Supplementary material

Carbon dots-doped Cu-MOF-based smart nanoplatform for enhanced immune checkpoint blockade therapy and synergistic multimodal cancer therapy

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[1] These authors contributed equally to the work.

Contents

1. DLS graphs of RCDs and Cu-MOF@RCD

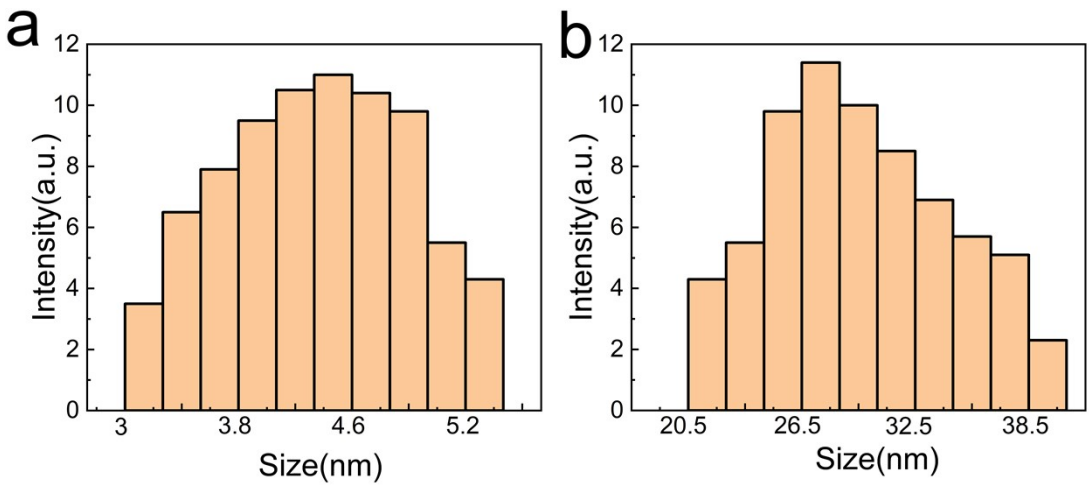


Fig. S1 a) DLS size of RCDs; b) DLS size of Cu-MOF@RCD.

2. XPS of Cu-MOF@RCD

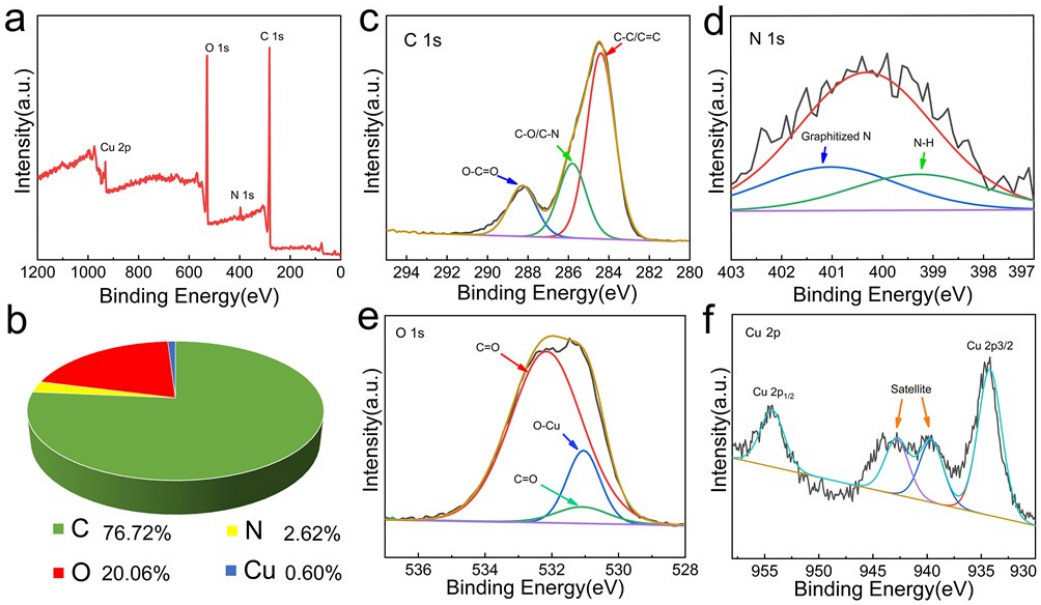


Fig. S2 a) X-ray photoelectron spectroscopy (XPS) of Cu-MOF@RCDs. b) Pie chart of the Cu-MOF@RCD's elemental content. c) High resolution XPS of C 1s. d) High resolution XPS of N1s. e) High resolution XPS of O1s. f) High resolution XPS of Cu2p.

3. The pH and ionic strength stability of Cu-MOF@CD

30 μL Cu-MOF@RCD aqueous solution (100 μg/mL) was added into a 4mL colorimetric dish

and diluted to 4mL with PBS at pH = 5.5, pH = 6.8, pH = 7.4, respectively. Zeta potential of the sample was measured using the Nanobrook Zeta-Pals zeta potential analyzer.

30 μL Cu-MOF@RCD aqueous solution (100 μg/mL) was added into a 4mL colorimetric dish and diluted to 4mL with 0 mM, 0.2 mM, 0.4 mM, 0.6 mM, 0.8 mM, 1.0 mM NaCl solution, respectively. The sample particle size was measured using the Nanobrook Zeta-Pals zeta potential analyzer.

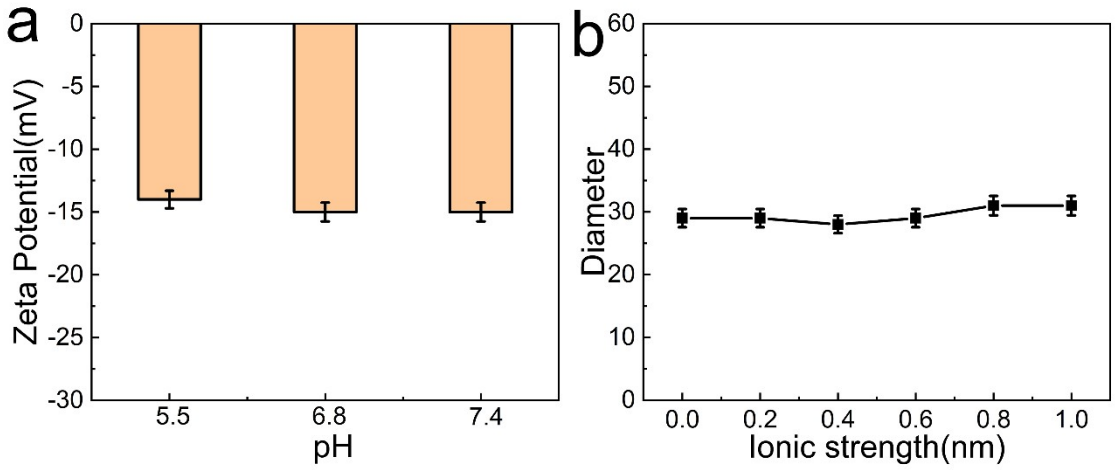


Fig.S3 a) Effect of pH (5.5, 6.8, 7.4) on diameter of Cu-MOF@RCD; b) Effect of ionic strength on diameter of Cu-MOF@RCD

4. Fluorescence emission spectrum of RCD at different excitation wavelengths

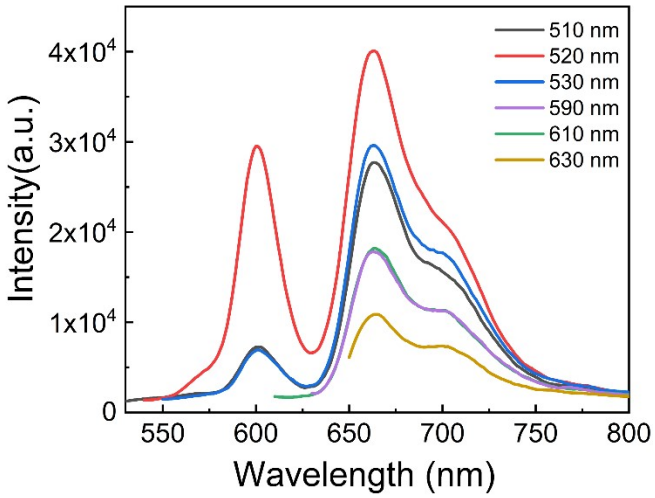


Fig.S4 Fluorescence emission spectrum of RCD at different excitation wavelengths

5. Nitrogen adsorption desorption test for Cu-MOF@RCD

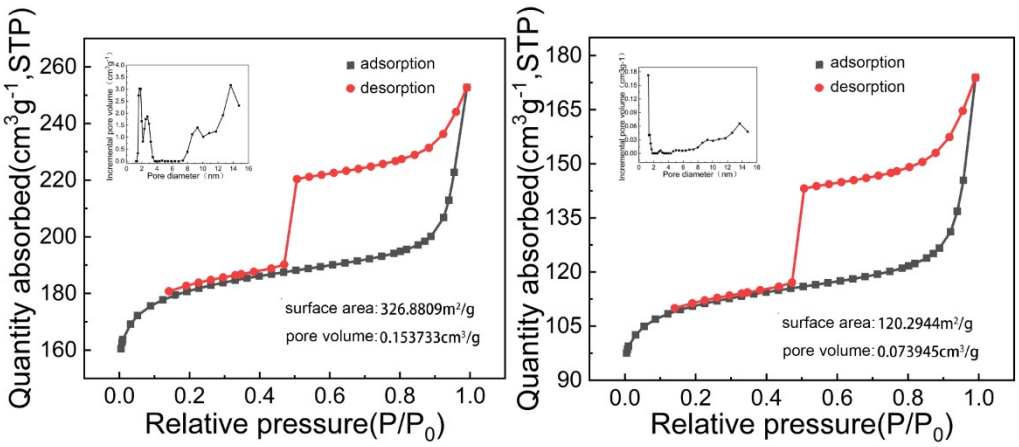


Fig.S5 a) Adsorption-desorption curve of Cu-MOF; b) Adsorption-desorption curve of Cu-MOF@RCD.

6. Cu-MOF@RCD's photothermal conversion efficiency calculation

200 μg/mL of Cu-MOF@RCD dispersion was added to a quartz cuvette and was exposed to NIR light (808 nm, 1.0 W/cm^2) for 10 min and cooled to room temperature, and the temperature was recorded with an IR thermometer to calculate the photothermal conversion efficiency η using equation (1).

$$\eta = \frac{hA(T_{Max} - T_{Surr}) - Q_{Dis}}{I(1 - 10^{-A\lambda})} \quad (1)$$

where h is the heat transfer coefficient and A is the surface area of the vessel; T_{Max} (48 °C) is the sample temperature and T_{Surr} (28 °C) is the ambient temperature; Q_{Dis} denotes the energy absorbed by the vessel (54 mW), and I denotes the laser power (1.0 W); A_λ is the absorption of the Cu-MOF@RCD solution at 808 nm (0.414). hA value can be calculated by equation (2).

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

where τ_s is the sample-system time factor and m_D is the mass of deionized water as a solvent (1 g). C_D is the specific heat capacity of deionized water (4.2 J/g). To calculate τs, the parameter θ for the temperature change versus the maximum temperature change can be obtained from equation (3) below.

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

where ΔT is the instantaneous temperature change, which is the temperature difference between the solution temperature and the ambient temperature. ΔT_{max} is the amount of temperature change at the maximum warming temperature. Therefore, the linear time data of the cooling cycle using ln(θ), τ_s

= 392.19 can be obtained from equation (4).

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

Therefore, the photothermal conversion efficiency is calculated as:$\eta = 32.7\%$.

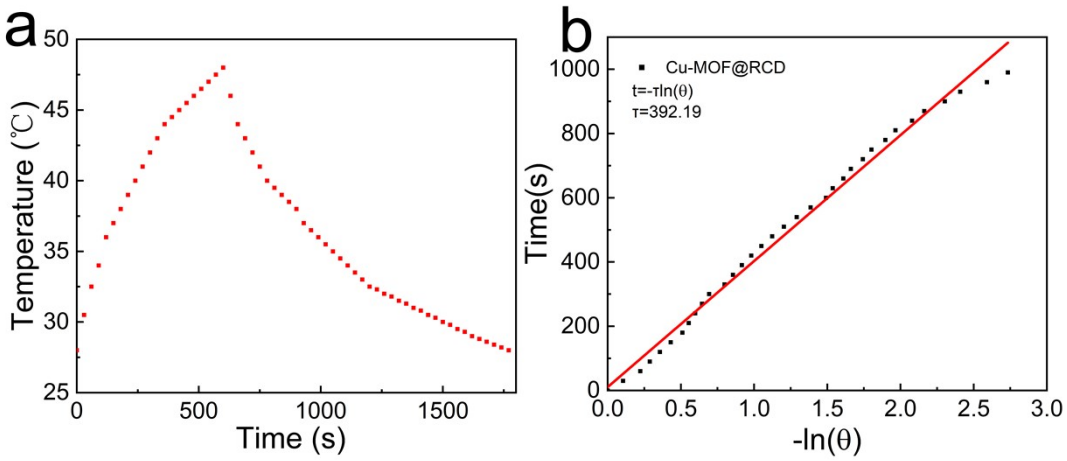


Fig. S6 a) Temperature variation of Cu-MOF@RCD solution (400 μg/mL) under laser irradiation of 808 nm (1.0 W/cm^2) for 10 min then with laser off; b) Plot of cooling time versus cooling cycle ln.

7. Singlet oxygen ($^1O_2$) quantum yield calculation for Cu-MOF@RCD

The $^1O_2$ quantum yield of Cu-MOF@RCD was determined using DPBF (1 mg/mL in DMF) as the $^1O_2$ trapping agent and MB as the reference sample. Firstly, the samples to be tested and the MB solution were prepared separately using DMF as the solvent, and the absorbance at 671 nm was fixed at 0.1 OD for both. The absorbance of the DPBF solution at 415 nm was measured at different times by irradiation with a 671 nm laser. Calculate the decay rate ln (A_0/A_t) with irradiation time and fit a first order linear equation. Calculate the $^1O_2$ quantum yield of the photosensitiser according to equation (5), where Φ_s is the $^1O_2$ quantum yield of Cu-MOF@RCD, t_s is the degradation time of sample, t_{MB} is the degradation time of methylene blue, and Φ_{MB} is the $^1O_2$ quantum yield of methylene blue (49%). According to equation (5) ,$^1O_2$ quantum yield of Cu-MOF@RCD was 32.17%.

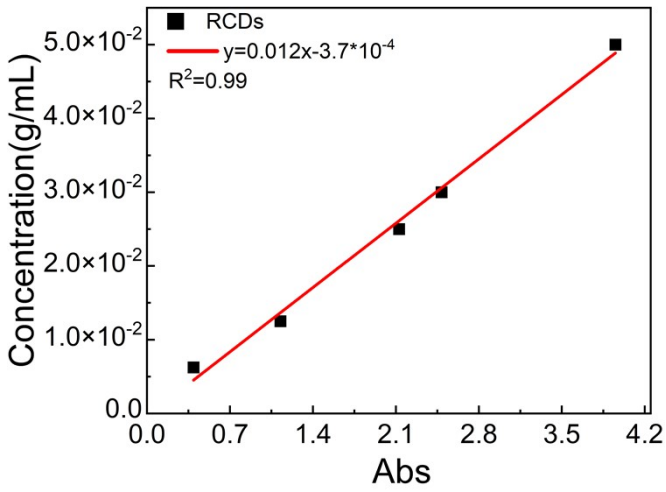
$$\Phi_s = \Phi_{MB}\frac{t_s}{t_{MB}} \quad (5)$$

Table S1 Recorded data of MB and Cu-MOF@RCD $^1O_2$

MB			RCD/CA@MOF		
t_S	A_t	$\ln(A_0/A_t)$	t_S	A_t	$\ln(A_0/A_t)$
0	1.302	0	0	1.295	0

10	1.281	0.03474	10	1.265	0.0234
20	1.244	0.06581	20	1.236	0.0466
30	1.211	0.09715	30	1.210	0.0679
40	1.176	0.14002	40	1.182	0.0913
50	1.135	0.1707	50	1.157	0.113
60	1.101	0.20476	60	1.131	0.135
70	1.066	0.24086	70	1.106	0.158
80	1.036	0.2671	80	1.082	0.180
90	1.003	0.30468	90	1.059	0.201
100	0.953	0.34002	100	1.036	0.223
110	0.920	0.37667	110	1.012	0.247
120	0.882	0.40678	120	0.986	0.273

8. RCD load factor calculation for Cu-MOF@RCD



The absorbance of RCD was measured at different concentrations (50, 30, 25, 12.5, 6.25 μg/mL)

Fig. S7 Standard curves for RCD

and the first order linear equation of absorbance versus concentration was plotted (Figure S7). 1 mg Cu-MOF (in 10 mL EtOH) was added to a 50 mL round bottom flask and 1 mL o-phenylenediamine carbon dots (0.1 mg/mL in EtOH) were added slowly dropwise at room temperature using a constant pressure dropping funnel, stirred for 24 h, centrifuged (10,000 rpm) for 10 min, washed three times with anhydrous ethanol, and combined the supernatants. The absorbance was measured by UV-Vis spectrophotometer, and the average value A was taken and substituted into the equation of the standard curve and repeated three times (Table S2). The amount of unloaded RCD after dilution, m_1, was calculated and substituted into equation (6), and the loading amount of RCD was calculated as 74. 1% by adding the mass of RCD, m. According to equation (7), the loading amount of 6.83% could be

calculated by the total mass of product, M.

$$\text{Load factor：LOAD} = (m - m_1) / m \times 100\% \qquad (6)$$

$$\text{Loading capacity：LOAD}_1 = (m - m_1) / M \times 100\% \qquad (7)$$

Table S2 Measured values of absorbance and load factor of RCD

	A_{RCD}/OD	m/mg	m_1/mg	M/mg	LOAD/%	$LOAD_1$/%
I	2.253	0.1	0.027	1.08	73	6.8
II	2.086	0.101	0.025	1.1	74.3	6.8
III	2.086	0.1	0.025	1.09	75	6.9
Average	2.141	0.1	0.026	1.09	74. 1	6.83

9. Intracellular GSH depletion in L929 cells of Cu-MOF@RCD

Logarithmically grown L929 cell lines were inoculated into 6-well culture plates and incubated at 37 ℃ in an incubator containing 5% CO_2 for 24 h. The medium was discarded, the cells were washed with PBS, treated with Cu-MOF@RCD solution and incubated for 24 h. The cells were treated with the Glutathione kit and their absorbance was measured on an enzyme marker and the GSH content was calculated according to the formula below.

$$W\% = (A_{Test}-A_{Blank})/A_{Control}-A_{Blank}) \qquad (8)$$

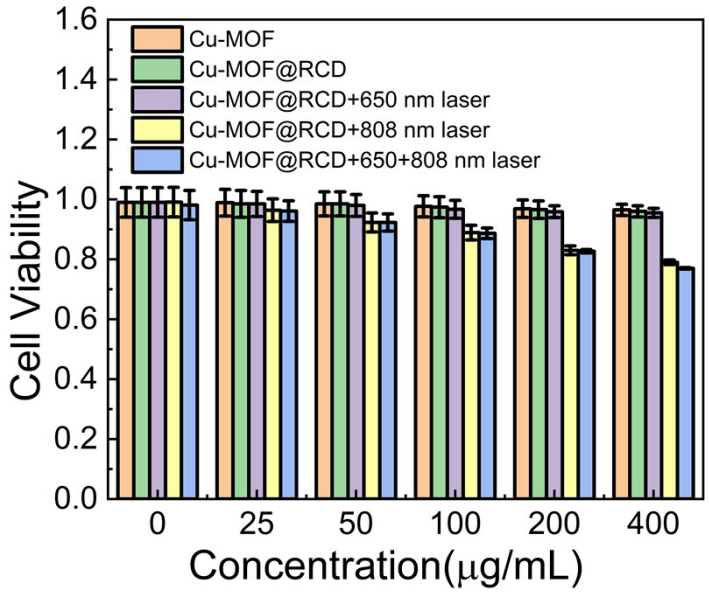


Fig.S8 GSH depletion in L929 cells