“Nano-lymphatic” of Photocatalytic Water-splitting for Relieving Tumor Interstitial Fluid Pressure and Achieving Hydrodynamics Therapy

Cong Cong, #a Cheng Rao, #b Zhenhe Ma, c Menghan Yu, c Yaqian He, a Yuchu He, a
Zining Hao, a Chunhui Li, a Hongming Lou, *b Dawei Gao* a

a State Key Laboratory of Metastable Materials Science and Technology, Applying Chemistry Key Lab of Hebei Province, Yanshan University, Qinhuangdao 066004, P. R. China.

b School of Chemistry and Chemical Engineering, Guangdong Provincial Engineering Research Center for Green Fine Chemicals, South China University of Technology, Guangzhou 510641, P. R. China.

c School of Control Engineering, Northeastern University at Qinhuangdao, Qinhuangdao 066004, China.

*Corresponding author: Prof. Dawei Gao, Tel: (+86)13930338376; Prof. Hongming Lou, Tel: (+86) 13620418395.

E-mail: dwgao@ysu.edu.cn; cehmLou@scut.edu.cn.

# These authors contributed equally to the work.
METHODS

Materials. Ferric chloride, Lactic acid, Luminol and doxorubicin HCl (DOX) were purchased from Aladdin Industrial Corporation (Shanghai, China). 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2-H-tetrazolium bromide (MTT) and 3,3′-dioctadecyloxacarbocyanine perchlorate (Dio) were purchased from Shanghai Beyotime Biological Technology Co. Ltd. Fluorescin diacetate (FDA), propidium iodide (PI), Hematoxylin and Eosin were purchased from Shanghai Aladdin Bio-Chem Technology Co., LTD. Absolute ethyl alcohol (Guangfu Fine Chemical Research Institute, China), 1,3-diphenylisobenzofuran (DPBF, Aladdin Bio-Chem Technology Co., Ltd) were used. Ultrapure water (18.2 MΩ cm) was prepared by Milli-Q Ultrapure Water Purification System (Millipore, Inc., USA). 2′,7′-dichlorofluorescin diacetate (DCFH-DA) reactive oxygen species assay kit was purchased from Beyotime Institute of Biotechnology (China).

The ELISA kits of CD44, PD-L, CD47, galectin-3, P-gp and HIF-1α were purchased from MSKBIO (Wuhan, China). Fluorochrome-conjugated antibodies against, CD80/B7-1 and CD62L/L-Selectin/SELL were purchased from Sino Biological (Beijing, China). The kits of Blood urea nitrogen (BUN), alanine aminotransferase (ALT) and aspartate transaminase (AST) were purchased from MSKBIO (Wuhan, China).

The HeLa cells (cervical cancer cell line) was purchased from Shanghai Tianjing Biological Technology Co. Ltd. (Shanghai, China). The Lsgc-7801 cells (gastric cancer cell line), U14 cells (mouse uterine cervix cancer cell line) and HepG2 (human liver cancer cell line) were purchased from Cell Bank of the Chinese Academy of Sciences (Shanghai, China).

Male Kunming mice (17-18 g, 4-6 weeks old) were purchased from Vital River Laboratory Animal Technology Co., Ltd (Beijing, China) in the armpit of the right forearm.
Synthesis of g-C$_3$N$_4$. The g-C$_3$N$_4$ was prepared by secondary exfoliation. 20g melamine was roasted at 550 °C for 4 h to obtain the blank g-C$_3$N$_4$, then roasted at 550 °C for 2 h to obtain the precursor of pale yellow g-C$_3$N$_4$, and finally roasted at 550 °C for 2 h to obtain the white g-C$_3$N$_4$ nanosheets.

Extraction of Tumor Membrane. The U14 tumor cytomembrane (CM) was isolated. Firstly, U14 cells (mouse uterine cervix cancer cell line) were collected and washed with phosphate buffer solution (PBS, pH 7.4) three times, the cells were resuspended in PBS containing 10 mM Tris, 10 mM KCl, 2 mM MgCl$_2$ and 1×EDTA-free protease inhibitor at 4 °C for 1 h, and then the solution was treated under the ice using Ultrasonic Cell Disruption System (UCDS, 200 W, 10 min) with the mode of 2 s-working and 2 s-resting. Followed, the cell homogenate was centrifuged in 3000 rpm for 5 min at 4 °C, then the supernatant was centrifuged at 13000 rpm for 1 h. Finally, the CM precipitation was resuspended with PBS at room temperature and stored at 4 °C.

Synthesis of “Nano-Lymphatic” system. 1 mg white g-C$_3$N$_4$ nanosheets were dispersed in PBS and ultrasound for 30 min using the UCDS with the 2 s-working and 2 s-resting mode (300W). Followed, 1 mg DOX was added to the above solution and mixed. Then 1 mL sodium bicarbonate solution of luminol (0.5 mg/mL) was slowly added in mixed solution under magnetic stirring at room temperature to obtain the DOX- g-C$_3$N$_4$-Luminol (DCL) nanosheets. After 1 h, the CM and DCL were shaken overnight in the air baths at 40 °C. Subsequently, the obtained solution was centrifuged in 10000 rpm for 30 min, then the supernatant solution was extruded for 15 times by Liposome Extruder with 100 nm filter and further centrifuged at 8000 rpm for 15 min. The resulting DCL@M nanosheets were resuspended in PBS and stored at 4 °C.

Photocatalytic water splitting experiment. The photocatalytic H$_2$ generation of obtained samples was performed using the gas-closed system with a 150 mL of outer Pyrex reaction cell at room
temperature. Typically, 10 mg of DCL@M was suspended in 100 mL of aqueous solution. In order to simulate the real tumor environment, 10 μmol ferric chloride (providing Fe$^{3+}$) and 1 mL lactic acid were added to the reaction system. Prior to reaction, the reaction vessel was deaerated by bubbling high purity N$_2$ to remove the air. Whereafter, the reaction vessel was irradiated by a 300 W Xenon lamp (PLS-SXE300, Beijing Perfect Light Co., Ltd, China) with a UV cutoff filter (> 420 nm). The reaction solution was maintained at room temperature using a flow of cooling water during the reaction for 12 h. The evolved gases were analysed using a gas chromatograph (GC7900, Techcomp Co., Ltd, China) equipped with a thermal conductive detector (TCD).

**Characterization.** The zeta potentials of CM, g-C$_3$N$_4$, DOX or DCL@M nano-flakes were measured using dynamic light scattering (DLS) examination (Malvern Instruments, UK). Surface morphology of the samples were characterized by atomic force microscope (AFM, Bruker Multimode 8, Germany), field emission scanning electron microscope (FESEM, SUPRA 55) and transmission electron microscope (TEM) operated at 80 kV. The Energy dispersive spectroscopy (EDS), which was employed in analyzing chemical composition of the DCL@M by JEM-2010 instrument at an accelerating voltage of 200 kV. At the same time, SHIMADZU UV2550 Ultraviolet–visible spectrophotometer (UV–Vis) was used to observe the absorption spectra of the samples to detect the synthesis of DCL@M. The electrochemical performance of samples was characterized using a three-electrode quartz cell and a CHI670E electrochemical workstation in 0.5 mol L$^{-1}$ Na$_2$SO$_4$ electrolyte, meanwhile, Pt foil used as a counter electrode and a Ag/AgCl as the reference. The rate of hydrogen production was measured by a chromatograph (Techcomp GC7900). Cell apoptosis and fluorescence intensity were tested by Flow Cytometry (FCM, BD FACS-Calibur, USA). The elemental composition and chemical valence states of samples were
detected by X-ray photoelectron spectroscopy (XPS) carried on an Escalab 250Xi (Thermo Fisher Scientific) with radiation from an Al Kα (1486.6 eV) X-ray source. UV–vis diffuse reflectance spectra (DRS) were obtained in air at room temperature in the 200-800 nm wavelengths range by means of a Shimadzu UV-2460 PC spectrophotometer, with BaSO₄ as the reference. Real-time fluorescence quantitative PCR instrument (ABI, Q1, USA)

**Zeta potential detection.** 2 mL of different samples (g-C₃N₄, DOX/g-C₃N₄, CM, DCL, DCL@M and DCL@M+100 μL of 30% H₂O₂) were placed in darkness and incubated at 37 °C for 2h, then, DLS was used to detect the point position of the samples.

**Drug loading efficiency.** Amounts of DOX on DCL@M were analyzed using absorbance at λ = 233 nm according to the corresponding standard calibration curve by subtracting the absorbance of DCL@M. The loading efficiency was calculated as follows:

\[
\text{DOX loading efficiency (\%) } = \frac{W_{\text{DOX}}}{W_{\text{DCL@M}}} \times 100\%
\]

**Detection of loading efficiency of luminol.** 0.5 mg white g-C₃N₄ nanosheets were dispersed in PBS and ultrasound for 30 min using the UCDS with the 2 s-working and 2 s-resting mode (300W). Followed, 0.5 mg DOX was added to the above solution and mixed. Then 1 mL sodium bicarbonate solution of luminol (0.25 mg/mL) was slowly added in mixed solution under magnetic stirring at room temperature to obtain the DOX/g-C₃N₄/luminol (DCL) nanosheets. After 1 h, the CM and DCL were shaken overnight in the air baths at 40 °C. Subsequently, the solution was extruded for 15 times by Liposome Extruder with 100 nm filter to obtain DCL@M, and then the solution was placed in a dialysis bag and placed in a beaker containing 2 mL of pH 7.4 PBS. The beaker was then placed in a water bath shaker (100 rpm) for 48 h, and then 2 mL PBS was removed. The HPLC was used for quantitative analysis of luminol amount in removed PBS. The HPLC conditions
were as follows: Velocity was 1 mL/min; Column temperature was 30°C; Detection wavelength was 356 nm; The chromatographic column was XDB-C18; Mobile phase was V (0.05 mol/L KH$_2$PO$_4$) : V (Methanol)=60 : 40.

**Detection of emission spectra of luminol with or without H$_2$O$_2$.** Firstly, 500 μL of H$_2$O$_2$ with a concentration of 10% and 500 μL of luminol solution (0.2 mg/mL) were added to different sample pools under the dark environment, respectively. Then the samples were rapidly mixed and the emission spectrum was detected by a fluorescence spectrograph (Hitichi, F-7000).

**Detection of absorption spectra of DCL@M.** The 0.5 mL of DCL@M was diluted to 5 times and 3mL diluent were taken into the sample pool under the dark environment, then the absorption spectrum of the DCL@M was measured with a varioskan flash.

**Chemiluminescent imaging.** Firstly, 100 μL of H$_2$O$_2$ with different concentration (0%, 5%, 10%, 20% and 30%) were added to the black plate under the dark environment, respectively. Then 100 μL of luminol solution (0.2 mg/mL) were rapidly added with a pipette into each hole with H$_2$O$_2$, the fluorescence signal of each hole were then detected under the IVIS system. In addition, 100 μL of H$_2$O$_2$ with a concentration of 10% were added to the black plate under the dark environment. Then the 100 μL of luminol solution (0.2 mg/mL) containing different concentrations of Fe$^{3+}$ (0, 10, 20, 30 and 40 mM of FeCl$_3$) was rapidly added with a pipette into each hole with H$_2$O$_2$, the fluorescence signal of each hole were then detected under the IVIS system.

**EIS of DCL@M in dark/light environment for charge conductivity.** The working electrode was prepared on fluorine-doped tin oxide (FTO) glass, which was cleaned by sonication in acetone and ethanol for 30 min. The FTO slide was dip coated with 10 μL of slurry, which was obtained from mixture of 2 mg DCL@M (according to the calculation of loading efficiency, the dose of luminol
was 0.184 mg and the dose of DOX was 0.704 mg), 1 mL ethanol and 5 μL 5% nafion under sonication for 0.5 h. The electrochemical performance of samples was characterized using a three-electrode quartz cell and a CHI670E electrochemical workstation in 0.5 mol L⁻¹ Na₂SO₄ electrolyte using Pt foil used as a counter electrode and a Ag/AgCl as the reference.

**Mole rates of photocatalytic water splitting to produce H₂ using DCL, DCL@M, DC@M and CL@M.** The photocatalytic water splitting reactions were performed in an outer top-irradiation photoreactor vessel with a closed gas circulation system. Briefly, 10 mg of the DCL@M were dispersed into 100 mL aqueous solution containing 10 μmol ferric chloride (providing Fe³⁺) and 1 mL lactic acid. A 300 W Xenon lamp source (PLS-SXE300, Beijing Perfectlight) with a 420 nm cutoff filter was used as the visible light source. Before irradiation, the suspension was thoroughly degassed to assure vacuum environment, and the reaction system was maintained at 30 °C by flowing cooling water during the reaction. The amount of H₂ product evolved was analyzed with an online gas chromatograph (GC9790II, Fuli) equipped with a TCD detector, and argon was used as the carrier gas. The detection of mole rates of photocatalytic water splitting to produce H₂ using DCL, DC@M and CL@M were the same as DCL@M, (according to the calculation of loading efficiency, the dose of luminol was 0.92 mg and the dose of DOX was 3.52 mg).

**Detection of reactive oxygen species production.** The production of ROS was measured by DPBF bleaching method. Briefly, The samples were diluted with PBS into a solution of equal concentration gradient (0.5 mg/mL, 0.25 mg/mL, 0.125 mg/mL and 0.625 mg/mL) and 10 mM DPBF was added to the samples (30 μL DPBF / ml sample) under shading. The absorbance of samples at 410 nm was measured.

**Identification of reactive oxygen species**
ROS were detected with using EPR method. In the procedure, 10 mg g-C$_3$N$_4$ sample was dispersed into 5,5-dimethyl-1-pyrroline-N-oxide (DMPO) solution (10μL including 1mL CH$_3$OH). After sample was illuminated for 5 min with using a 300 W Xenon lamp source with a 420 nm cutoff filter, and DMPO- O$_2^-$ was examined with EPR instrument to identify •O$_2^-$. When water was used instead of methanol, the DMPO-•OH signal was detected to identify hydroxyl radicals (•OH).

**Cell culture.** The Hela cells, Lsgc-7801 cells and HepG2 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) containing 10% fetal bovine serum and 1% penicillin/streptomycin at 37 °C containing 5% CO$_2$.

**Intracellular Reactive Oxygen Species Detection.** Intracellular ROS production was detected by 2’ , 7’-dichlorofluorescin diacetate (DCFH-DA) ROS assay kit. HeLa cells were inoculated into the 12-well plates for 24 h. Different samples (1 mL of Saline, 1 mL of g-C$_3$N$_4$@M, 1 mL of CL@M, 1 mL of CL@M + 100 μL of 30 % H$_2$O$_2$, 1 mL of DC@M, 1 mL of DCL@M, 1 mL of DCL@M+100 μL of 10 % lactic acid and 1 mL of DCL@M+100 μL of 30 % H$_2$O$_2$, the dose of DCL@M was 2 mg/mL, the dose of luminol was 0.184 mg/mL and the dose of DOX was 0.704 mg/mL) were added to each well respectively. After 2 h, Dulbecco’s Modified Eagle Medium (DMEM) was removed and the wells were washed twice with PBS, and then 1 mL DCFH-DA (0.01 mM) was added to each well and incubated for 20 min. DCFH-DA was removed and washed with PBS for three times. Then, fluorescence images of the treated cells were obtained by fluorescence microscopy.

**Homologous Targeting Effect Evaluation.** ELISA measurements of programmed cell death-Ligand 1 (PD-L1), CD47, galectin-3 and CD44 for the samples (DCL@M, CM and tumor cells) were performed according to the instructions of the kits. The HeLa cells (cervical cancer cell line),
Lsgc-8701 cells (gastric cancer cell line) and HepG2 cells (human liver cancer cell line) were incubated with 1 mL of Dio-dyed DCL@M (the dose of DCL@M was 2 mg/mL, the dose of luminol was 0.184 mg/mL and the dose of DOX was 0.704 mg/mL) for 2 h respectively, and then observed under the fluorescence microscope. In addition, Hela cells were incubated with Dio-dyed DCL@M (1 mg/mL, the dose of luminol was 0.092 mg/mL and the dose of DOX was 0.352 mg/mL)) for 1, 2 and 4 h, and then fluorescence intensity of cell uptake was quantitatively analyzed by flow cytometry.

**Antitumor Effect in vitro.** HeLa cells were incubated with 200 µL of different samples (1: g-C$_3$N$_4$, 2: Saline+100 µL of 30 % H$_2$O$_2$ 3: DOX, 4: DL@M, 5 :DCL@M, 6: DL@M+100 µL of H$_2$O$_2$, 7: DCL@M+100 µL of 30 % H$_2$O$_2$, the dose of DCL@M was 1 mg/mL, the dose of luminol was 0.092 mg/mL and the dose of DOX was 0.352 mg/mL), for 24 h, and the cell activities of groups were evaluated using the MTT method. The HeLa cells were cultured with 1 mL of different samples (Saline, DOX, CL@M, DCL, DCL@M and DCL@M+100 µL of 30 % H$_2$O$_2$, the dose of DCL@M was 2 mg/mL, the dose of luminol was 0.182 mg/mL and the dose of DOX was 0.704 mg/mL), and then the live and dead cells were detected by FDA/PI staining under the inverted fluorescence microscope.

**Animal models.** The mice acclimated to standard laboratory environment for a week, U14 cells (2.0 × 10$^6$ cells) were injected subcutaneously into the right buttock of the mice. Tumor-bearing mice were randomly divided into 6 groups (6 animals in each group) and treated differently: Saline, DOX, DC@M, CL@M, DCL and DCL@M (the dose of DOX in the samples was 1.5 mg/kg), when the average tumor size reached about 100 mm$^3$. 200 µL samples were injected into the tail vein of each mouse every 2 d. Next, the relative tumor volume ($V/V_0$, $V_0$ is the initial volume and $V$ is the
real time measurement volume) and body weight were monitored every other day.

**Depth of penetration.** The two groups of mice with similar tumor volumes (about 4 mm in radius) were intravenously injected with DC@M and DCL@M. After 12 h, the tumors were carefully removed from mice. At 0, 1, 2 and 3 mm away from the tumor center (in radius), 1 mm³ tissue block was taken, respectively. The fresh tumor tissue was cut and placed into RPMI-1640 culture medium with sterile operation. The tumor tissue was first cut into pieces with scissors and gently ground to obtain small cell masses. Then add 5 mL of enzymolysis liquid (collagen enzyme 0.2%, 0.01% hyaluronate enzyme and 0.002% hyaluronidase DNA enzymes in the RPMI-1640 culture medium), then mixed solution was kept warm in a water bath at 37 °C to digest 60 min, mixed the cells with a pipette once every 5 to 10 min. Subsequently, RPMI-1640 culture medium containing serum was added to termination of digestion, repeatedly mixed with a pipette for single cell suspension. Then, 100 mesh sieve filtration was used to collect filtrate twice, 1000 rpm centrifugal for 5 min, and then the cells were collected by serum free medium after 1 to 2 times. Finally, the flow cytometer was used to detect DOX fluorescence intensity in the cells.

**Biosecurity in vivo.** The fresh eyes blood with anticoagulant of the mice was bathed in sterile water at 37 °C for 4 h, and then centrifuged for 3 times to obtain supernatant. ELISA measurement was used to determine the levels of biochemical indicators (AST, ALT and BUN) in the mice treated with 1 mL of different injection samples (saline and DCL@M).

**Measurement of Tumor Interstitial Fluid Pressure.** The TIFP level was measured with the WIN technique. Briefly, the pressure detector was calibrated before each animal pressure was measured. For TIFP, the needle was attached to a pressure transducer via a PE-50 polyethylene tube filled with sterile heparinized saline. The anesthetized animals, whose body temperature was maintained at
37.5 °C with a heating pad, were carefully peeled off the skin above the tumors. In saline treated mice, two measurements were obtained by introducing two needles into the central area of the tumor. The DCL@M injected mice were divided into two groups, one of which was continuously monitored for TIFP within 2 h of the injection, in the another group, the DCL@M was injected and measuring with a separate needle 2 h later, and then the results were compared with those of saline tumors of similar size.

The tumor-bearing mice injected with DCL@M were anesthetized, and photoacoustic imaging system was used to detect blood vessels and blood flow in tumor sites at 20, 40, 60 and 80 min. Photoacoustic Imaging system (PAI) includes pulse laser, glass slices, balance detector, circulator, laser diode, translation platform, lens, mirror, etc. The imaging principle of this PAI system is that the photoacoustic signal generates water film vibration on the sample surface, and then the photoacoustic image is reconstructed by using the light interference principle to detect the tiny vibration on the water surface, which is a new detection method for the photoacoustic signal. The detection principle of PAI is based on the optical absorption of hemoglobin to the laser with a wavelength of 560 nm, and the location of blood vessels and blood perfusion can be determined according to the distribution of hemoglobin in the tumor sites. When the blood perfusion is stronger and the blood flow is faster, accompanying the stronger absorption of hemoglobin and the stronger signal. Through software processing and simulation, the signal is converted into images. The color from red to yellow represents the signal from weak to strong. The following figure shows the schematic of the PAI experimental setup, and the following table shows the Technical data of PAI system.
Table 1. Technical data of PAI system

<table>
<thead>
<tr>
<th>Component</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>Laser (DTL-419QT, Russia, Laser-export Co. Ltd)</td>
<td>Central wavelength 527 nm</td>
</tr>
<tr>
<td>Glass slices (NDM2, Thorlabs)</td>
<td>50% Laser attenuation</td>
</tr>
<tr>
<td>Laser diode (LSDL131-4-S-O-1-SMFA)</td>
<td>Central wavelength 1310 nm; Power 4 mW</td>
</tr>
<tr>
<td>Acquisition card(PCI 6713, National Instruments)</td>
<td>36 mm</td>
</tr>
<tr>
<td>High-pass filter(EF505, Thorlabs)</td>
<td>Camera link</td>
</tr>
<tr>
<td>Software</td>
<td>LABVIEW/ MATLAB</td>
</tr>
</tbody>
</table>

Measurement of Tumor Water Content. The fresh weight ($T_1$) of tumor was weighed immediately, and the dry weight ($T_0$) was weighed after drying in a drying oven at 50 °C for 5 days.

The calculation formula of tumor water content ($W_C$) is as follows:

$$W_C(\%) = \frac{T_1 - T_0}{T_1} \times 100\%$$

Measurement of Hypoxia-Inducible Factor. The fresh eyes blood samples with anticoagulant, which were obtained from the mice in different treatment groups (Normoxia, Hypoxia, Hypoxia...
and DCL@M injection), were bathed in sterile water at 37 °C for 4 h, and then centrifuged for three
times to obtain serums. ELISA measurement was used to determine the expression levels of
hypoxia-inducible factor (HIF-1α) and P-glycoprotein (P-gp) of the samples.

**Measurement of mRNA expression of HIF-1α and P-gp.**

**Primer design:**

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequences (5’→3’)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mHIF-1a-158-F</td>
<td>CATTCCCCCTCTCTGTAAGCA</td>
</tr>
<tr>
<td>mHIF-1a-158-R</td>
<td>TTCAGACTCTTTGCTTCGCC</td>
</tr>
<tr>
<td>mP-pg-132-F</td>
<td>GCGGAGTCAGACAGAAACAAGA</td>
</tr>
<tr>
<td>mP-pg-132-R</td>
<td>CCTTCTTACTCCATTTCCCCCTTT</td>
</tr>
<tr>
<td>GAPDH-127F</td>
<td>CCAGGTGGTGTCCTCTGTA</td>
</tr>
<tr>
<td>GAPDH-127R</td>
<td>GCTGTAGCCAAAATCGTTGT</td>
</tr>
</tbody>
</table>

500 L Trizol was added to the cell sample, RNA was extracted using a kit, and then the RNA
concentration was measured using a nucleic acid protein detector. RNA amplification was
performed using real-time fluorescence quantitative PCR instrument with a cycle number of 40 and
a temperature of 60 °C. The mRNA of HIF-1α and P-gp can be quantitatively analyzed by Ct value
and standard curve.

All animal experiments were performed in accordance with the statute of the Experimental Animal
Ethics Committee of Yanshan University.
References

Table S1. Analysis of free Luminol in removed PBS

<table>
<thead>
<tr>
<th>Retention time (min)</th>
<th>Area (mV•s⁻¹)</th>
<th>Concentration/Area</th>
<th>Concentration(mg/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2.481</td>
<td>688.44391</td>
<td>$6.37145 \times 10^{-5}$</td>
<td>$4.386383 \times 10^{-2}$</td>
</tr>
</tbody>
</table>
Figure S1. FESEM analysis of DCL@M.
Figure S2. EDS analysis of DCL.
Figure S3. The standard curve of amount of luminol.
**Figure S4.** Molecular structure of luminol and Doxorubicin hydrochloride.
Figure S5. ROS production of DCL@M (0.0625, 0.125, 0.25 and 0.5 mg/mL) detected via UV-vis absorption of the DPBF
Figure S6. ROS production of DPBF, g-C$_3$N$_4$-luminol and luminol+DPBF detected via UV-vis absorption of the DPBF.
**Figure S7.** ROS production in HeLa cells treated with saline, g-C₃N₄@M, CL@M, CL@M+H₂O₂ and DC@M+H₂O₂, which were control group to compare the ROS production.
**Figure S8.** ROS production in HeLa cells treated with saline and DCL@M+ Lactic acid in hypoxia.
Figure S9. Fluorescent microscopic images of HeLa cells treated with DCL@M at 4 h.

Red: DOX; Blue: Nucleus.
**Figure S10.** Schematic diagram of tissue block selection (in radius) method in deep penetration experiment.
Figure S11. Amplification curves of QPCR of HIF-1α and P-gp.
Figure S12. Melting curves of QPCR of HIF-1α and P-gp.