Supplementary material

Copper nanocrystalline-doped folic acid-based super carbon dots for enhanced antitumor effect in response to tumor microenvironment stimuli

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Content

1. Study of Photothermal effect

The photothermal conversion efficiency was evaluated by recording the temperature change of FA-CD@Cu^x (400 µg/mL) with prolonged time under 808 nm laser irradiation at a power density of 1.0 W/cm^2. The photothermal conversion efficiency η was calculated by equation (1), referring to previous literature

$$\eta = \frac{hA(T_{max} - T_{surr}) - Q_{Dis}}{I(1 - 10^{-A_\lambda})} \quad (1)$$

where h is the heat transfer coefficient and A is the surface area of the vessel. T$_{max}$ (50.2 °C) and T$_{surr}$ (25 °C) denote the equilibrium temperature and ambient temperature, respectively. Q_{Dis} denotes the heat dissipation of light absorbed by the quartz cuvette as 54.0 mW. I is the incident laser power (1.0 W/cm^2), and A_λ is the absorbance of FA-CDs@Cu^x at 808 nm (0.445). The hA value can be calculated from equation (2).

$$\tau_s = \frac{m_D C_D}{HA} \quad (2)$$

where τ_s is the sample system time constant, m$_D$ and C$_D$ are the mass (1 g) and heat capacity (4.2 J g^{-1}) of deionized water as a solvent, respectively. To calculate τ_s, the dimensionless parameter θ should be obtained from the following equation (3).

$$\theta = \frac{\Delta T}{\Delta T_{max}} \quad (3)$$

where ΔT is the temperature variation, which is the temperature difference between the solution temperature and the ambient temperature. ΔT$_{max}$ is the amount of temperature change at the maximum steady-state temperature Therefore, using the linear time data of the cooling period for ln(θ) (Fig.S1), $\tau_s = 261$ can be determined by equation (4).

$$t = -\tau_s ln(\theta) \quad (4)$$

Therefore, it can be calculated that:

$$hA = \frac{1 \times 4.2 \times 1000}{261} = 16.09$$

$$\eta = \frac{16.09 \times (50.2 - 25) - 54}{1000 \times (1 - 10^{-0.445})} = 54.3\%$$
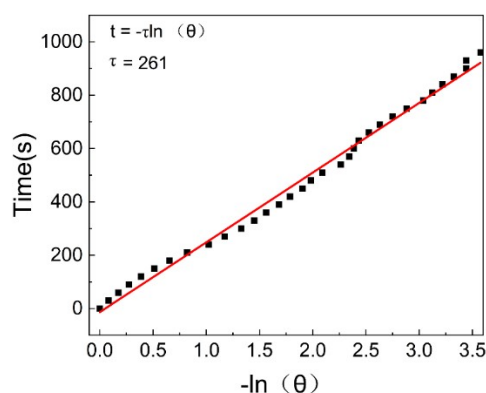

Fig.S1. Plot of cooling time versus negative natural logarithm of the temperature driving force obtained from the cooling period.

The time constant τ_s for heat transfer of the system was determined to be 261.
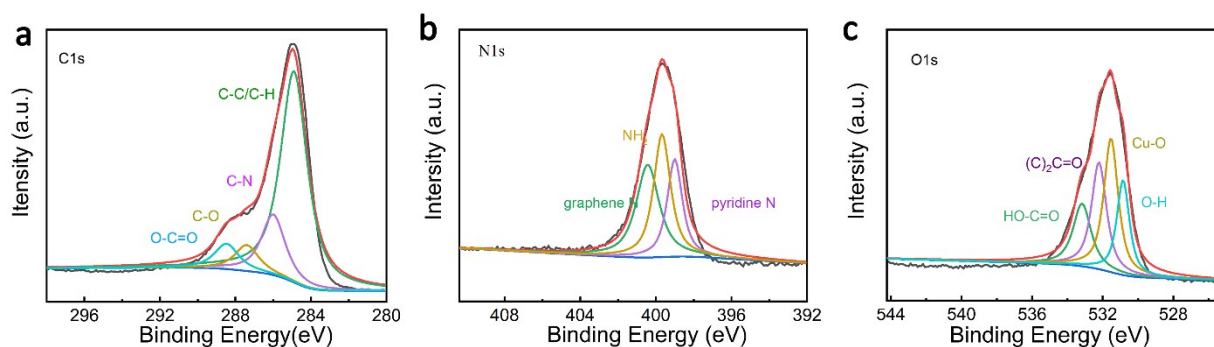
2. High resolution XPS spectra of FA-CDs@Cu^x

Fig.S2. a) XPS-deconvoluted C1s; b) XPS spectrums as depicted in N 1s; c) XPS spectrums as depicted in O 1s

Carbon has four binding states of C=C/C-C, O=C-O, C-O, C-N (Fig. S2a). Nitrogen has three binding states: 398.7eV belonging to the pyridine N, 399.3 eV belonging to the -NH$_2$, 401.1 eV belonging to the graphitized N (Fig. S2b). Oxygen has four binding states: O-H, O=C-O, Cu-O, and HO-C=O (Fig.S2c).

3. Singlet oxygen quantum yield test

Test procedure: 1,3-diphenylisobenzofuran (DPBF, 1 mg/mL dissolved in DMF) was used as $^1O_2$ trapping agent, and the change of absorbance at 415 nm after oxidation of DPBF by ROS was detected by UV-Vis spectrometer (UV-Vis) to calculate the $^1O_2$ quantum yield of FA-CDs@Cu^x. Methylene blue (MB) was used as the control sample. First, the absorption of FA-CDs@Cu^x and MB at 671 nm

was fixed at about 0.10 OD, and then DPBF (30 μL) was added to each of the above solutions and irradiated with a 670 nm laser at a power density of 0.1 W/cm^2. The decay rate of DPBF solution at 416 nm with irradiation time was calculated. The experimental data were listed in Table S1. We calculated the $^1O_2$ quantum yields of CDs using Eq. (5).

$$\Phi_{FA-CDs@Cu^x} = \Phi_{MB}\frac{T_{MB}}{T_{FA-CDs@Cu^x}} \quad (5)$$

where $T_{FA\text{-}CDs@Cu}x$ and T_{MB} are the time for the DPBF absorption drop in the presence of CDs and MB (Fig. 3c), respectively. Φ_{MB} is the $^1O_2$ quantum yield of MB in DMF, given as 49%. The $\Phi_{FA\text{-}CDs@Cu}$ was calculated as 56.8%.

Table S1 The record data for the calculation of $^1O_2$ for different samples.

Light(670 nm)						Light(808 nm)		
The absorption of DPBF at 416 nm (Containing MB)			The absorption of DPBF at 416 nm (Containing FA-CDs@Cu^x)			The absorption of DPBF at 416 nm (Containing FA-CDs@Cu^x)		
t_s	A_t	$Ln(A_0/A_t)$	t_s	A_t	$Ln(A_0/A_t)$	t_s	A_t	$Ln(A_0/A_t)$
0	1.038	0	0	1.038	0	0	1.05	0
10	0.967	0.0705	10	0.949	0.0894	10	0.904	0.1496
20	0.919	0.1219	20	0.905	0.1374	20	0.849	0.2123
30	0.889	0.1554	30	0.858	0.1901	30	0.809	0.2605
40	0.838	0.2139	40	0.857	0.1913	40	0.769	0.3111
50	0.808	0.2507	50	0.806	0.2532	50	0.729	0.3645
60	0.798	0.2633	60	0.767	0.3020	60	0.689	0.4208
70	0.767	0.3020	70	0.727	0.3561	70	0.649	0.4805
80	0.707	0.3842	80	0.726	0.3575	80	0.599	0.5606
90	0.673	0.4340	90	0.706	0.3857	90	0.563	0.6224
100	0.651	0.4661	100	0.684	0.4176	100	0.544	0.6567
110	0.624	0.5088	110	0.671	0.4370	110	0.519	0.7037
120	0.586	0.5723	120	0.634	0.4928	120	0.490	0.7631
130	0.555	0.6254	130	0.617	0.5202	130	0.450	0.8482

4. Hemolysis test of Fa-CDs@Cu^x

Take 20 mL of fresh blood from rabbits and add 1 mL of 2% potassium oxalate saline solution to make fresh anticoagulant blood. Take 8 mL of anticoagulated blood and add it to 10 mL of saline to dilute. Then take 1μg of Fa-CDs@Cu^x and add it to 10 mL of saline, preheat it in a water bath at 37 °C for 30 min, then add 0.2 mL of diluted anticoagulated blood, mix it slowly and heat it in a water bath at 37 °C for 60 min. Take 10 mL of distilled water as a positive control, preheat it in a 37 °C water bath for 30 min, add 0.2 mL of diluted anticoagulant blood and mix slowly and heat it in a 37 °C water bath for 60 min. Take 10 mL of saline as a negative control, preheat it in a 37 °C water bath for 30 min, add 0.2 mL of diluted anticoagulant blood and heat it in a 37 °C water bath for 60 min. for 5 min (2500 rpm). The absorbance (OD) at 545 nm was recorded with a spectrophotometer and the hemolysis rate was calculated using the formula:

$$HR = (As - A_1)/(A_2 - A_1) \times 100\%.$$

Where HR denotes the Hemolysis rate, As denotes the absorbance of the experimental group, A1 denotes the absorbance of the negative control group, and A2 denotes the absorbance of the positive control group. The experiment was repeated three times, and the blood of different rabbits was taken each time. The mean absorbance of each group and the hemolysis rate of Fa-CDs@Cu^x are listed in Table S2. No erythrocyte rupture was observed under the microscope by taking a small amount of the lower fluid containing erythrocytes

Table S2 The absorbance of each group and hemolysis rate of Fa-CD@Cu^x

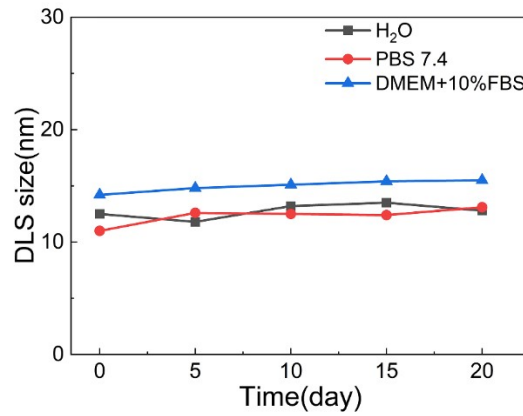| Experience	Absorbance(OD)		
	A_s	A_1	A_2
I	0.014	0.013	0.097
II	0.015	0.014	0.099
III	0.013	0.012	0.098
HR（%）	1.18%		

5. Fig.S3

Fig.S3. Change in size of FA-CDs@Cu^x in H$_2$O, PBS (pH 7.4), DMEM (10%FBS).
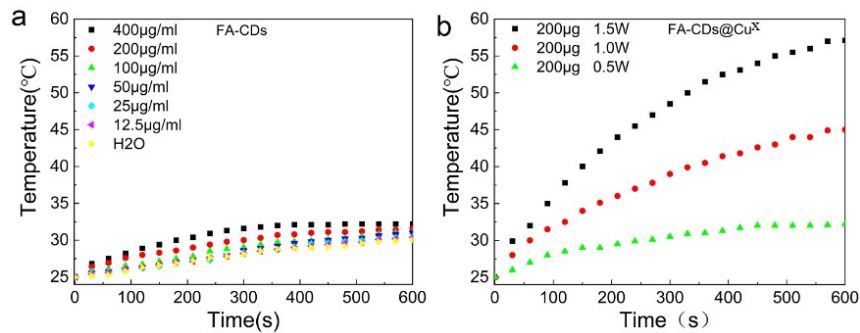
6. Fig.S4

Fig.S4. a) Temperature change curves of FA-CDs irradiated by light (808 nm, 1 W/cm^2) at different concentrations;

b) Temperature change curve of 200 µg/mL FA-CDs@Cu^x solutions under different light source irradiation
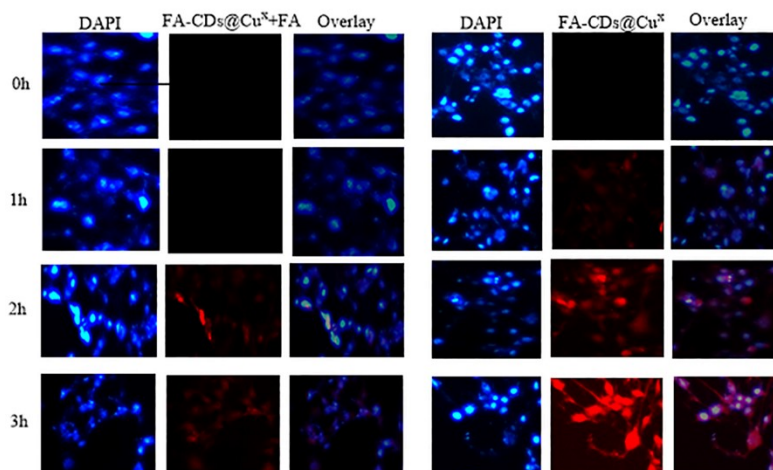
7. Fig.S5

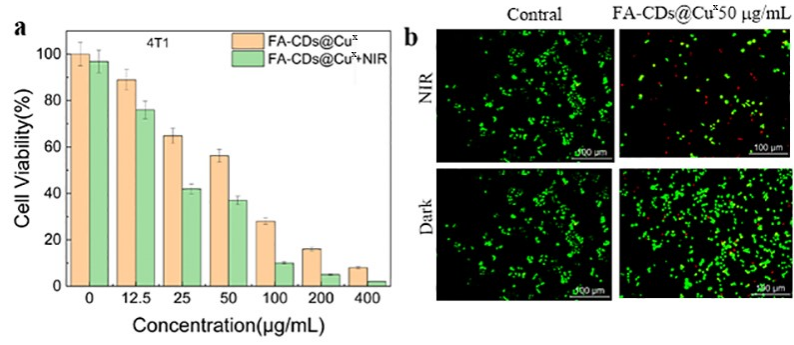Fig.S5. 4T1 Cytophagy and targeting of Fa-CDs@Cu^x

8. Fig.S6

Fig.S6. a) MTT assay to detect the toxicity of FA-CDs@Cu^x on 4T1 cells; b) Live-dead cell staining of 4T1 cells
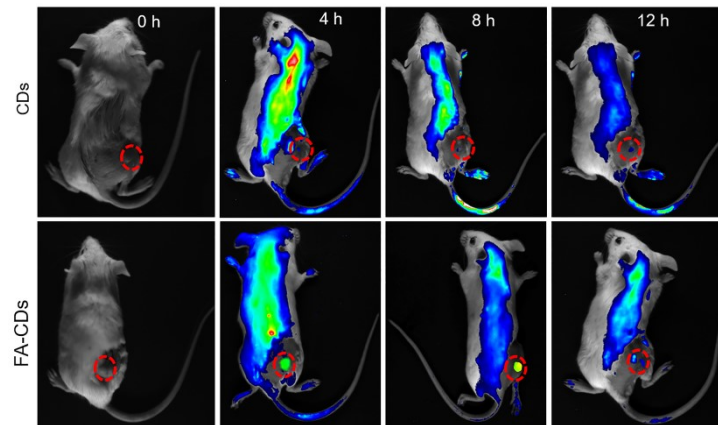
9. Fig.S7

Fig. S7 In vivo fluorescence imaging of CDs and FA-CDs at different intervals (0,2,4,8,12 h)