Supporting Information

Protein-Directed One-Pot Synthesis of Ag Microspheres with Good Biocompatibility and Enhancement of Radiation Effects on Gastric Cancer Cells

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Experimental Section

Materials

Silver nitrate (AgNO₃), hydrazine monohydrate (N₂H₄·H₂O) and ethanol (CH₃CH₂OH) were purchased from Sinopharm Company (China) and used as received without further purification. Bovine serum album (BSA, molecular mass ca. 68 kDa) was obtained from Xiamen Sanland Chemicals Company Limited, China. Milli-Q water was used in the preparation of all solutions.

Preparation of Ag@BSA Microspheres

In a typical experiment, 5 mL of silver nitrate solution (50 mM) was added into 10 mL of BSA solution (3.0 mg/mL) with vigorous magnetic stirring at room temperature. Thereafter, the mixed solution was vacuumized and kept static under nitrogen protection for 2 h. Then 0.2 mL of hydrazine monohydrate was injected into the vacuumed solution under magnetic stirring. After reaction, the resulting mixed
solution was aged under ambient conditions for 24 h, and then was separated by centrifugation at 10,000 rpm for 15 min. The collected solid state products were washed using double distilled water and ethanol for three times, respectively, and dried for further characterization.

**Characterization of Ag@BSA Microspheres**

The UV-visible spectra were recorded in a Shimadzu UV-2450 UV-visible spectrophotometer from 250 to 600 nm. Deionized water was used as the blank. The CD spectra (190~250 nm) were recorded on a Jasco J-815 spectropolarimeter. The same samples were repeated three times. A cell with a path length of 0.1 cm was used. The parameters used were as follows: bandwidth, 1 nm; step resolution, 0.1 nm; scan speed, 50 nm/min; response time, 0.25 s. Each spectrum was obtained after an average of six scans. SEM images were taken on a ZEISS-ULTRA 55 scanning electron microscope equipped with an X-ray EDS at an accelerating voltage of 20 kV. For transmission electron microscopy (TEM), a drop of aqueous solution containing the silver nanomaterials was placed on the carbon coated copper grids and dried under an infrared lamp for 30 min. The micrographs were obtained using a JEOL JEM-2010 transmission electron microscope operating at an accelerating voltage of 200 kV. Electron diffraction patterns were also recorded for the selected area. The fourier transform infrared (FT-IR) spectra were acquired using a Bruker EQUINOX 55 FTIR Spectrometer in the wavenumber range of 4000–400 cm⁻¹. The size distribution and surface charge of Ag@BSA microspheres were performed on NICOMP 380ZLS (Zeta potential/Particle sizer) system. Thermal-gravimetric analysis (TGA) curves were
recorded on a Perkin Elmer TGA 7 thermogravimetric analyzer.

**In vitro cytotoxicity assessment**

MGC803 cells were available in the cell store of Chinese Academy of Science. MGC803 cells (3 × 10^3 cells per well) were seeded in 96-well plates and incubated overnight at 37 °C in a humidified 5% CO₂ atmosphere. After being rinsed with PBS (pH 7.4), the cells were incubated with 100 μL of varying concentration of Ag@BSA microspheres prepared above for 24 h at 37 °C. Rinsed with PBS again, the cells were incubated another 24 h. Cell viability was determined by MTT assay. Meanwhile, one part of the cells incubated with 50 μg/mL Ag@BSA microspheres was collected, embedded and made into TEM specimens, and then observed via a TEM (JEOL JEM-1230) at 100 kV.

**X-ray irradiation enhanced assay**

A standard radio-oncology linear accelerator (Siemens Oncor Avant-Garde, Siemens Medical Solutions, Concord, CA) with electron beam energy of 6 MeV was used and yielded a mean dose rate 1 Gy/min. The cell culture plate was set up at source to surface distance (SSD) of 100 cm using a 25 × 25 cm² electron applicator. The cells were exposed to total doses of 6 Gy in a single fraction and the corresponding to irradiation times is 6 min. Finally, the cells were incubated another 24 h. Cell viability was determined by MTT assay.
Fig. S1 EDX spectrum of Ag@BSA microspheres.

<table>
<thead>
<tr>
<th>Element</th>
<th>Weight%</th>
<th>Atomic%</th>
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<tbody>
<tr>
<td>C K</td>
<td>3.65</td>
<td>17.22</td>
</tr>
<tr>
<td>N K</td>
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<tr>
<td>O K</td>
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<tr>
<td>Ag L</td>
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<td>45.75</td>
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<tr>
<td>Totals</td>
<td>100.00</td>
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Fig. S2 SEM images of Ag microspheres synthesized through the mediation of BSA: (A) the low-magnification, (B) the higher-magnification.
**Fig. S3** TGA curve of Ag@BSA microspheres. The weight of the BSA is 8.673 wt%.

**Fig. S4** Dynamic light scattering (DLS) histogram of Ag@BSA microspheres.
**Fig. S5** FT-IR spectra of (a) pure BSA, (b) BSA-Ag⁺, and (c) Ag@BSA microspheres.

**Fig. S6** UV-vis spectra of pure BSA, BSA-Ag⁺, Ag@BSA microspheres.
Fig. S7 The investigation of the stability of Ag@BSA microspheres. The prepared Ag@BSA microspheres exhibited good stability and dispersibility in a wide scope of different solutions such as serum, PBS buffer (pH 7.4), and deionized distilled water.

Fig. S8 Zeta potential of Ag@BSA microspheres.