Supporting information for

Injectable and NIR-Responsive Hydrogels Encapsulating Dopamine-Stabilized Gold Nanorods with Long Photothermal Activity Controlled for Tumor Therapy

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1. Characterizations of AuNRs-PDA

AuNRs were prepared in two steps using seed-mediated growth as following: (1) Au nanoparticle (AuNP) seeds formation: Freshly prepared $NaBH_4$ solution (0.6 mL, 0.01 M) was quickly added into a $HAuCl_4 \cdot 3H_2O$ (0.25 mL, 0.01 M) and CTAB (9.75 mL, 0.1 M) mixed solution by vigorous stirring for 2 min. After keeping the solution at room temperature for at least 2 h, AuNPs (as seeds solution) were obtained. (2) Seeds growing: Growth of AuNRs was prepared by firstly mixing $HAuCl_4 \cdot 3H_2O$ (0.5 mL, 0.01 M) and $AgNO_3$ (0.1 mL, 0.01 M) in CTAB solution (10 mL, 0.1 M). After 0.08 mL ascorbic acid solution (0.1 M) was added into the above solution, color of the mixed solution changed from yellow to colorless with blending completely. Followed by adding 0.2 mL HCl solution (1.0 M), 0.024 mL seeds solution was also added into the resultant solution with gentle inversion for 10 s. Finally, the solution was left stationary for AuNRs growing. AuNRs were purified by centrifugation for three times at 7500 r/min for 10 min and kept for further use.

The purified AuNRs were then dispersed into 5 mL SH-PEG-CH_3 aqueous solution (2 mg/mL, Mw = 2000) with ultrasonic shaking for 3 min to completely disperse AuNRs, and then the dispersion liquid was stirred for 16 h at room temperature. AuNRs-PEG was obtained by centrifuged three times at 7500 r/min for 10 min. AuNRs-PEG was further coated with polydopamine (PDA) by dispersing AuNRs-PEG into 5 mL DA buffer solutions at room temperature for 30 min.

X-ray photoelectric spectroscopy (XPS) of the AuNRs-PEG was employed to analyze the surface characteristic bonds of the nanorods via the binding energies, as shown in Figure S1. The analysis of the Au 4f spectrum showed a very broad signal, which was similar with the previously reported adsorption of sulfur on gold. Thereinto, two main peaks were observed for the Au 4f signal. The first peak located at ~83.8 eV was corresponding to Au $4f_{7/2}$, and a second doublet for Au $4f_{5/2}$ component was also observed at ~87.6 eV, which indicated the existence of then Au-S bond in the surface of AuNRs.

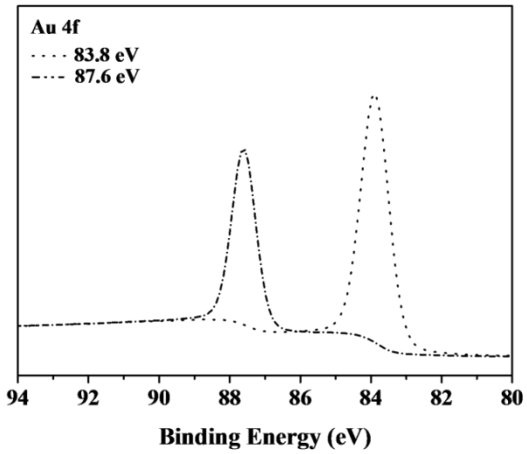


Figure S1. XPS analysis of AuNRs-PEG (Au 4f binding energies)

Raman spectrum of AuNRs-PEG by excited at 785 nm is shown in Figure S2. A Raman band at 310 cm^{-1} was contributed to the S-Au interaction, suggesting the coat layer formation of PEG-SH on the Au NRs.

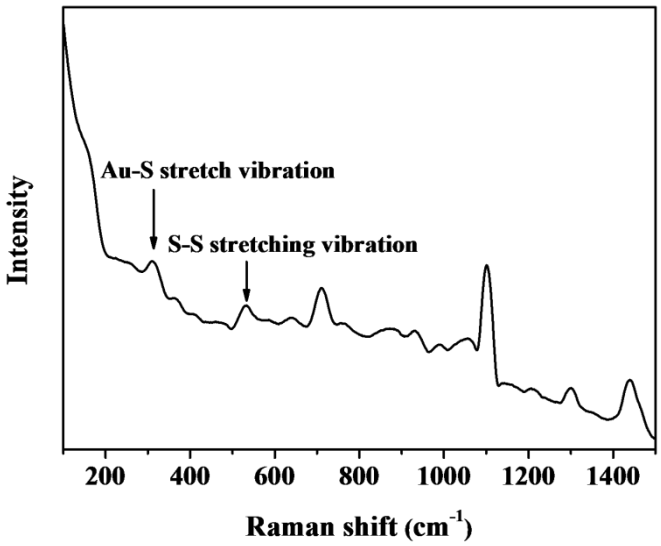


Figure S2. Raman spectrum of AuNRs-PEG with 785 nm of excitation

Diameters of the nanorods were determined by dynamic laser light scattering (Figure S3). The average hydrodynamic diameter of AuNRs-PEG in water was 82 nm, which was similar with AuNRs (70 nm). After modification of PDA, the average hydrodynamic diameter of AuNRs-$PDA_{0.1}$, AuNRs-$PDA_{0.25}$, AuNRs-$PDA_{0.5}$ were 101, 127, and 164 nm, respectively. The average diameter and size distribution of Au NRs-PDA were increased with various deposition thicknesses of PDA.

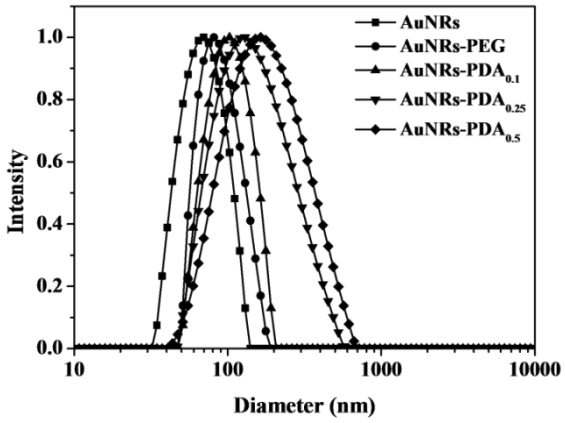
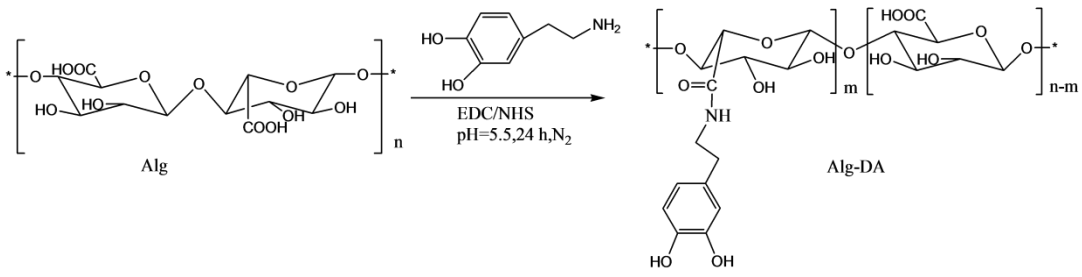


Figure S3. Hydrodynamic size distributions of Au NRs, Au NRs-PEG and Au NRs-PDA.

2. Synthesis and characterization of dopamine-modified alginate (Alg-DA)

Alg-DA was synthesized by grafting dopamine onto alginate by amidation reaction under the assistance of the EDC/NHS as described previously. Briefly, 2 g Alg (10.1 mmol) was dissolved completely in 100 mL PBS buffer solution (pH = 5.5, 0.2 M) under stirring vigorously. Then, EDC (20.1 mmol) and NHS (40.2 mmol) were added into the Alg aqueous solution to activate carboxylic groups on Alg molecules adequately for 45 min. Subsequently, predefined amount of DA was added into the above mixture (Scheme S1). Double-rowed pipe was utilized for three times to prevent the oxidization of DA. And then, the above mixture was stirred for 24 h at room temperature under N_2 protection. Finally, the mixture was precipitated and washed 3 times using ethanol. The final samples were dialyzed for 2 days against water, and followed by lyophilization. In order to obtain Alg-DA with various DA contents, different molar ratios of alginate to dopamine (1:1, 1:3, 1:5) were designed, noting as Alg-DA_1, Alg-DA_3, and Alg-DA_5, respectively.



Scheme S1. Schematic representation of the synthesis process of dopamine modified alginate (Alg-DA)

The structure analyses of Alg-DA were by FT-IR and ^{1}H-NMR spectra (Figure

S4a) and elemental analysis. Figure S4a shows FTIR spectra of Alg and Alg-DA with various DA contents. Characteristic absorption band at 1650-1800 cm^{-1} assigned to the C=O (-COOH) in Alg. After introducing DA, amide bonds (C=O) at 1730 cm^{-1} and C-O stretch vibration band at 1253 cm^{-1} were found in Alg-DA, owing to the reaction between carboxylic acid groups of Alg and primary amino groups of DA and the phenol groups in DA. ^{1}H-NMR spectra of Alg and Alg-DA with various DA contents were recorded in D_2O (δ 4.8) at room temperature and shown in Figure S4b. Chemical shifts at δ 3.0~6.0 were assigned to the protons of the β-D-mannuronic and α-L-guluronic units in Alg. The characteristic peak of benzene also appeared in the ^{1}H-NMR spectra of Alg-DA at δ 7.33~6.93. All the results from FTIR and ^{1}H-NMR spectra suggested the successful introduction of DA into Alg.

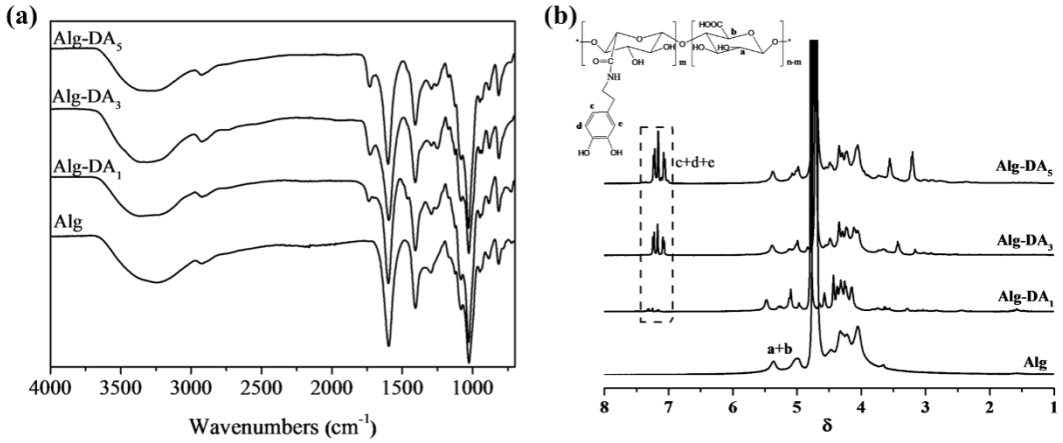


Figure S4. FTIR (a) and ^{1}H-NMR (b) spectra of Alg and Alg-DA with various DA contents.

By comparison of the peak areas between the benzene groups (δ 7.33~6.93) and the methylene groups in Alg (δ 4.8~5.7), the contents of DA in the Alg were 9.8%, 33.0%, and 47.3% (Table S1) for the feed molar ratios of DA to Alg at 1:1, 1:3 and 1:5, respectively. Moreover, element analysis was used to detect the DA contents, as listed in Table S1, which was similar with the results from ^{1}H-NMR spectra. For clarity, these DA modified Alg polymers were abbreviated as Alg-DA_1, Alg-DA_3 and Alg-DA_5.

Table S1. Graft ratio of Alg-DA on ^{1}H-NMR and elemental analysis.

Molar ratio of Alg to DA	1:1	1:3	1:5
^{1}H-NMR (%)	9.8	33.0	47.3
Elemental analysis(%)	9.0	27.9	49.8

3. Characterization of the hydrogel

Figure S5 shows the gelation time of the CGP/Alg thermo-sensitive hydrogels with various mass ratios of CGP:Alg (5:0, 4:1, 3:1, 2:1, 1:1). The gelation time was decreased gradually from 40 min to 30 min and even to only 2 min with the increment of the Alg compositions, because of the hydrogen-bond and electrostatic interactions between CS and Alg.

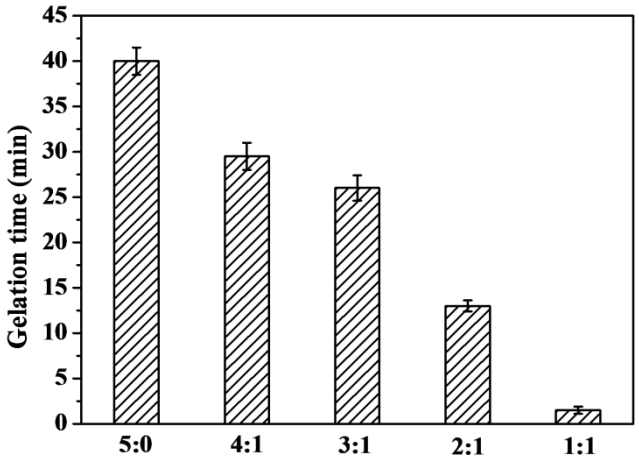


Figure S5. Gelation time of CGP/Alg thermo-sensitive hydrogel

Optical photographs showed that the CGP/Alg hydrogel with white color, and the CGP/Alg-DA/AuNRs nanocomosite hydrogels were dark red in the presence of PDA. Microtopography of the CGP/Alg-DA/AuNRs hydrogel was observed by SEM image (Figure S6). The CGP/Alg hydrogel and CGP/Alg-DA/AuNRs nanocomposite hydrogels showed almost the same pore structure, independing on the DA contents.

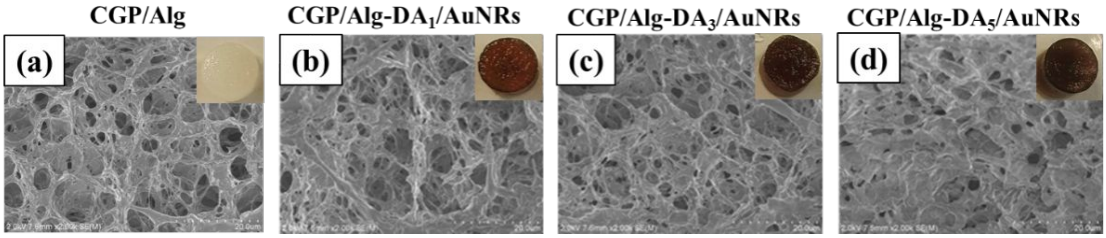


Figure S6. SEM images of CGP/Alg (a), CGP/Alg-DA_1/AuNRs (b), CGP/Alg-DA_3/AuNRs and CGP/Alg-DA_5/AuNRs composite hydrogels. The inset images are optical photographs of the hydrogels.

Figure S7 showed the UV-vis spectra of AuNRs and the supernatants of the CGP/Alg-DA/AuNRs gel after immersed in PBS buffer solution for 1 day and 2 weeks. AuNRs showed a special absorbance at about 800 nm. However, there was no

absorbance for the supernatant of the hydrogel even immersed after 2 weeks, indicating no AuNRs leaked from the CGP/Alg-DA/AuNRs composite hydrogel.

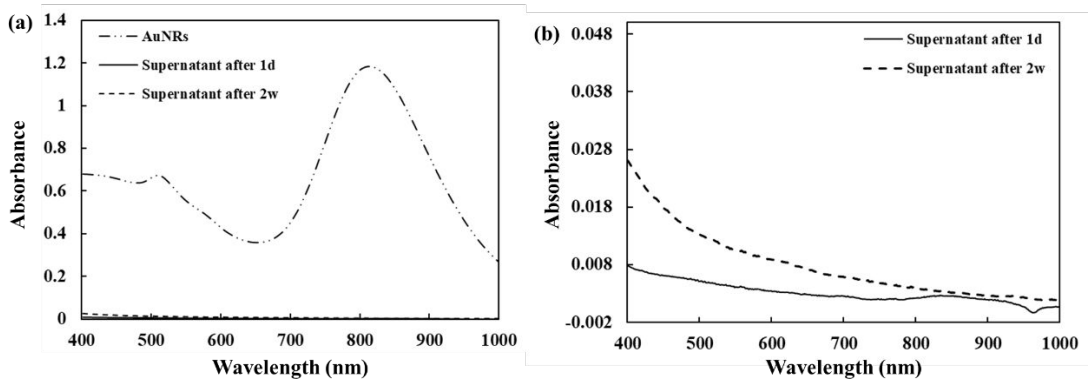


Figure S7. (a) UV-vis spectra of the AuNRs, the supernatant of the CGP/Alg-DA_1/AuNRs composite hydrogels after immersed in PBS for 1 day and 2 weeks, and (b) the magnified UV-vis spectra of the upernatant of the CGP/Alg-DA_1/AuNRs composite hydrogels after immersed in PBS for 1 day and 2 weeks.

Compression strength of the hydrogels in the equilibrated swelling state was recorded at 60 % strain with 1.0 mm/min of compresses rate using a mechanical testing machine (WDW-1E, Jinan). The results were expressed as means of three parallel replicates, and shown in Figure S8a. The compression strength of the hydrogels was above 30 KPa and increased to 63 KPa with increasing DA contents. After immersing the hydrogel in PBS for 15 days, the strengths declined slightly, about 10% less than the original hydrogels. Since CS and Alg are biodegradable biopolymers, CGP/Alg-DA/AuNRs hydrogels might also degrade in PBS, resulting in the decrement of compression strength. To confirm this presumption, the degradation behavior of the hydrogel was investigated in PBS (pH 7.4) at 37 ^{o}C. As shown in Figure S8b, within the first period of 2 weeks, the weights of the hydrogels declined around 10 %, and to almost half after 8 weeks. This weight loss was well corresponding to the declined strength of the hydrogels. Accordingly, the decreased strength was confirmed to be induced by the degradation of hydrogel, not the leakage of AuNRs. With prolonging the degradation time, the wight loss increased and to almost less than half of the wight after 8 weeks. For the pure CGP and CGP/Alg hydrogels, they almost completely degraded after 8 weeks. However, the weight of the CGP/Alg-DA/AuNRs hydrogels

remained about 40%~50% after 8 weeks, exhibiting lower degradation rate than the hydrogels without AUNRs-PDA. There might be two reasons: the one was undegradation of AuNRs, and another was the strong interactions between DA and polymers or PDA. Hence, the degradation of the hydrogels could be controlled to a suitable time to support AuNRs in the tumor site stably for high and long-time PTT efficacy.

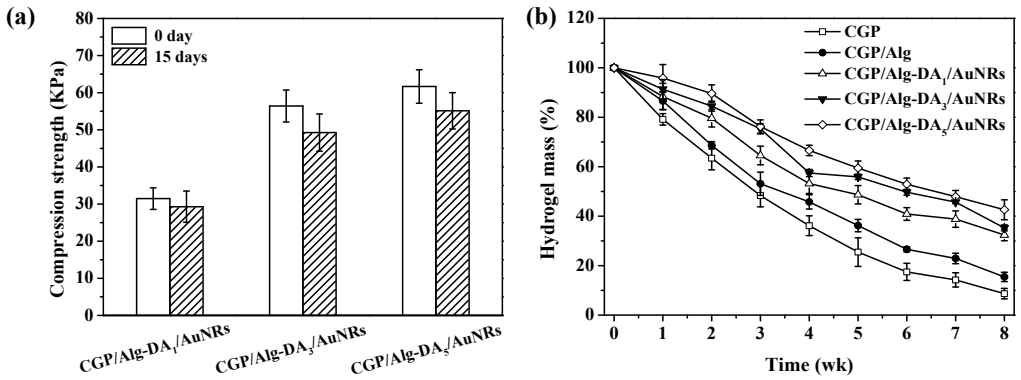


Figure S8. (a) Compression strengths of hydrogels treated before and after 15 days immersion. (b) Degradation rate (pH=7.4, 37 °C) of CGP/Alg-DA/AuNRs hydrogel

The body weight was monitored as an indicator of physical health of mice during implantation of foreign material, laser irradiation and repeated PTTs, as shown in Figure S9. The mice in all groups maintained their normal growth characteristics without sharp reduction or increase in 21 days, suggesting that multiple PTTs were tolerated well with negligible side effects.

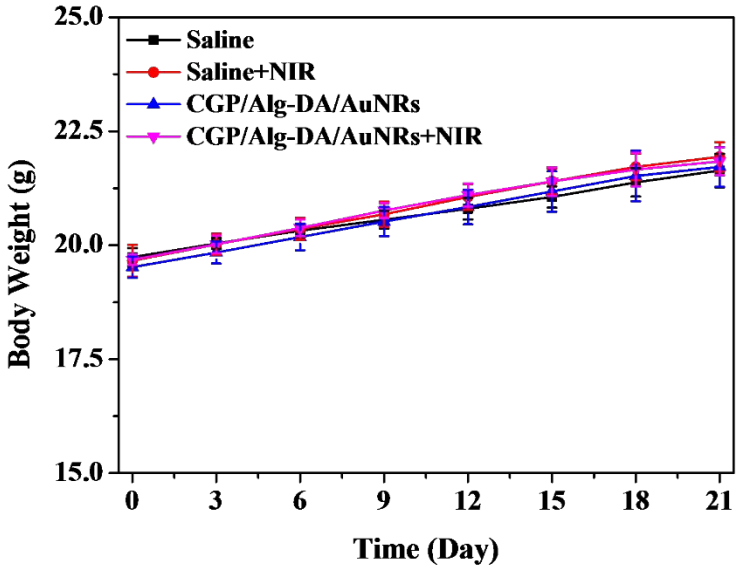


Figure S9. Body weight of HepG2-bearing Balb/c mice upon various treatments.