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Supporting Information

Skin-Safe Photothermal Therapy Enabled by Responsive Release of Acid-Activated Membrane-Disruptive Polymer from Polydopamine Nanoparticle upon Very Low Laser Irradiation

Rui Zhu [§ † $], Feng Gao [§ † $], Ji-Gang Piao [* § † ¶],Lihua Yang [* § †]

[§] CAS Key Laboratory of Soft Matter Chemistry, [†] School of Chemistry and Materials Science, University of Science and Technology of China, Hefei, Anhui 230026 China

[¶] Hangzhou Branch of Technical Institute of Physics and Chemistry, Chinese Academy of Sciences, Hangzhou, Zhejiang， China

* corresponding authors: (L. Y.) lhyang@ustc.edu.cn; (J.-G. P.) piaojigang@sina.com

[$] These authors contributed equally to this work.

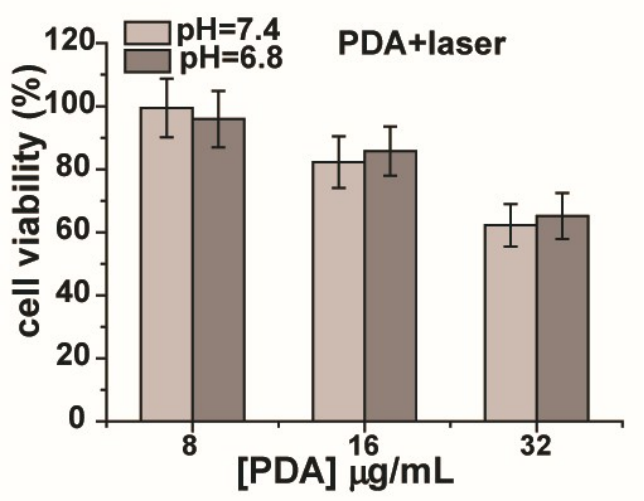

Figure S1. In vitro cytotoxicity of bare PDA nanoparticle to 4T1 cells at different pH (7.4 and 6.8). The cells were treated with PDA nanoparticle at varying PDA concentration for 4 h in FBS-absent DMEM at either pH 6.8 or 7.4, followed by 5-min irradiation with an 850-nm laser at 0.4 W/cm^2.

Material and methods

Synthesis of Acid-Activated Hemolytic Polymer (aHLP). CPADB (4.17 mg), azodiisobutyronitrile (AIBN) (0.492 mg), and monomers (300 mg) at a molar ratio of APBA : HMA : MAA : DMAEMA = 10 : 30: 30 : 30 were dissolved into DMF (2 mL) in a reaction tube. The resulting mixture was subsequently degassed with three freeze-pump-thaw cycles, sealed under vacuum, and placed in an oil bath thermostated at 70 °C. After 9 h, the tube was opened (i.e., broken at top) and the resulting reaction mixture was added into excess amount of anhydrous diethyl ether, which yielded the product as white solid precipitate. After removing the supernatant, excess amount of anhydrous diethyl ether was added to re-suspend the as-prepared product, followed by standing still for a few minutes and supernatant removal; this step was repeated once, to help remove molecules and/or polymer chains with small molecular weights from the product. The as-obtained precipitate was dried in a vacuum oven, which yielded the final product as yellow solid. The yield of polymer is 30.4 %. ^{1}H NMR (d-DMSO 400MHz):

δ12.54 (s, 1H), δ11.26 (s, 1H), δ7.95-7.24 (m, 4H), δ4.3 (s, 2H), δ3.87 (s, 2H), δ1.78(s, 3H), δ1.29-0.88(d, 2H).

Synthesis of Polydopamine (PDA) Nanoparticle. PDA nanosphere is synthesized *via* redox polymerization with a previously reported procedure.[14] Ethanol (40 mL) and deionized (DI) water (90 mL) was mixed in a round-bottom flask, followed by addition of ammonia aqueous solution (2 mL, NH4OH, 28-30%) and sealing. The resulting mixture was subsequently kept in a water bath at 30 °C with mild stir for 30 min, injected with dopamine hydrochloride (0.5 g) pre-dissolved in DI water (10 mL), and kept in the above water bath for another 24 h; the color of the reaction mixture, which was originally colorless clear, immediately became pale yellow upon addition of dopamine hydrochloride, turned into dark brown a few minutes later, and changed to dark black after 24 h. The reaction mixture was subsequently centrifuged (5,000 rcf) and the resulting pellet was washed with DI water for three times, which yielded the expected polydopamine (PDA) nanoperticle as black solid.

Preparation of aHLP-PDA Nanoparticle. Our aHLP-PDA was prepared by conjugating acid-activated hemolytic polymer (aHLP) onto PDA nanoparticle. The as-prepared aHLP (10 mg) pre-dissolved in ethanol (5 mL) was mixed with PDA nanoparticle (5 mg) pre-dispersed in 500 μL ethanol, followed by pH adjustment with NaOH solution (2 M, 500 μL). The resulting reaction mixture was kept in a water bath with stir at 30 °C for 48 h and subsequently centrifuged (5,000 rcf). The resulting pellet was washed with DI water for three times, which yielded the expected aHLP-PDA nanoparticle as black solid.

Characterizations on the Nanoparticles. Morphologies of the nanoparticles were characterized using a transmission electron microscope (TEM) (H-7650, Hitachi) operating at 100 kV. Briefly, one drop of a nanoparticle suspension in DI water was added onto a carbon-

coated copper grid and dried under ambient conditions, followed by TEM imaging. Hydrodynamic diameters and zeta potentials of the nanoparticles were measured using a nanoparticle analyzer (Nano-ZS90, Malvern) at 25 ºC. Hydrodynamic diameter measurements were carried out with nanoparticle dispersions in PBS (pH = 7.4), while zeta potential measurements were performed with nanoparticle dispersions in PBS at differing pH values; PDA concentration was kept constant at 50 μg/mL for both of them. UV-vis-NIR absorption spectra were recorded with a UV-vis spectrometer (Cary 60 UV-vis, Agilent), using nanoparticle dispersions in PBS (pH = 7.4) (PDA content of 50 μg/mL).

***In Vitro* Photothermal Characterizations.** In vitro photothermal effects of the nanoparticles were characterized by monitoring the temperature rises of nanoparticle dispersions (500 μL) in phosphate buffered saline (PBS) during a 5-min irradiation with an 850-nm laser at power density of 0.4 W/cm^2 and a subsequent cooling naturally to room temperature (i.e., waiting for 10 min after irradiation suspension); PDA concentration of 50 μg/mL was used for aHLP-PDA dispersion.

***In Vitro* Release of aHLP from aHLP-PDA Nanoparticle.** aHLP-PDA nanoparticle (400 μg) was dispersed into 2 mL Millipor water, at different time intervals, the resulting dispersion was centrifuged (5,000 rcf, for 5 min). The asa-obtained supernatant (1 mL) was taken for measuring optical density at 280 nm (OD_{280}), which yields the content of released aHLP by using a calibration curve of OD_{280} versus aHLP content. To determine how heat generated by PDA nanoparticle via photothermal effects affects aHLP release, we performed comparable assays but, at 120 min after assay initiation, applied 5 or 10-min irradiation with an 850-nm laser at 0.4 W/cm^2.

To determine the total content of aHLP grafted on the PDA nanoparticle surface, we dispersed aHLP-PDA nanoparticle (400 μg) into 2 mL Millipore water and subsequently heated the resulting dispersion at 70 ºC for 12 h, followed by centrifugation (5,000 rcf, 5 min). The resulting supernatant (1 mL) was measured for OD_{280}, which indicates 100% aHLP release by using the calibration curve of OD_{280} versus aHLP content.

***In Vitro* Cell Viability Assays.** 4T1 cells were used as representative cancer cells,. In vitro cytotoxicity of PDA and aHLP-PDA nanoparticle was assessed with MTT assays, using 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide (MTT, 98%, Beyotime). Briefly, approximately 10,000 cancer cells in DMEM supplemented with FBS (10%, v/v) were seeded into a well of a 96-well plate, incubated at 37 ºC for 24 h till reaching ~80% confluence, washed with 1× PBS twice, treated with PDA or aHLP-PDA nanoparticle at varying PDA concentration for 4 h in FBS-absent DMEM at differing pH (i.e., 6.8, or 7.4), and subsequently irradiated with an 850-nm laser at 0.4 W/cm^2 for 5 min, followed by addition of MTT (5 mg/mL, 10 μL) in PBS. The as-treated cells were subsequently incubated for another 4 h, followed by addition of dimethyl sulfoxide (DMSO, 150 μL). Relative cell viabilities were determined by monitoring the optical densities at 490 and 570 nm with a microplate reader (Varioskan, Thermo).

Confocal Microscopy Imaging. 100 000 4T1 cells were seeded into a culture dish specifically designed for use with a confocal microscopy, incubated at 37 °C under 5% CO_2 conditions for 24 h until reaching ~80% confluency, treated with 32 μg/mL aHLP-PDA when pH=6.8 for 3 h, and subsequently irradiated with a 850 nmlaser at 1 W/cm^2 for 5 min or not. The irradiated cells were then stained with Calcein AM and propidium iodide (PI) (Molecular Probes) in dark for 30 min, washed with PBS and imaged under fluorescence confocal microscope (Axioskop2 plus, Carl-ZEISS). Laser lines of 485and 535 nm at approximately 20%

of their maximum intensitywere used to excite Calcein AM and PI, respectively. Comparabl eassays using the pristine PVP-AuNCs are included for parallel comparisons. Controls are those assayed similarly but without aHLP-PDA addition.

Biodistribution Studies. BALB/c mouse bearing 4T1 murine breast tumor was used as the mouse model for biodistribution studies. Sixteen BALB/c mice (3-4 week) were obtained from the Animal Center at Anhui Medical University and, into the left rear flank of each mouse, injected subcutaneously with 3×10^5 4T1 cells in 100 μL PBS, and cultured till formation of visible primary tumor. Sixteen mice bearing 4T1 tumor were used and randomly divided into two groups (n = 8 per group). Each mouse in one group was injected with aHLP-PDA dispersion in PBS through the tail vein, while that in another group was injected with the pristine PDA dispersion in PBS (100 μL); for both cases, 100 μL nanoparticle dispersions at PDA dosage of 5 mg/mL were injected intravenously through the tail vein. At 24 h after injection, four mice from each group were randomly selected and sacrificed, and their livers, kidneys, spleens, lungs, hearts, and tumors were collected for subsequent PDA content determination by measuring absorbance at 800 nm (OD_{800}). All animal experiments were conducted in compliance with the guidelines for the care and use of research animals established by the Animal Care and Use Committee at University of Science and Technology of China. The experiment was also approved by the Animal Care and Use Committee at University of Science and Technology of China.

***In Vivo* Photothermal Characterizations.** BALB/c mouse bearing 4T1 murine breast tumor was used as the mouse model for in vivo photothermal characterizations, and the kinetics in temperature rise of a mouse during a 10-min NIR irradiation was monitored by recording its

thermographs with an infrared thermal camera (ICI 7320, Infrared Camera Inc.). Six BALB/c mice (3-4 week) were obtained from the Animal Center at Anhui Medical University and, into the left rear flank of each mouse, injected subcutaneously with 3×10^5 4T1 cells in PBS (100 μL), cultured till formation of visible primary tumor, and then randomly divided into three groups. Each mouse was injected intravenously with 100 μL of either aHLP-PDA dispersion in PBS, the pristine PDA dispersion in PBS, or PBS through the tail vein; nanoparticle dosages were kept constant at PDA concentration of 4 mg/mL. At 48-h after injection, each mouse was irradiated with an 850-nm laser at 0.4 W/cm^2 for 10 min; the NIR laser was coupled to a 100 mm-core fiber with the spot size of the laser beam adjusted to cover the entire tumor regions. During the irradiation, thermographs of mice were recorded with an infrared thermal camera (ICI7320, Infrared Camera Inc.) and, based on the as-obtained thermographs, temperatures at tumor sites were obtained.

***In Vivo* Cancer Treatment.** Fifteen BALB/c mice (3-4 week) were obtained from the Animal Center at Anhui Medical University and, into the left rear flank of each mouse, injected subcutaneously with 3×10^5 4T1-R cells in PBS (100 μL), cultured till formation of visible primary tumor, and then randomly divided into three groups (n = 5 per group). Among these three groups, two groups were injected with either the aHLP-PDA nanoparticle or the pristine PDA counterpart in PBS (PDA content of 5 mg/mL, 100 μL), while the other group were injected with 100 μL PBS; all injections were administered intravenously through the tail vein. At 48-h after injection, all mice were anesthetized with 3% neodorm, and their tumors were irradiated with an 850-nm laser at 0.4 W/cm^2 for 10 min; the NIR laser was coupled to a 100 mm-core fiber with the spot size of the laser beam adjusted to cover the entire tumor regions. The NIR irradiation was repeated once a day ever since over a span of 21 days. During this span

of 21 days, tumor lengths and widths were measured with a caliper and mouse body weights were measured using a scale-balance every other day. Tumor volume was calculated as the volume $V = (\text{tumor length}) \times (\text{tumor width})^2/2$.

Histology Analysis. On the 21 th day, one mouse from each group was randomly selected and sacrificed for histology analysis; Five major organs including liver, spleen, kidney, heart, and lung, as well as tumor of these mice were collected, fixed in 10% neutral buffered formalin, processed routinely into paraffin, sectioned at 8 μm, stained with hematoxylin and eosin (H&E), and examined using an inverted fluorescence microscope (IX81, Olympus).

Statistical Analysis. Statistical comparisons were carried out by performing student t test analysis with the statistical software package BioMedCalc (version 2.9). p values of < 0.05 and < 0.01 indicate statistical difference and statistically significant difference, respectively.