Supporting Information
for

Tumor-targeted nanoplatfrom with stimuli-responsive cascaded activities for multiple model tumor therapy

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1. Materials

Hyaluronidase (HAase) was purchased from Sigma-Aldrich, USA. Gambogic acid (GA), cysteamine (95%), 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 98.5%), and N-hydroxysuccinimide (NHS, 98%) were obtained from Macklin Inc. Dopamine hydrochloride (98%), Doxorubicin hydrochloride (DOX, 98%), butyl nitrite and methacrylic anhydride (MA, 95%) were purchased from Energy Chemical (Shanghai). Dimethyl sulfoxide (DMSO), ammonium persulfate (APS, 98%) and dibasic sodium phosphate dehydrate were purchased from Tianjin Tianli Chemical Reagent Co., Ltd. Absolute ethanol (99.7 %) were purchased from Tianjin HengXing Chemical Reagent Co., Ltd. (Tianjin, China). Tris(hydroxymethyl)aminomethane (Tris), sulfanilamide and N-(1-naphthyl)ethylenediamine dihydrochloride were obtained from Tianjin Fuxing Chemical Reagent Co., Ltd (Tianjin, China). Rhodamine B and sodium dihydrogen phosphate were purchased from Tianjin Kemiou Chemical Reagent Co., Ltd (Tianjin, China). MilliQ water was prepared using a MilliQ system (Bedford, MA, America).

2. Synthesis of polydopamine (PDA) nanoparticles

PDA nanoparticles were fabricated by the chemical oxidation of dopamine hydrochloride, as presented in previous reports [1]. Briefly, dopamine hydrochloride (0.9 g, 4.7 mmol) was dissolved in 450 mL of deionized water, and then 4.75 mL
(0.475 mmol) of 1 N NaOH solution was added dropwisely under stirring. The solution gradually changed to dark brown over reaction time indicating the generation of PDA nanoparticles. The PDA nanoparticles were collected by centrifugation (12 000 rpm, 5 min) and the precipitation was rinsed with ultrapure water for several times. The formation of PDA nanoparticles were monitored using an ultraviolet–visible spectrometry at 280 nm (GENESYS 150; Thermo Scientific, USA).

3. Synthesis of gambogic acid functionalized hyaluronic acid (HA-GA)

3.1 Synthesis of methacrylated-hyaluronic acid (HA-MA)

Methacrylic anhydride (MA) was added dropwisely to 2 wt% HA solution with ice bath. Then the pH value of the solution was changed between 9 and 11 before overnight stirring at 4 °C to produce methacrylated-hyaluronic acid (HA-MA). The resulting HA-MA was then precipitated twice in cold ethanol followed by dialysis (molecular weight cut-off 3500; Spectrum Laboratories, Rancho Dominguez, CA) against water for at least 72 h. Purified HA-MA was lyophilized and stored desiccated at room temperature until use [2].

3.2 Synthesis of amino-functionalized hyaluronic acid (HA-NH$_2$)

HA-MA (20 mg) and thioethanolamine (76 mg) were dissolved in water (5 mL). The solution was then degassed with nitrogen under stirring at ambient temperature for 1 h. Then, APS (1.132 mg) was added to the mixture to trigger the click reaction, which
was finished in 1 h under nitrogen atmosphere at 80 °C. The resulting mixture was purified by dialysis against an excess volume of water for at least three days (water was changed more than three times a day).

### 3.3 Synthesis of gambogenic acid-functionalized hyaluronic acid (HA-GA)

HA-NH$_2$ (25 mg) was dissolved in water with magnetic stirring to prepare solution (1 wt%, Solution i). Separately, GA (4 mg) was dissolved in DMSO (1 mL) under stirring. Then 200 uL of EDC aqueous solution (10 mg·mL$^{-1}$) and 100 uL of NHS aqueous solution (10 mg·mL$^{-1}$) were added, followed with vigorous stirring at 37 °C for 1 h (solution ii). After that, the solution ii was poured to solution i. The mixture was allowed to stir overnight at room temperature. The resulting HA-GA was purified by dialysis against an excess volume of water for at least three days (water was changed more than three times a day).

### 4. Synthesis of carboxylated-S-nitrosothiol (SNO-COOH)

Butyl nitrite (500 μL) was cooled to 0 °C, and then mercapto acetic acid (250 μL) was added dropwisely to the solution. The formation of TGA-SNO was noticed by immediate appearance of a deep red color. The reaction was continued at 0 °C for 2 h in dark. After that, the solution was placed in the freezer section of the refrigerator before use.

### 5. Fabrication of S-nitrosothiol (SNO) functionalized polydopamine with HA-
GA coating (PDA-SNO-GA-HA)

The as-prepared PDA nanoparticles (10 mg) were dispersed in a mixed solvent (water/DMSO, 1/2 v/v) by 30 min of sonication. Then 150 μL of excess TGA-SNO solution, 75 mg of excess N-(3-(dimethylamino)propyl)-N’-ethylcarbodiimide (EDC) and 35 mg of N-hydroxysuccinimide (NHS) were gradually added into the suspension, followed with stirring for 4 h to generate PDA-SNO nanoparticles via the amidation/esterification reaction. After that, the resulting PDA-SNO nanoparticles were collected via centrifugation and washed several times by ultrapure water.

As to form PDA-SNO-GA-HA, the as-synthesized HA-GA (10 mg) was dissolved in water with magnetic stirring, resulting in a 10% solution (Solution i). Separately, PDA-SNO nanoparticles (10 mg) were dispersed in water under agitation (Solution ii). After stirring for 1 h, the solution i was added into solution ii. The mixture was allowed to react for 4 h in dark. The resulting PDA-SNO-GA-HA nanoparticles was collected by centrifugation and washed several times by ultrapure water.

6. Fabrication of DOX loaded PDA-SNO-GA-HA (PSGHD) nanoparticles

PDA-SNO-GA-HA nanoparticles were firstly resuspended in Tris-HCl buffer (20 mM) at pH 8.0. Then, DOX at different weight ratios with PDA-SNO-GA-HA nanoparticles (DOX:PDA=0.5, 1,2 and 4) was added. After stirring for 6 h in dark, the suspension was centrifuged and washed with Tris-HCl buffer solution until the
supernatant is colorless to get purified PSGHD nanoparticles. The product was lyophilized and stored in the freezer section of the refrigerator before use.

7. Measurement of photothermal performance of PSGHD nanoparticles

To investigate the photothermal conversion performance of the PSGHD nanoparticles, 1 mL PSGHD nanoparticles at different concentrations (0-160 μg·mL⁻¹) were added into a quartz cuvette and exposed to an 808 nm NIR laser at different power density (2.0 W·cm⁻², 2.4 W·cm⁻², 3.6 W·cm⁻² and 4.0 W·cm⁻²) for 5 min, respectively. The temperature change of the PSGHD nanoparticles was recorded using an IR thermal camera (testo 882 Thermal imager, Germany) every 30 s.

8. Measurement of NO release in aqueous solution

Considering the released NO molecules could be readily converted into nitrite ions, the NO release behaviors of the PSGHD nanoparticles were measured using a Griess method. It could effectively detect the presence of nitrite ions in solution, due to the rapid reaction of nitrite ions with the Griess agent to form a diazo compound with a pinkish color. The absorption at 540 nm was determined via UV–Vis spectroscopy to quantitatively measure the amount of released NO.

9. Characterization

¹H NMR spectrum was recorded using an AVANCE III HD 600 MHz spectrometer
with Deuterium Oxide (D\textsubscript{2}O) or dimethyl sulfoxide-d\textsubscript{6} (DMSO-d\textsubscript{6}) as the solvent at 293 K. Tetramethylsilane (TMS) was used as the internal standard. Scanning electron microscope (SEM) images and elemental analysis were obtained using a TESCAN MAIA3LMH scanning electron microscope equipped with energy dispersive spectrometer (EDS). Transmission electron microscopy (TEM) images were recorded on a JEOL JEM-2100Plus Transmission electron microscope operated at 200 kV. For the TEM observation, samples were obtained by dropping 10 μL of solution onto carbon-coated copper grids. All the TEM images were visualized without staining. The infrared (IR) spectra was measured by Nicolet iS50 FT-IR using KBr pellets. The ultraviolet-visible (UV-Vis) spectra was measured with dilute aqueous solution in a 2 mm thick quartz cell using a Thermoscientific GENESYS 150 spectrophotometer. All pH value measurements were carried out on a METTLER TOLEDO FiveEasy plus FE 28 pH meter. X-ray photoelectron spectroscopy (XPS) analysis was carried out on a Thermo Fisher ESCALAB Xi\textsuperscript{+} spectrometer (all the peaks corrected with reference to the C signal (284.8 eV)).

10. Cell Culture

HN6 cells (tongue squamous cell carcinoma) and HaCat cells (human keratinocyte cell) used in this study were obtained from the National Institutes of Health (NIH). These cells were cultured in Dulbecco's modification of Eagle’s medium (DMEM,
Invitrogen, USA) supplemented with 10% fetal bovine serum (FBS, Gibico, USA) and penicillin-streptomycin (100 U·mL⁻¹ and 100 μg·mL⁻¹, Gibico, USA), and incubated at 37 °C in 5% CO₂.

11. **In vitro cytotoxicity analysis**

HN6 cells and HaCat cells were seeded at 3 × 10³ per well in 96-well plate for 24 h before treatment, respectively. The cells were exposed to PDA-HA, PDA-GA-HA, PDA-SNO-HA, PDA-HA-DOX, PDA-SNO-HA-DOX and PDA-SNO-GA-HA-DOX (PSGHD nanocomplex) respectively for 48 h, in the absence or presence of 10 min near-infrared (NIR) light irradiation. Cell viability was measured by Cell Counting Kit 8 (CCK-8, Dojindo Co., Ltd. Japan) proliferation assay according to the manufacture’s protocol. The absorbance of the wells was read at 570 nm by using Varioskan Flash multimode reader (Thermo Fisher Scientific, USA).

12. **In vivo Antitumor Efficacy**

All experimental protocols were approved by the Ethics Committee of Fourth Military Medical University, Xi’an China. To set up the tumor xenograft model, BALB/c female nude mice (5 weeks old) were prepared. A total of 5 × 10⁶ HN6 cells were injected subcutaneously into the back next to the right hind limb, and permitted to grow until palpable. When the tumor reached approximately 50 mm³ in volume, 100 μL of aqueous solution of PSGHD nanocomplex (2 mg·mL⁻¹) was intravenously injected into the tail
vein of WSU-HN6 tumor-bearing nude mice. Twenty-four hours post injection, the tumorous areas were exposed to 808 nm NIR irradiation (0.75 W) for 2 min to investigate the photothermal effect of the PSGHD nanocomplex in vivo using an IR thermal camera (testo 882 Thermal imager, Germany).

To further examine the antitumor therapy effect in vivo, twenty-eight tumor-bearing mice were randomly divided into seven groups (n=4). The seven groups mice were injected via the tail vein with PBS (i), DOX (ii), PDA-SNO-GA-HA-DOX (PSGHD nanocomplex, iii), PDA-HA+NIR (iv), PDA-HA-DOX+NIR (v), PDA-SNO-HA-DOX+NIR (vi), PDA-SNO-GA-HA-DOX+NIR (PSGHD nanocomplex+NIR, vii), respectively. The weight of the tumor-bearing mice and the tumor volume were measured every three days for 21 days. The tumor size was calculated as $V=W^2 \times L/2$ (W: the width of the tumor; L: the length of the tumor). At Day 21, the tumors were excised for hematoxylin and eosin (H&E) staining. Four mice per group were monitored every 3 days until the mice were either naturally died or were sacrificed when the tumor volume grew to 2000 mm$^3$ for survival analysis, according to the animal ethical requirement.

Reference

Figure S1. (a) $^1$H NMR spectra of HA-MA, HA-NH$_2$ and HA-GA.
Figure S2. UV-Vis spectrum of pure butyl nitrite, pure thioglycolic acid (TGA) and S-nitrosothiol-functionalized thioglycolic acid (TGA-SNO).
Figure S3. Fluorescence spectrum of PDA-SNO-GA-HA nanoparticles and PDA-SNO-GA-HA-DOX (PSGHD) nanoparticles.

Figure S4. PSGHD nanocomplex before (a) and after (b) 48 h incubation with HAase.
**Figure S5.** (a) Temperature elevation of water and PDA-SNO-GA-HA-DOX aqueous solutions with different concentrations as a function of irradiation time. (b) Temperature change of PDA-SNO-GA-HA-DOX solution at a concentration of 160 ug·mL⁻¹ with 808 nm laser irradiation under various power densities over time.

**Figure S6.** NO release profiles of PDA-SNO-GA-HA-DOX under dark conditions or 808 nm laser irradiation (1 W·cm⁻², 10 min).
Figure S7. (a) Western blot images and (b) quantified Hsp90 expression levels with GAPDH as an internal reference.

Figure S8. TUNEL-stained sections of tumor and nearby normal tissues after treated by PDA+NIR (55 °C, a) and PDA-GA+NIR (43 °C, b)

Figure S9. Body weight change of the tumor bearing mice during the whole treatment by different formulations.
Figure S10. The histological analysis of main organs after 21 d treatment of PSGHD nanoparticles and PBS (control) by HE staining.