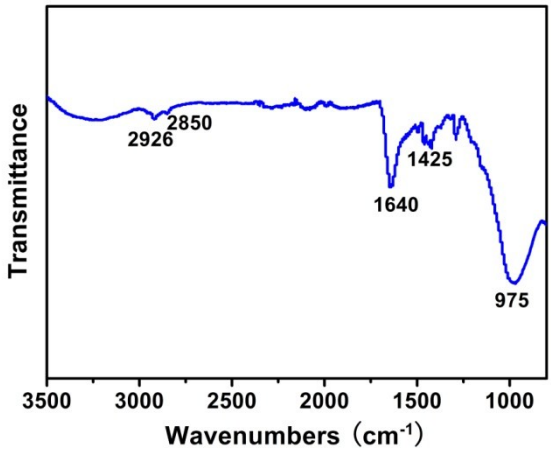

Fig.S4 FTIR spectra of porous $Se@SiO_2$ nanospheres.

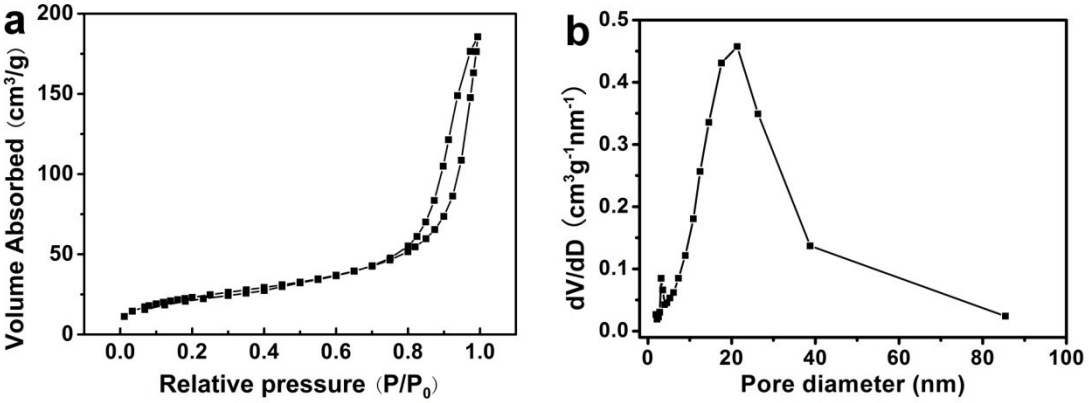

Fig.S5 (a) N_2 adsorption-desorption isotherms and (b) the pore diameter distribution of porous $Se@SiO_2$ nanospheres.

Tab. S1 The IC_{50} value of porous $Se@SiO_2$, DOX and $Se@SiO_2$/DOX

materials	porous $Se@SiO_2$	DOX	$Se@SiO_2$/DOX
IC_{50} value	375 μg·mL^{-1}	4.43 μg·mL^{-1}	26.9 μg·mL^{-1} of $Se@SiO_2$ + 1.79μg·mL^{-1} of DOX

References

1. X. Liu, X. Wang, B. Zhou, W. C. Law, A. N. Cartwright and M. T. Swihart, *Adv. Funct. Mater.*, 2013, **23**, 1256-1264.