Supporting information

Facile Construction of Highly Luminescent and Biocompatible Gold Nanoclusters by Shell Rigidification for Two-photon pH-Edited Cytoplasmic and *In Vivo* Imaging

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Reagent and materials

All chemicals are analytical grade, Tetrachloroauric acid trihydrate ($HAuCl_4 \cdot 3H_2O$), Sodium hydroxide, 6-aza-2 thiothymine (ATT) was purchased from Aladdin Reagent Co. Ltd. (Shanghai, China). PVP was purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai, China). Rhodamine B was bought from Solarbio Science & Technology Co., Ltd. (Beijing, China). Deionized water (18.2 MΩ cm^{-1}) was used during all experiment steps.

Characterization

TEM was conducted by Talos F200X G2. Fluorescence excitation and emission spectra were recorded by SF2000 (Shimadzu, Japan). UV-vis spectra were recorded by UV-1800 spectrophotometer (Shimadzu, Japan), DLS (dynamic light scattering) measurements were conducted by nanoparticle size analyzer (OMNI, USA), and XPS (X-ray photoelectron spectroscopy) spectra were obtained by using AXIS Ultra DLD (Shimadzu, Japan). Fluorescence Lifetime measurement was excited by 470 nm picosecond diode laser (PicoQuant, Germany) and detected with SPC 150 TCSPC module (Becker & Hickl GmbH, Germany).

Synthesis of ATT-AuNCs, Arg-AuNCs and GSH-AuNCs

ATT-AuNCs, Arg-AuNCs and GSH-AuNCs were synthesized as previously described [1, 2]. ATT (0.33 mL, 80 mM) containing 0.2 M NaOH was added to a $HAuCl_4$ solution (1 mL, 10 mM), and the mixed solution was continuously stirred in the dark at room temperature for 1 hour. The synthesized ATT-AuNCs were purified by ultrafiltration (Millipore, 50 kDa) under 6000 rpm for 10 minutes. The Arg-AuNCs were obtained by addition of 0.2 mL L-arginine (40 mM) to 0.9 mL synthesized ATT-AuNCs solution. The synthesis of GSH-AuNCs is that $HAuCl_4$ (20 mM, 0.50 mL) and GSH (100 mM, 0.15 mL) were mixed with 4.35 mL of ultrapure water at 25 °C. The reaction mixture was heated to 70 °C under gentle stirring (500 rpm) for 24 hours.

MTT assay

MTT test was conducted to determine cell viability under different PVP-AuNCs concentrations. Human hepatocellular carcinomas (HepG2) cells were plated in 96-well plate (200 μL per well) at a density of about 6000 cells per well and cultured in DMEM culture medium for 12 hours. Then the culture medium was replaced by 200 μL medium containing different concentrations of PVP-AuNCs (0, 20, 40, 60, 80, 100, 120, 140 μg/mL). After incubation for 24 hours, 10 μL of MTT solution (5 mg/mL) was added to every well and then was incubated for 4 hours. Then the mixture was removed and 150 μL DMSO was added to dissolve formazan.

Quantum yield

The QY of the nanoclusters was calculated by the following equation.

$$QY_s = QY_f * \frac{F_s}{F_f} * \frac{A_f}{A_s} * \frac{\eta_s^2}{\eta_f^2}$$

In this equation, F are the integrated areas of fluorescence intensity between 450 nm and 650 nm, A are the absorbance at 470 nm, η are the solvent refractive index. The subscript "s" and "f" are "sample" and "reference" respectively. And the η_s is 1.33 (water) and the η_f is 1.36 (ethanol). The standard reference we chose is Rhodamine B dissolved in ethanol, and QY is about 71%.

Two-photon absorption cross-section calculation

The two-photon absorption cross-section (TPACS) of the nanoclusters was calculated by the following equation.

$$\sigma_s = \frac{F_s}{F_f}\left[\frac{\phi_f C_f \eta_f}{\phi_s C_s \eta_s}\right]\sigma_f$$

where F_s and F_f are the two-photon fluorescence signals of PVP-AuNCs and the reference dyes Rhodamine B respectively. ϕ_s is the quantum yield of PVP-AuNCs. ϕ_f is the quantum yield of Rhodamine B in methanol (0.55). C_s is the concentration of PVP-AuNCs, C_f is the concentration of Rhodamine B. η_s is the refractive index of the water. η_f is the refractive index of methanol. σ_2 is the known TPA cross section of the reference dye Rhodamine B.

Fluorescence Lifetime measurement

We use the double-exponential fitting function.

$$I(t) = I_0 + A_1 e^{\frac{-t}{\tau_1}} + A_2 e^{\frac{-t}{\tau_2}}$$

$$\tau_{avg} = \frac{A_1 {\tau_1}^2 + A_2 {\tau_2}^2}{A_1 \tau_1 + A_2 \tau_2}$$

Table. S1 The parameter of fluorescence lifetime measurement

	Value		Value		Value
			pH=4.5		
τ_1	4.35	A_1	39.52	τ_{avg}	48.38
τ_2	50.34	A_2	76.77		
			pH=7		
τ_1	4.35	A_1	61.07	τ_{avg}	40.67
τ_2	42.36	A_2	134.76		
			pH=9		
τ_1	4.86	A_1	61.54	τ_{avg}	30.89
τ_2	34.23	A_2	68.13		

QYs and spectral properties for the AuNCs that this article mentioned

Table.S2 Optical properties of ATT-AuNCs, Arg-AuNCs and GSH-AuNCs and PVP-AuNCs

	QY	λ_{ex}	λ_{em}	Ref.
ATT-AuNCs	1.8%	400 nm	523 nm	1
Arg-AuNCs	65%	400 nm	523 nm	1
GSH-AuNCs	15%	365 nm	610 nm	2
PVP-AuNCs	39%	474 nm	505 nm	This article

Supplementary figures

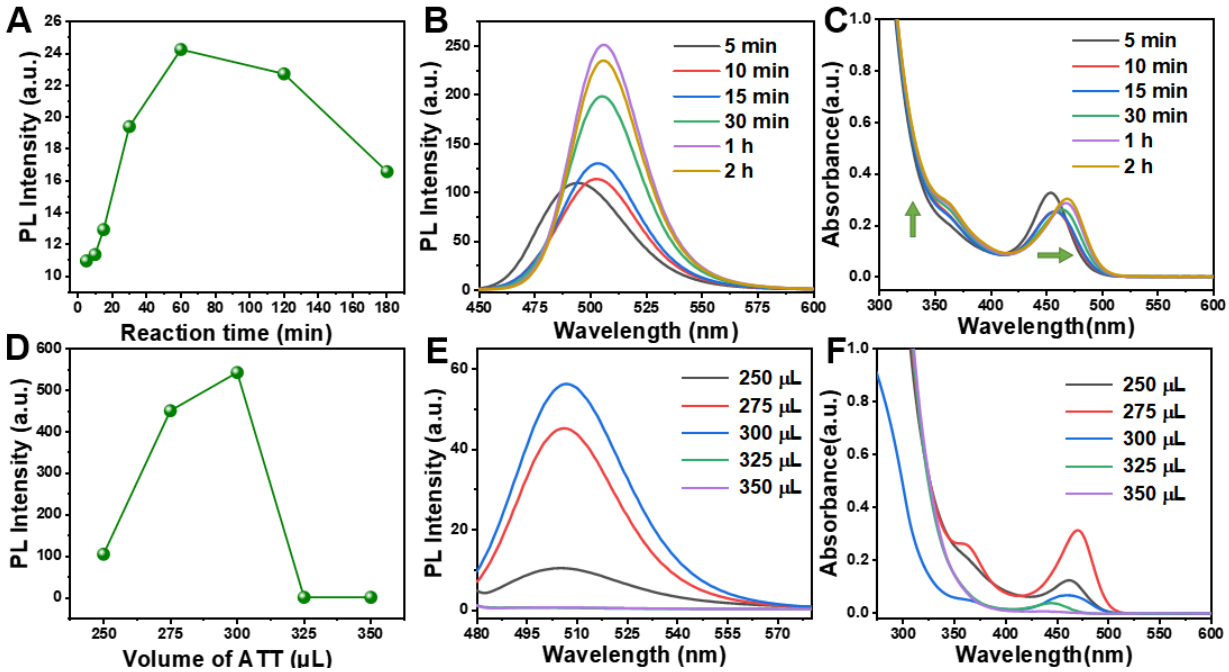

Fig. S1 The influence of different reaction time on PL intensity (A), PL spectra (B), UV absorption spectra (C); The influence of different volume of ATT on PL intensity (D), PL spectra (E), UV absorption spectra (F).

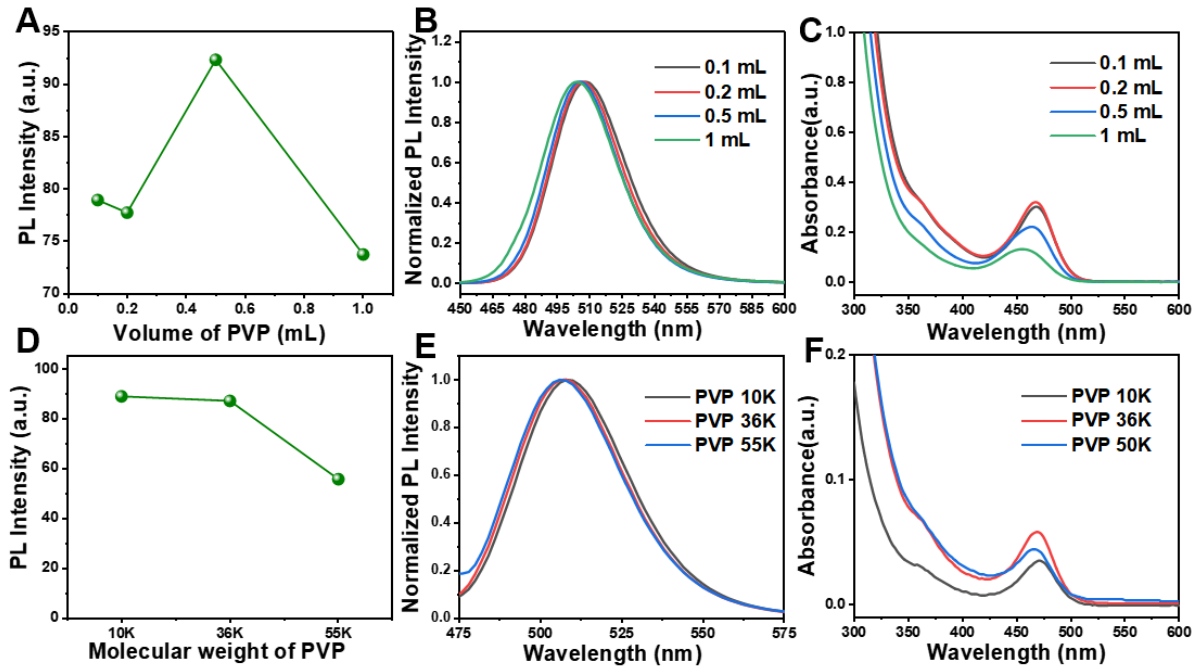

Fig. S2 The influence of different volume of PVP (10 k) on PL intensity (A), normalized PL spectra (B), UV absorption spectra (C). The influence of different molecular weight of PVP on PL intensity (D), normalized PL spectra (E), UV absorption spectra (F).

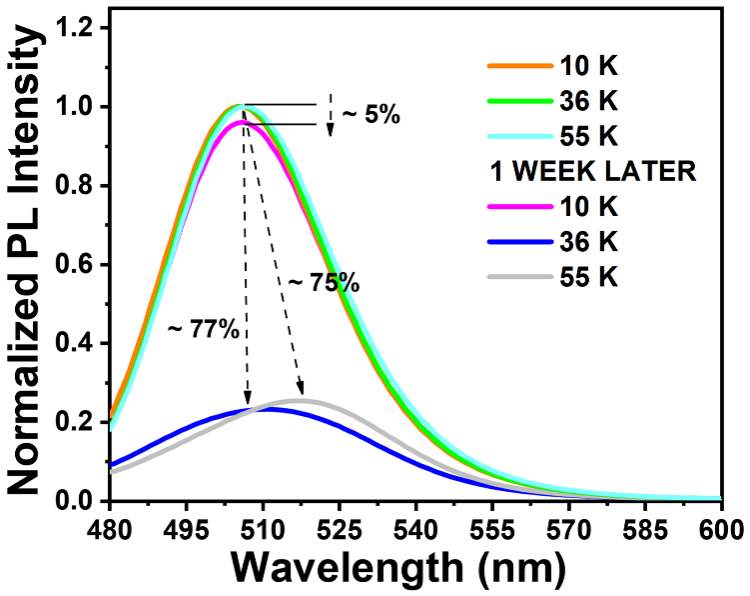

Fig. S3 The stability of gold nanoclusters with different PVP molecular amount.

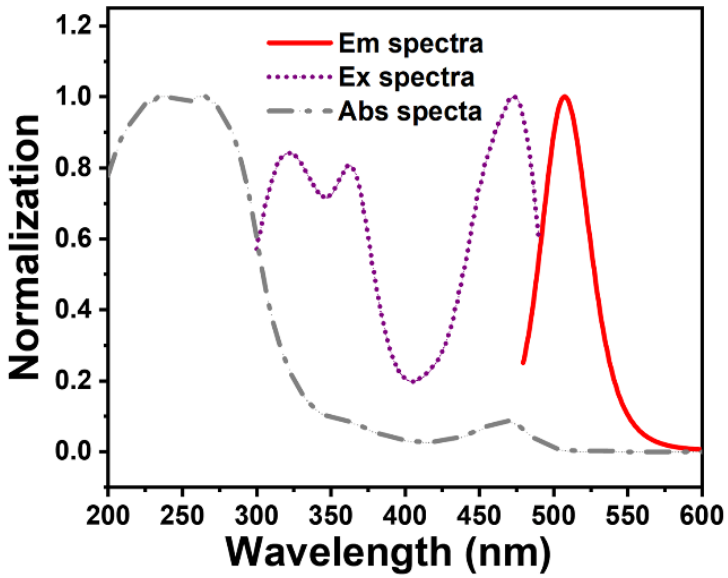

Fig. S4 UV absorption (black curve), PL excitation (purple curve) and emission spectra (red curve) of PVP-AuNCs.

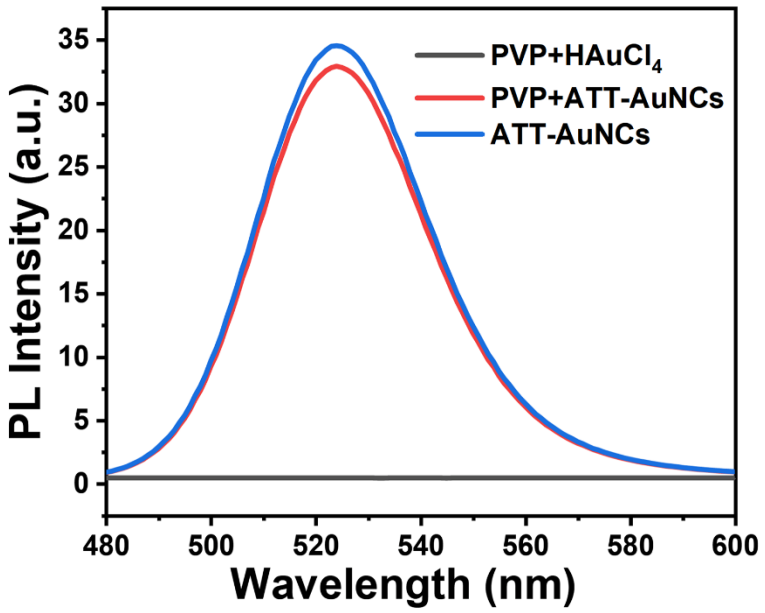

Fig. S5 The PL emission spectra of PVP+AuNCs, PVP+$HAuCl_4$ and ATT-AuNCs.

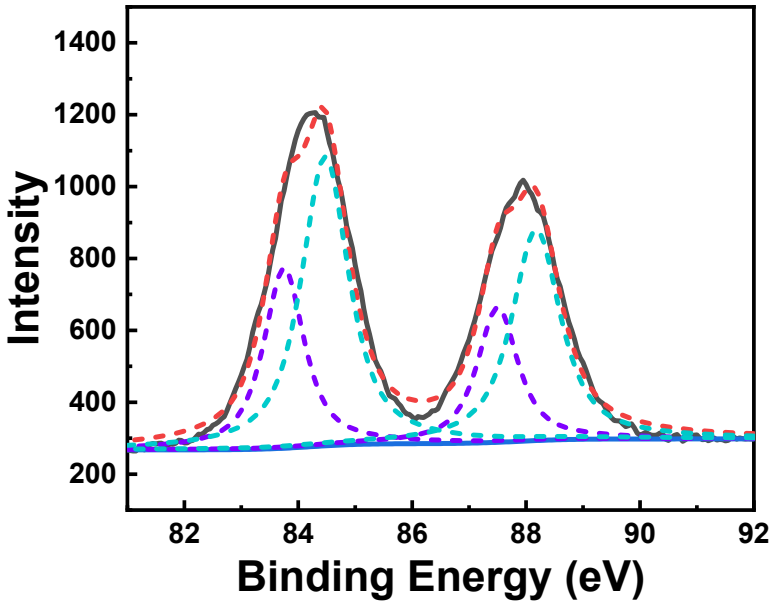

Fig. S6 The XPS spectra of Au in PVP-AuNCs.

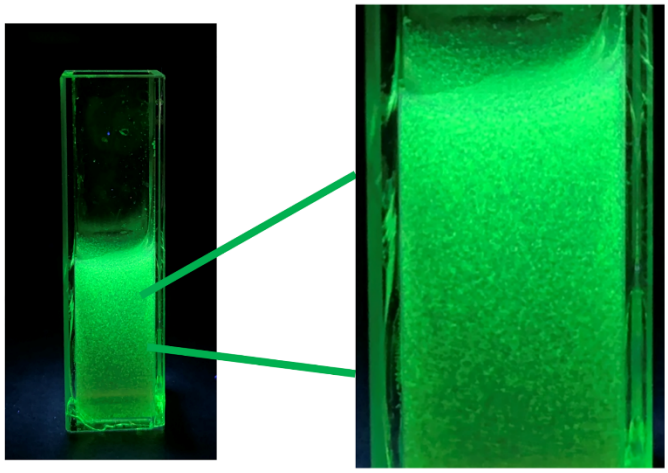

Fig. S7 The fluorescence imaging of ATT-AuNCs incubated in DMEM culture medium after 1 day.

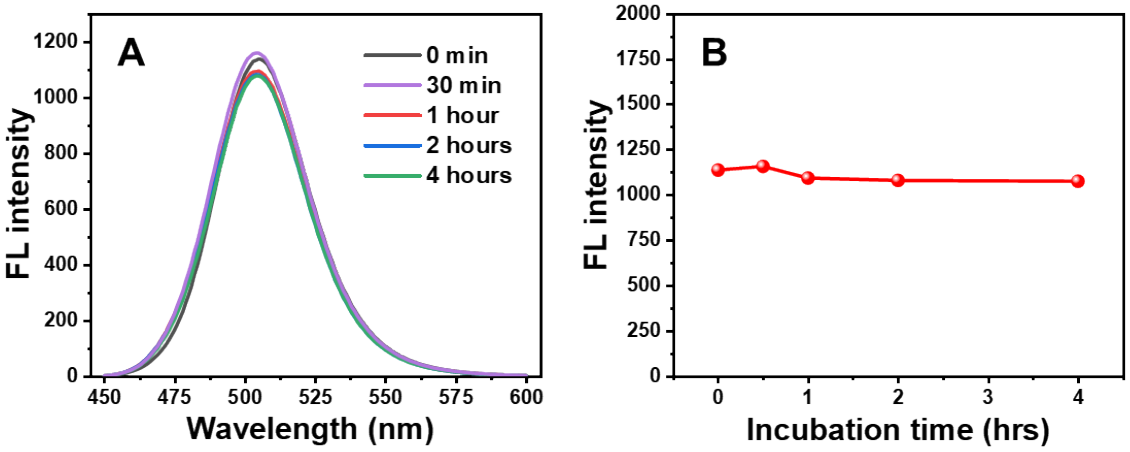

Fig. S8 (A) The fluorescence spectra of PVP-AuNCs after different incubation time in ATP; (B) the trend of fluorescence intensity after different incubation time in ATP.

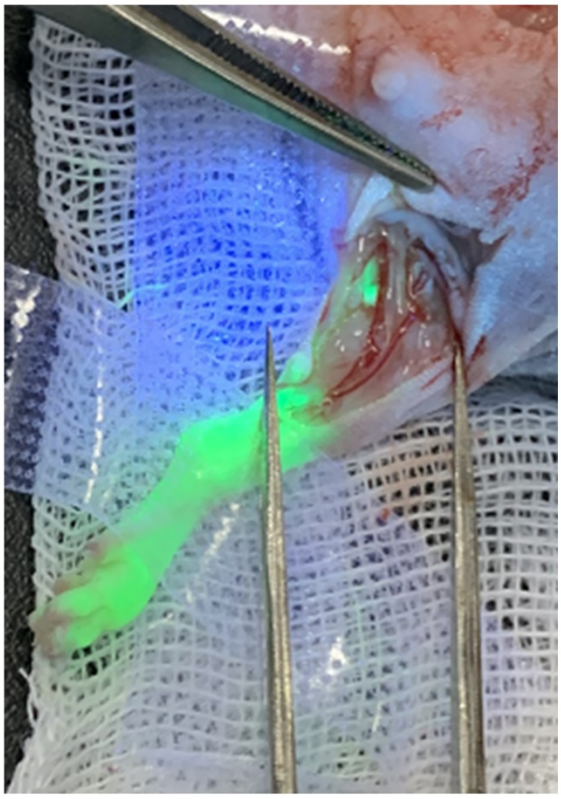

Fig. S9 The fluorescence image of the SLN in the mouse leg under UV lamp.

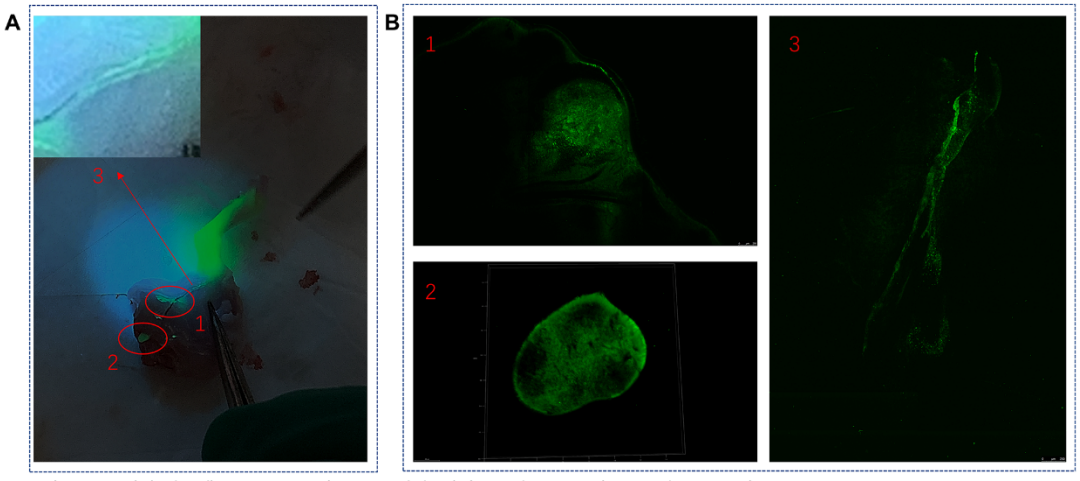

Fig. S10 (A)The fluorescence image of the injected mouse leg under a UV lamp. (B) Two-photon fluorescence imaging of SLN (1), SCLN (2), Lymphatic vessels (3).

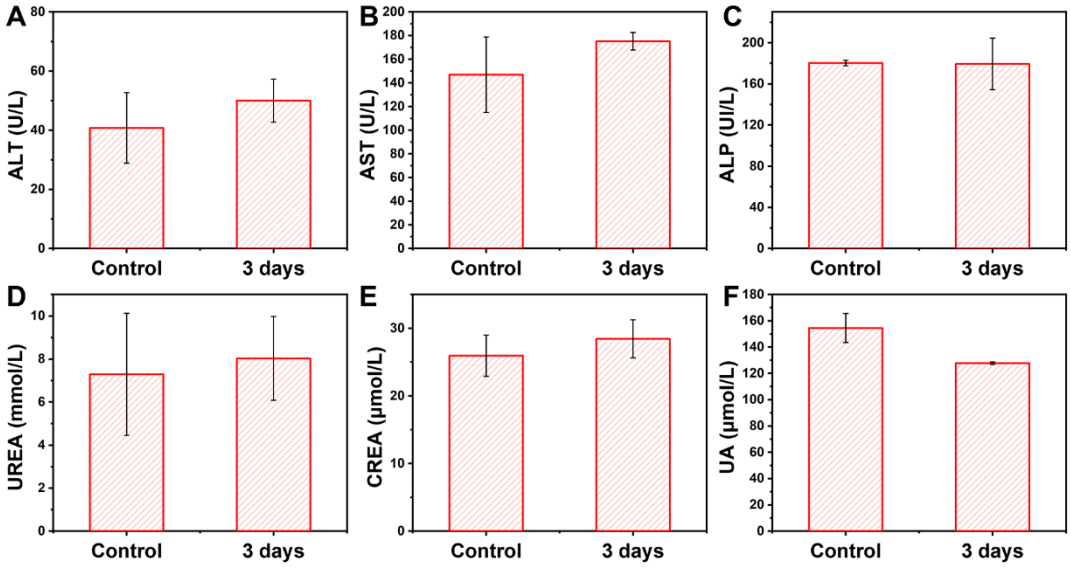

Fig. S11 Blood biochemistry assays of (A-C) liver function markers: ALT, AST, and ALP, (D-F) kidney function markers: UREA, CRE and UA.

References:

1 H.-H. Deng, X.-Q. Shi, F.-F. Wang, H.-P. Peng, A.-L. Liu, X.-H. Xia and W. Chen, Chem. Mater., *2017*, **29**, 1362–1369.

2 Z. Luo, X. Yuan, Y. Yu, Q. Zhang, D. T. Leong, J. Y. Lee and J. Xie, J. Am. Chem. Soc., *2012*, **134**, 16662–16670.