Electronic Supplementary Material (ESI) for Biomaterials Science.

Supporting Information

Theranostic Nanoagent of Mo_2C for Multi-modal Imaging-Guided Cancer Synergistic Phototherapy

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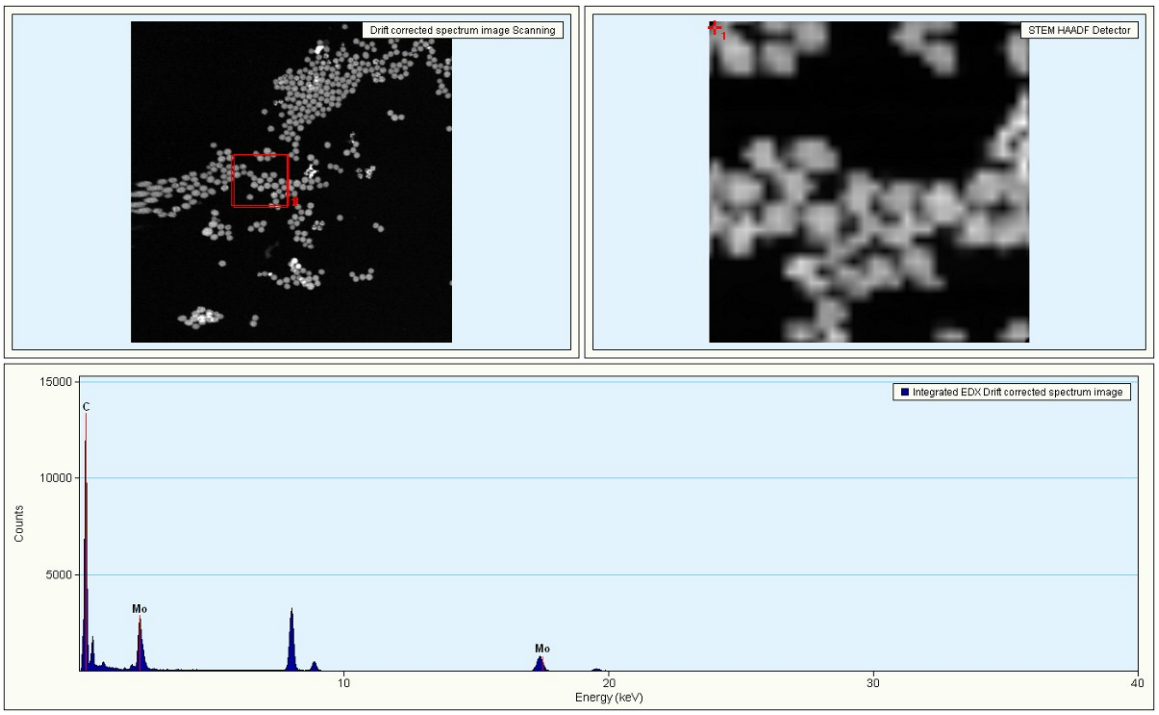

Figure S1. STEM image and EDX spectra of Mo_2C.

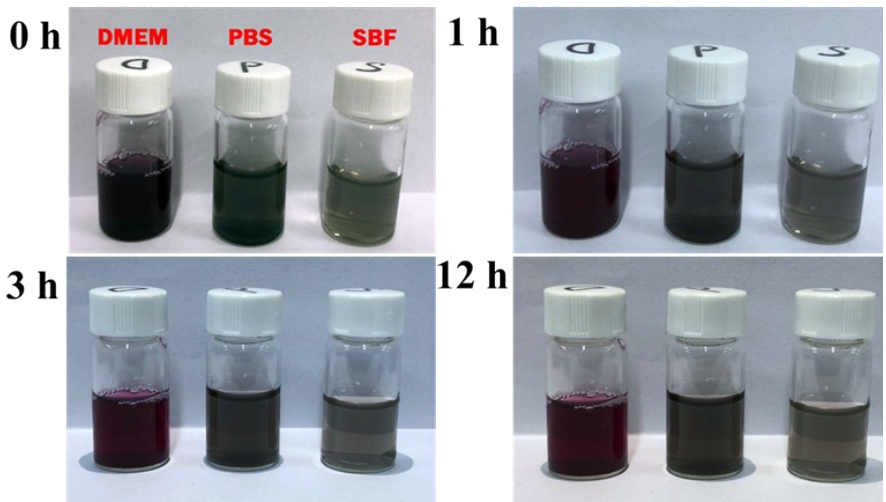

Figure S2. Photographs of Mo_2C dispersed in PBS, Dulbecco's Modified Eagle Medium (DMEM) and simulated body fluid (SBF).

Calculation of photothermal conversion efficiency.

$$\eta = \frac{hA(T_{max} - T_{surr}) - Q_{dis}}{I(1 - 10^{-A_{\lambda}})} \qquad \textbf{Equation S1}$$

The photothermal conversion efficiency could be determined by **Equation S1**, in which *h* is the heat transfer coefficient, S is the surface area of the container, T_{max} and T_{Surr} are the equilibrium temperature and ambient temperature of the surroundings, respectively. Q_{dis} (11.3 mW) is heat dissipated from light absorbed by the quartz sample cell itself, I is the incident laser power (2.0 W cm^{-2}) and A (=0.456) means the optical absorbance at 1064 nm.

The hS could be determined by the **Equation S2**. m, C_p are the mass (0.3 g), heat capacity of water (4.2 $J \cdot g^{-1}$), respectively.

$$hS = \frac{\sum mDCD}{\tau s} \qquad \textbf{Equation S2}$$

To obtain the value of τ_s, we introduced a dimensionless driving force temperature (θ).

$$\theta = \frac{T - T_{surr}}{T_{max} - T_{surr}} \qquad \textbf{Equation S3}$$

$$t = -\tau s \ln(\theta) \qquad \textbf{Equation S4}$$

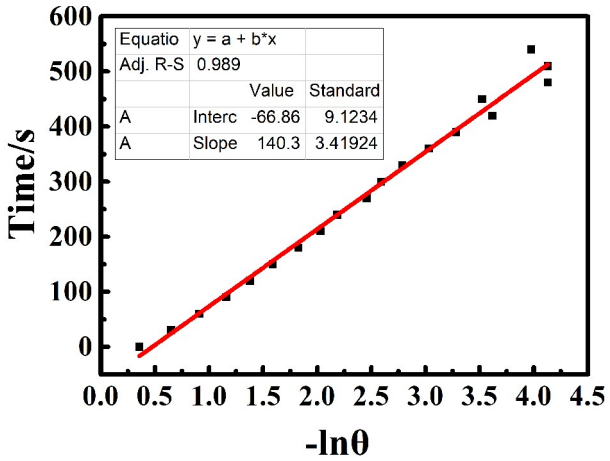

Figure S3. The curve of time vesus –ln (θ)

According to the **Figure S3**, the τ_s is determined to be 140 s and then the hs is obtained as 9 $mW \cdot {}^{o}C^{-1}$. Finally, the photothermal conversion efficiency η is 24.9 %.

Table S1. The DFT calculated atomic Mulliken populations.

Atom Species	s	p	d	Net Charge (e)
C1	1.43	3.18	0	-0.61
C2	1.43	3.18	0	-0.61
C3	1.43	3.18	0	-0.61
C4	1.43	3.18	0	-0.62
C5	1.43	3.18	0	-0.61
C6	1.43	3.18	0	-0.61
C7	1.43	3.18	0	-0.61
C8	1.43	3.18	0	-0.61
Mo1	2.26	6.42	5.02	0.29
Mo2	2.26	6.43	5.02	0.29
Mo3	2.26	6.43	5.03	0.29
Mo4	2.26	6.42	5.02	0.29
Mo5	2.26	6.42	5.02	0.29
Mo6	2.26	6.43	5.02	0.29
Mo7	2.26	6.43	5.03	0.28
Mo8	2.26	6.42	5.02	0.3
Mo9	2.26	6.42	4.99	0.33
Mo10	2.26	6.42	5.00	0.32
Mo11	2.26	6.42	5.00	0.32
Mo12	2.26	6.42	5.00	0.32
Mo13	2.26	6.42	4.99	0.33
Mo14	2.26	6.42	5.00	0.32
Mo15	2.26	6.43	5.00	0.32
Mo16	2.26	6.42	4.99	0.33

Calculation of singlet oxygen quantum yield.

$^1O_2$ generation of Mo_2C was quantified by using 1,3-diphenylisobenzofuran (DPBF) as the $^1O_2$ trapping agent and ICG as the reference ($^1O_2$ quantum yield Φ_{ICG} = 0.0020, at 808 nm). The singlet oxygen quantum yield at 808 nm can be determined by the following formula:

$$\Phi\Delta_{808nm} = \Phi^{Std}_{\Delta\ 808\ nm} \frac{k_{808nm} \cdot I^{Std}_{abs}}{k^{Std} \cdot I_{abs\ 808nm}} \qquad \textbf{Equation S5}$$

Where Φ^{Std}_{Δ} is the singlet oxygen quantum yield for the standard ICG. k_{808nm} and k^{Std} are the DPBF photobleaching rates in the presence of Mo_2C and standard ICG at 808 nm, respectively. $I_{abs\ 808\ nm}$ and I^{Std}_{abs} are the rates of light absorption by the Mo_2C and standard ICG at 808 nm, respectively.

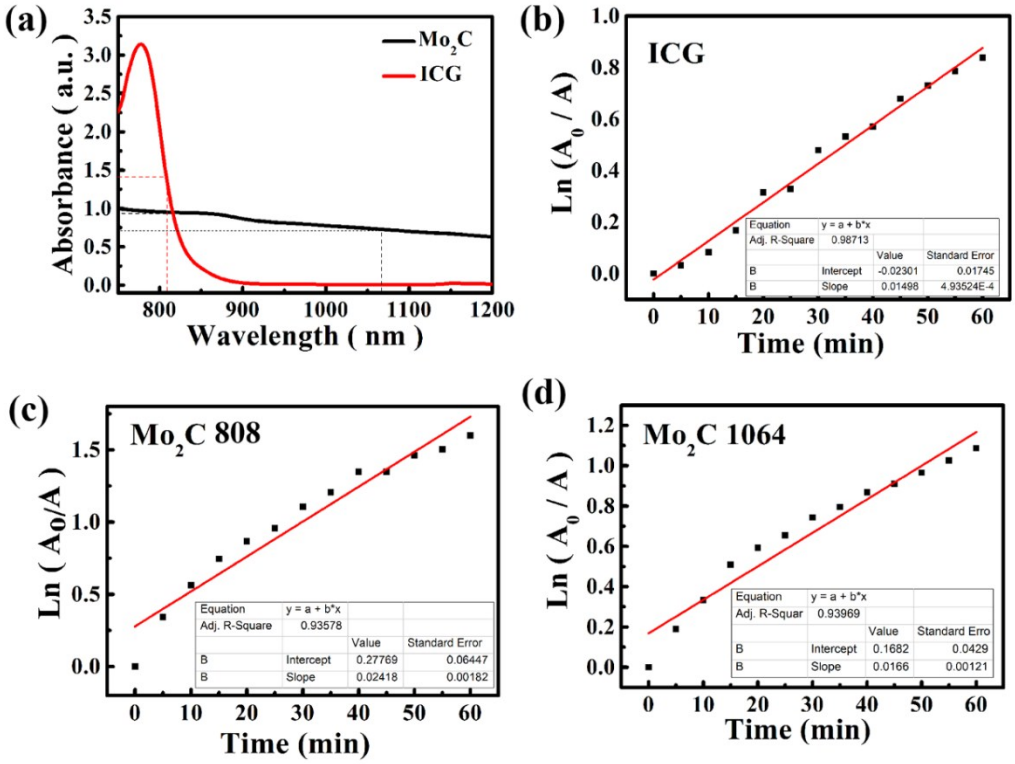

Figure. S4 (a) UV-Vis-NIR absorption spectra of Mo_2C and ICG. (b) Rate constants for DPBF decomposition by stardard ICG at 808 nm. (c)-(d)) Rate constants for DPBF decomposition by Mo_2C at 808 and 106 nm.

By this way, we firstly obtained the Φ_{808}=0.0049. Then, the singlet oxygen quantum yield at 1064 nm was finally determined by below formula, which is Φ_{1064}=0.0044.

$$\Phi\Delta_{1064nm} = \Phi_{\Delta 808\ nm} \frac{k_{1064nm} \cdot I_{abs\ 808nm}}{k_{808nm} \cdot I_{abs\ 1064nm}} \qquad \textbf{Equation S6}$$

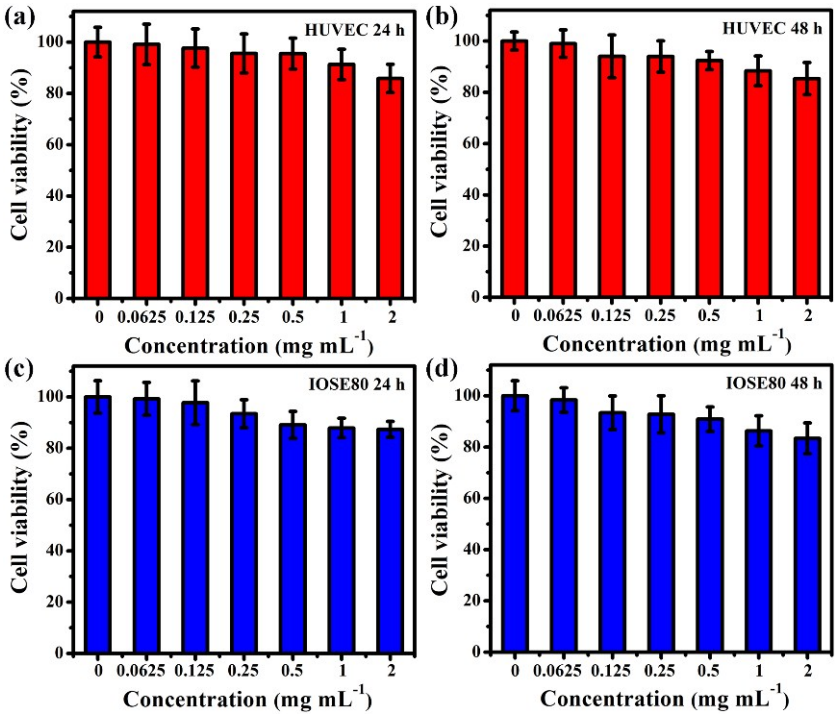

Fig. S5 *In vitro* relative cell viability incubated with various concentrations of Mo_2C dispersion for normal cell lines. (a) HUVEC for 24 h, (b) HUVEC for 48 h, (c) IOSE80 for 24 h and (d) IOSE80 for 48 h.

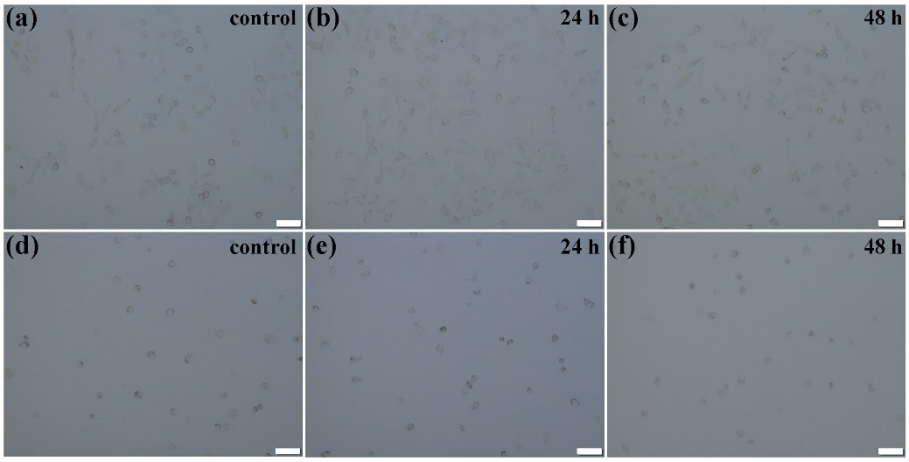

Fig. S6 Cell bright field images before and after treatment with 250 μg mL^{-1} Mo_2C dispersion. (a-c) HUVEC, (d-f) IOSE80 (scale bar = 100 μm).

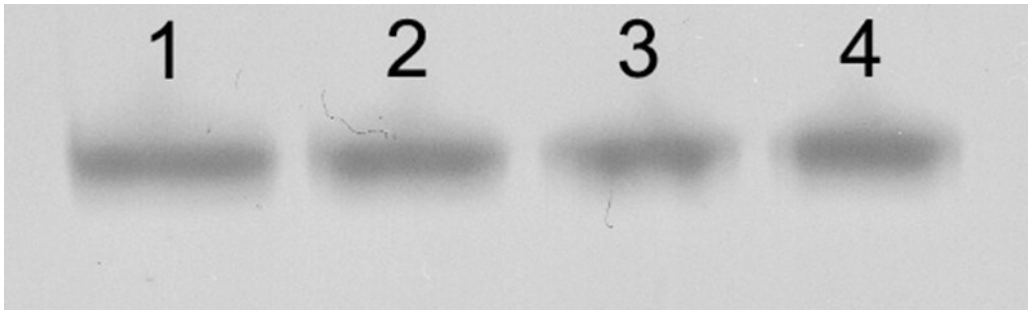

Figure S7 Native PAGE gel characterization of single strand DNA incubated with Mo_2C nanoagent of different concentrations for 48 hours. Lane 1: Control DNA; Lane 2: DNA incubated with Mo_2C of 0.25 $mg \cdot mL^{-1}$; Lane 3: DNA incubated with Mo_2C of 0.5 $mg \cdot mL^{-1}$; Lane 4: DNA incubated with Mo_2C of $mg \cdot mL^{-1}$.

Experimental process of PAGE

(Single-stranded DNA was purchased from Suzhou Synbio Tecnologies, China, and diluted in MilliQ water. Same amount of DNA (5 μM) was incubated with Mo_2C nanoagent of different concentrations at 37 ^oC for 48 hours. The product obtained was characterized by native PAGE (15%) in TBE buffer at a constant voltage of 150 V at 4 °C for 2 h, and then stained for analysis. For control experiment, MilliQ water was added to the DNA to achieve the same volume. Single strand DNA was employed to evaluate the toxicity of Mo_2C nanoagent. Same amount of DNA was incubated with Mo_2C nanoagent of different concentrations for 48 hours and then characterized with native PAGE.

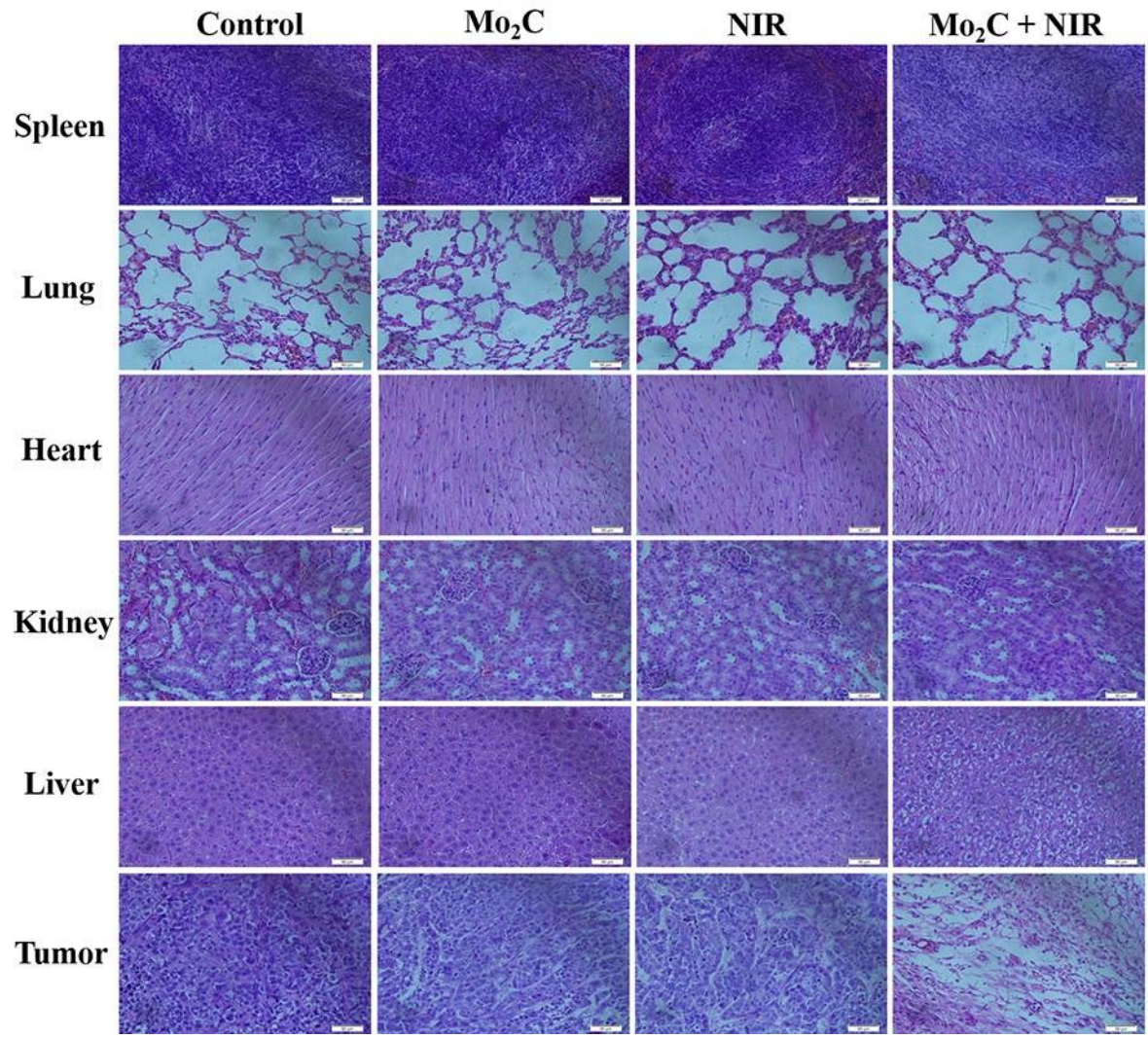

Figure S8. H&E staining results of tumors and major organs from the mice after different treatments.

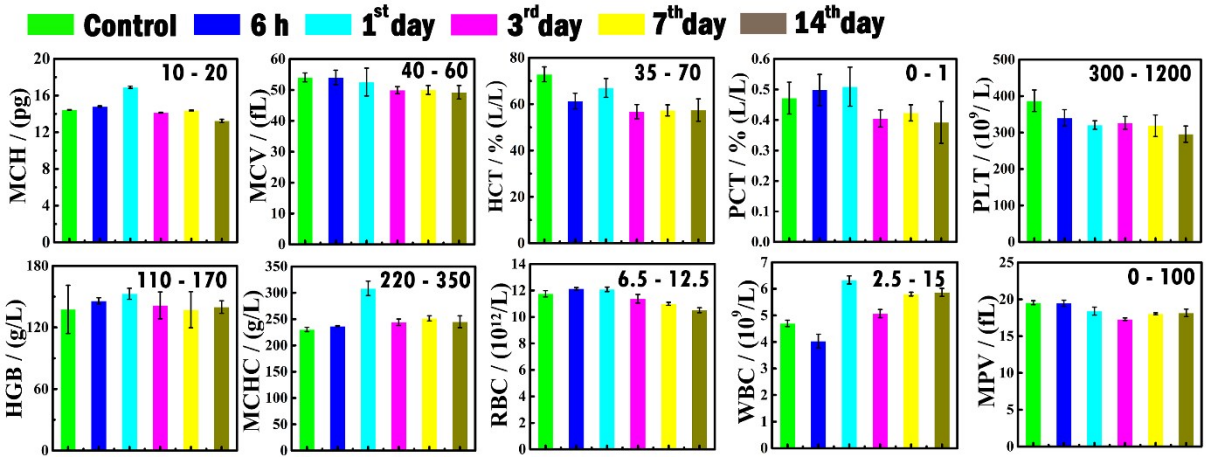

Figure S9. Hematological data were collected from the control nude mice or mice injected with Mo_2C (1 mg mL^{-1}) at 1, 3, 7 and 14 days after injection. (The inset of figure presents the normal range of blood indicators).

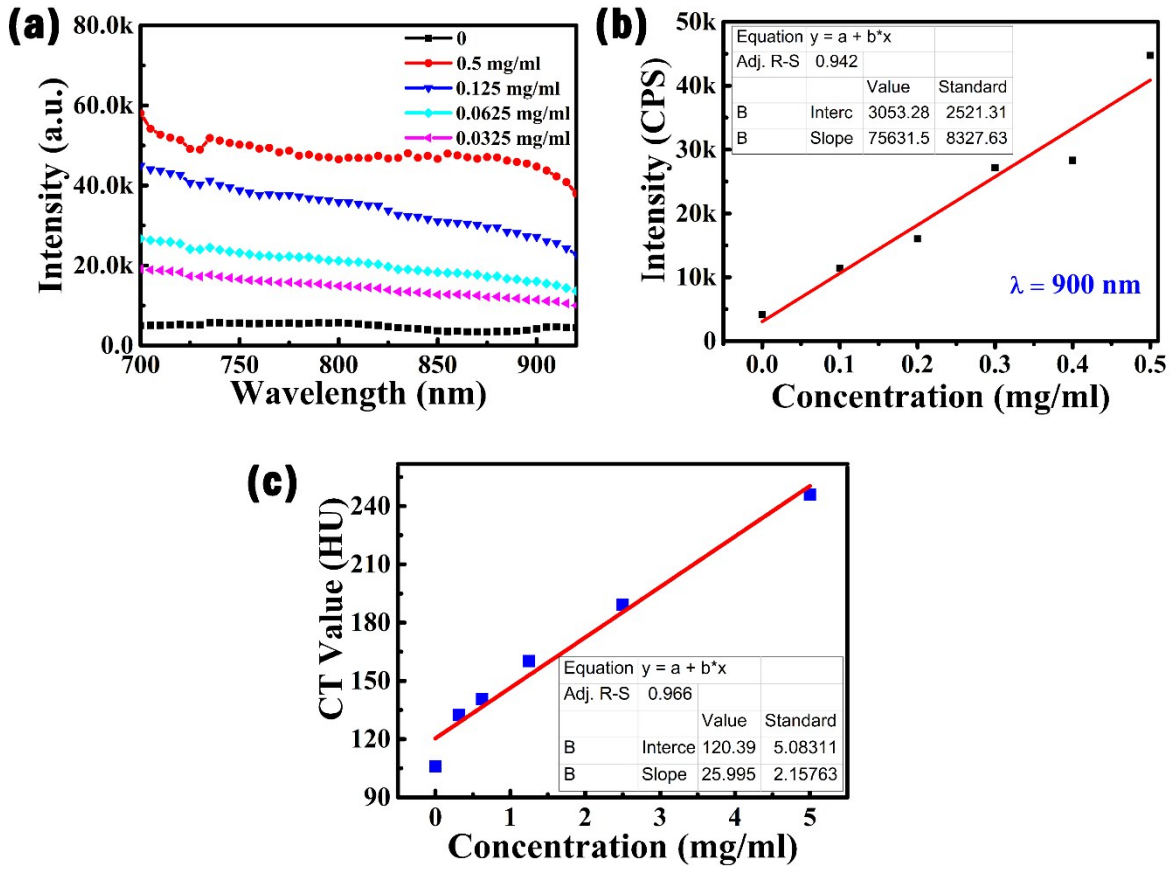

Figure S10. (a) *In vitro* PA signal intensity of Mo_2C dispersion of different concentration. (b) *In vitro* PA signal intensity at 900 nm versus Mo_2C concentration. (c) *in vitro* CT HU value versus Mo_2C concentration.

***In vitro* simulation of Mo_2C biodegradation**

The Mo_2C was dispersed into the PBS, DMEM and simulated body fluid (SBF) to form a 30 mg/L dispersion, respectively. Then, the resulting Mo_2C dispersions were kept in 37 ^oC oven. At different time intervals, the Mo_2C dispersions were centrifuged and the concentration of Mo ions in the supernatant was tested by ICP-MS measurement. The releasing rate of Mo ion from Mo_2C was used to evaluate the degradation performance, which is calculated as (Mass of Mo ions)/(Molybdenum content input)"

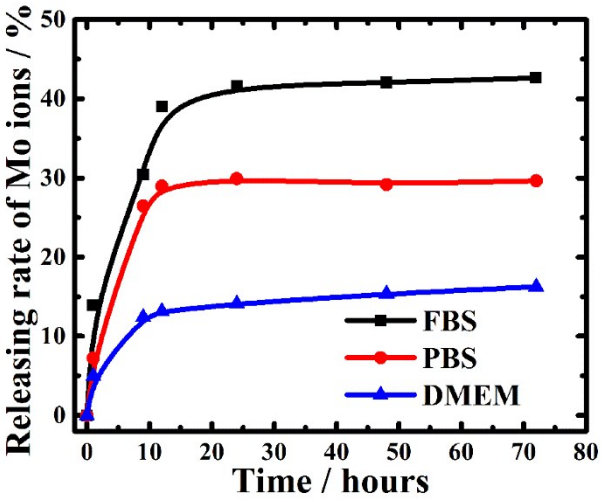

Figure S11 The releasing of Mo ions from the Mo_2C at different medium of PBS, DMEM and SBF.

Biodistribution studies.

The mice were intravenously injected with Mo_2C solution (1 mg·mL^{-1}, 100 μl). At different time internals post injection, the mice were sacrificed for collecting the organs and blood (n = 5 for each trial). For the *in vivo* excretion study, the feces and urinary of mice were collected every day. The obtained organs, blood, feces and urinary were boiled in HNO_3 (68 wt%) until the solution became clear. Then, the ICP-MS measurement was performed to quantify the content of Mo element. The blood half-life of Mo_2C is determined by pseudo-first order reaction kinetics ($t_{1/2}$=ln2/k, k is elimination rate constant and determined as slope in the Figure S6d)

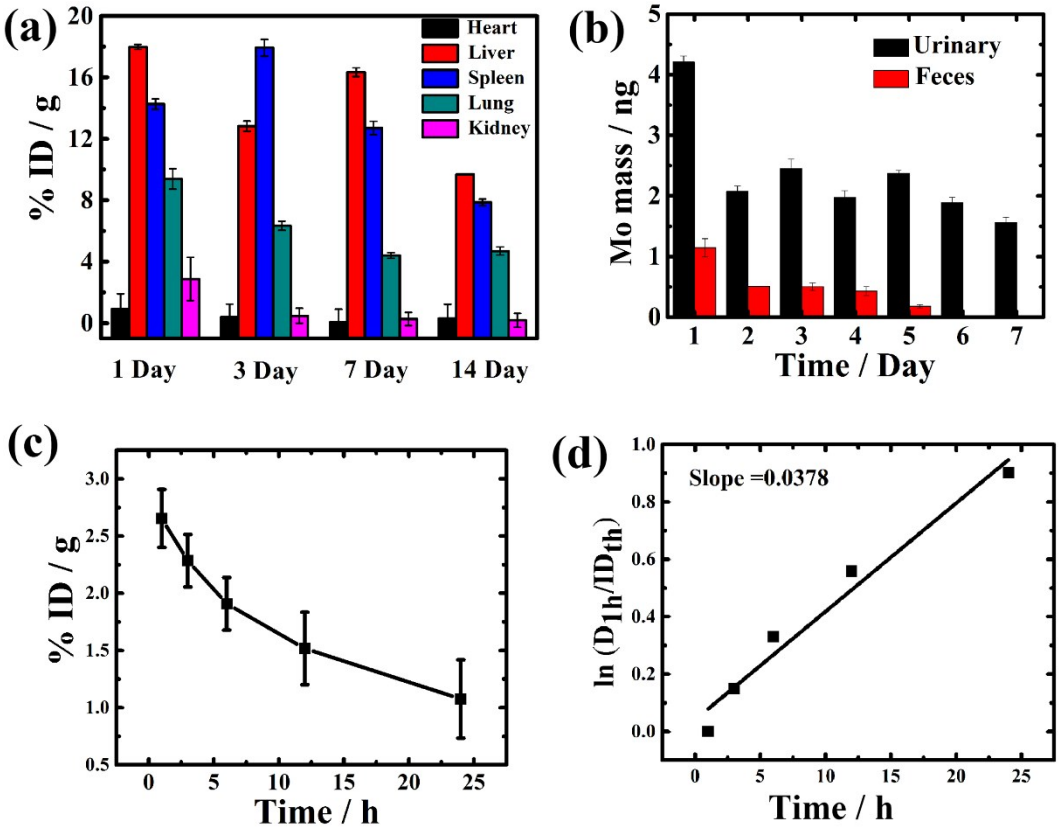

Figure S12 (a) Biodistribution of Mo_2C in major organs, (b) Mo mass in feces and urine, and (c) Mo content in the blood after intravenous injection of Mo_2C. The curve of ln (%ID_{1h}/ID_{th}) versus time post injection of Mo_2C for determining the blood-circulation half-life time.