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# Novel phthalocyanine-based micelles/PNIPAM composite hydrogels: spatial/temporal-controlled drug release triggered by NIR laser irradiation

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**Characterization:**

The optical characteristics of 4OCSPC micelles were investigated by UV−vis absorption spectra (TU1901 UV−vis absorption spectrophotometer, Beijing PuXi general instrument co., LTD, China). The morphological structures of the hydrogels were observed using scanning electron microscopy (SEM, JSM6390LA, JEOL, Japan) at 10 kV. The fluorescent images of the cancer cells were obtained by fluorescence microscope (Olympus, Japan). Hydrogel shrinkage was under 808 nm laser irradiation (MDL-III-808-2W, 808nm infrared semiconducto laser, Changchun New Industries Optoelectronics Tech. Co., Ltd, China).

**Materials:**

*N*-isopropylacrylamide (NIPAM, purchased from Sigma-Aldrich) was purified by recrystallization. Methylene bisacrylamide (MBAm), ammonium persulfate (APS), *N,N,N′,N′*-tetramethylethylenediamine (TEMEDA) and Pluronic 127 were all purchased from Sigma-Aldrich. Dulbecco's modified Eagle's medium (DMEM), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltertra-zolium bromide (MTT), fetal bovine serum (FBS)，penicillin/streptomycin mixture, phosphate-buffered saline (PBS), TrypLE Express enzyme (1×) and 4′,6-diamidino-2-phenylindole (DAPI) were purchased from Life Technologies (Singapore). Doxorubicin hydrochloride (DOX・HCl, MW: 580) was purchased from Dalian Meilun Biotechnology, Co., Ltd. All other solvents and reagents used in this study were certified analytical reagent grade.

**Synthesis of H0 hydrogel:**

NIPAM (1.78 g，$1.6\times10^{-2}$ mol), MBAm (0.97 mL, $10^{-4}$ mol), TEMEDA (0.05 mL, $3.3\times10^{-4}$ mol) and 0.105 mL of deionized water were added sequentially. The solution was degassed with three freeze−pump−thaw cycles, and APS solution (0.1 mL, $4\times10^{-5}$ mol) was added to initiate the polymerization. The prepolymer was purged with nitrogen, placed in an airtight bottle, allowed to polymerize for 12 h.

**Synthesis of H1 hydrogel:**

NIPAM (1.78 g，$1.6\times10^{-2}$ mol), MBAm (0.97 mL, $10^{-4}$ mol), TEMEDA (0.05 mL, $3.3\times10^{-4}$ mol) and 0.105 mL of deionized water were added sequentially. The solution was degassed with three freeze−pump−thaw cycles, and APS solution (0.1 mL, $4\times10^{-5}$ mol) was added to initiate the polymerization. This pregel mixture was injected the 4OCSPC (0.043 mL, $7.22\times10^{-9}$ mol) micelles, purged with nitrogen, placed in an airtight bottle, allowed to polymerize for 12 h.

**Synthesis of H2 hydrogel:**

NIPAM (1.78 g，$1.6\times10^{-2}$ mol), MBAm (0.97 mL, $10^{-4}$ mol), TEMEDA (0.05 mL, $3.3\times10^{-4}$ mol) and 0.105 mL of deionized water were added sequentially. The solution was degassed, and APS solution (0.1 mL, $4\times10^{-5}$ mol) was added to initiate the polymerization. The 4OCSPC (0.075 mL, $1.24\times10^{-8}$ mol) micelles and anticancer drug DOX • HCl (1.22 mL, $1.05\times10^{-5}$ mol) were added this pregel mixture. It was then purified with nitrogen, placed in a sealed bottle under ultrasonic treatment and

allowed to polymerize for 12 h. (4OCSPC/H2, $8.42\times10^{-5}$, m/m, DOX • HCl/H2, $1.71\times10^{-2}$, m/m)

**Degree of shrinkage of hydrogels:**

For the shrinkage ratio studies, the weight of hydrogel in the initial state is ($m_d$), and the hydrogel will produce obvious shrinkage and deformation after being exposed to laser intensity of 1.0 W/cm$^2$ for 20min. At this time, the weight is ($m_t$). The same experiment was carried out for 5 times. The shrinkage ratio (SR) of the hydrogels was defined as follows:

$$SR = \frac{m_d - m_t}{m_d}$$

**Cytotoxicity evaluation *in Vitro:***

HeLa cells were seeded in a 96-well plate and observed until the cells were evenly distributed. Approximately $1\times10^6$ cells per hole were cultivated. The DMEM containing 10% FBS and 1% penicillin/streptomycin in each well was 200 μL. Then, the H0, H1 and H2 hydrogels were cut into small pieces and placed in a 96-well plate. The HeLa cells were cultured at 37 ℃ with 5% $CO_2$ in a humidified incubator for 24 h or 48 h respectively. Only the cell group, H0, and H1 was used as a control. Thereafter, 20 μL of 5 mg/mL MTT was added to the mixture and incubated for another 4 hours, and then the DMEM in the 96-well plate was replaced with DMSO. Cell viability was measured at 490 nm by colorimetric assay (Rayto RT-2100C, Shenzhen, China).

**Cell labeling with fluorescent dyes：**

HeLa cells were seeded in a 6-well plates. The H2 hydrogels were cultured for 2 h or 12 h with and without laser irradiation (1 W/cm$^2$, 808 nm, 20 min irradiation). The medium was then removed and washed three times with PBS. The HeLa cells were fixed with formalin solution and washed three times with PBS. The nuclei were stained for 10 min with DAPI and then washed three times with PBS. The condition of the cell nucleus was observed by a fluorescence microscope.

**Statistical Analysis:**

Each experiment was repeated three times. Data was presented as mean ± standard deviation (SD). Statistical analyses were performed by one-way ANOVA. Differences with a $P<0.05$ were considered significant.

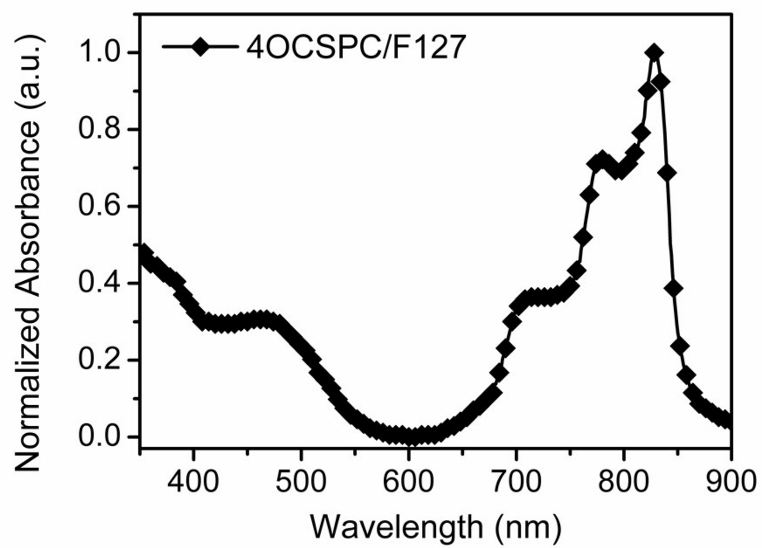



**Figure S1.** Absorption spectra of 4OCSPC/F127 ($C_{4OCSPC}$ = 20.0 μg/mL, dissolve in DI water).

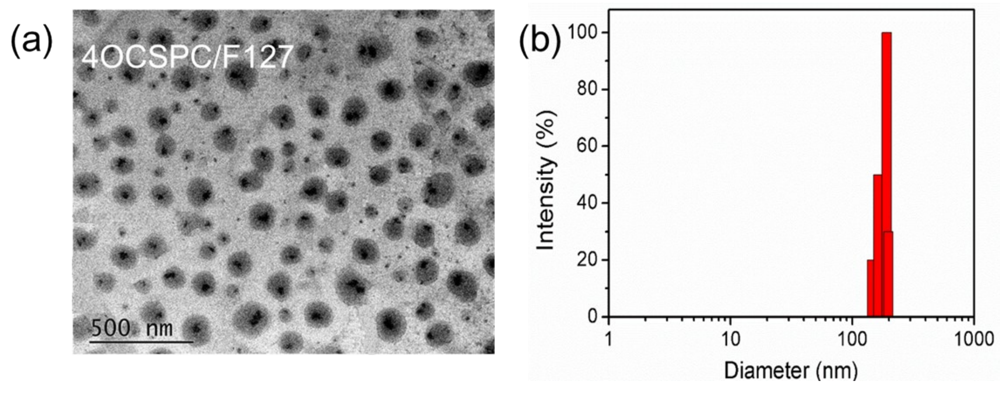



**Figure S2.** TEM image (a) and DLS spectrum (b) of the 4OCSPC/F127 micelles.

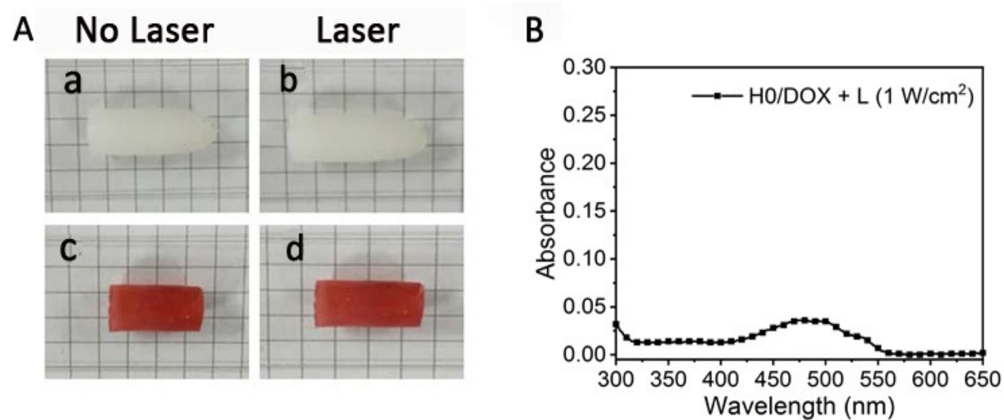



**Figure S3.** (A) a (H0), c (H0 + DOX), b, d, before and after laser irradiation (808 nm, 1.0 W/cm$^2$), the grid size of the background was $0.5 \times 0.5$ cm$^2$; (B) UV-Vis absorption spectra of release DOX from the hydrogel under 808 nm laser irradiation at the power density of 1.0 W/cm$^2$.

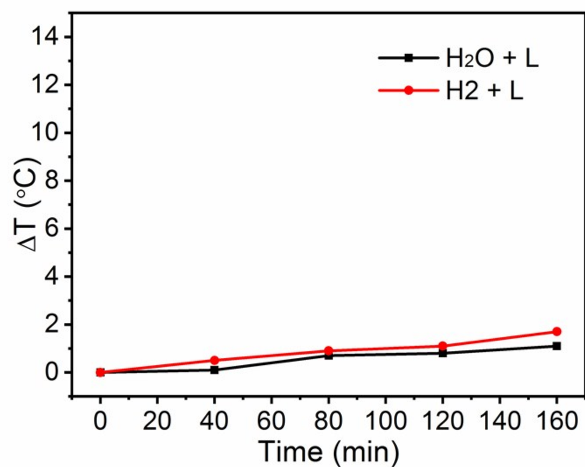



**Figure S4.** The photothermal heating curves of ambient solution under 808 nm continuous laser irradiation at the power density of 1.0 W/cm$^2$.

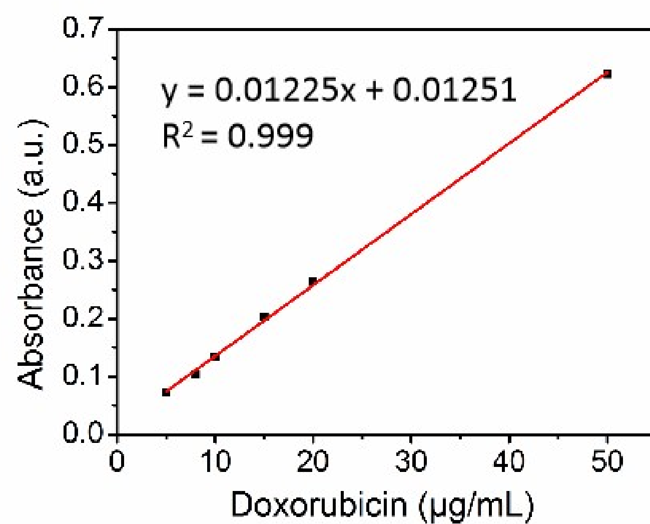



**Figure S5**. The calibration curve of DOX in water using the UV-Vis absorbance intensity at the wavelength of 490 nm.

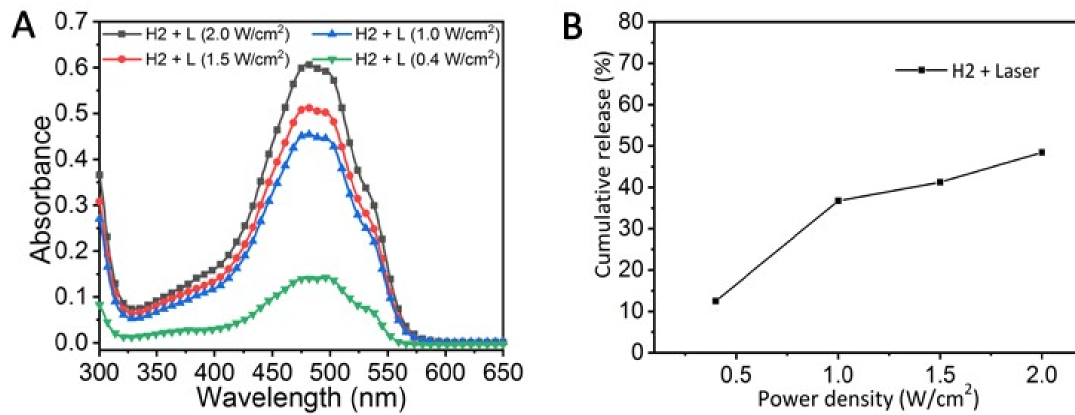
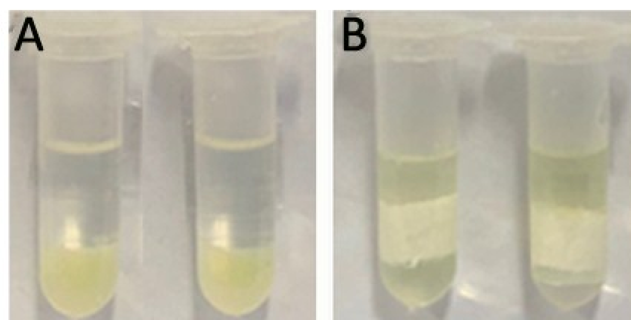



**Figure S6**. (A) UV-Vis absorption spectra of DOX released from H2 with laser irradiation at different power density. (B) Drug release contents of H2 with laser irradiation at different power density.



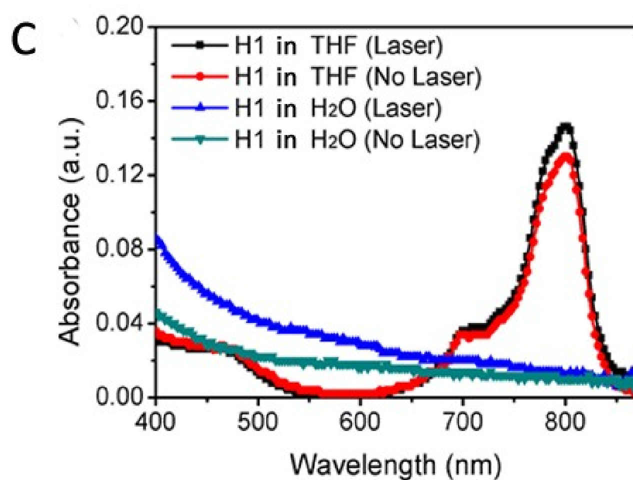




**Figure S7**. Digital photographs of **H1** hydrogels (A, B). The H1 hydrogels soaked in distilled water (A) and THF (B) before and after 808 nm laser irradiation at the power density of 1.0 W/cm$^2$ for 20 min. (C) UV-Vis absorption spectra.

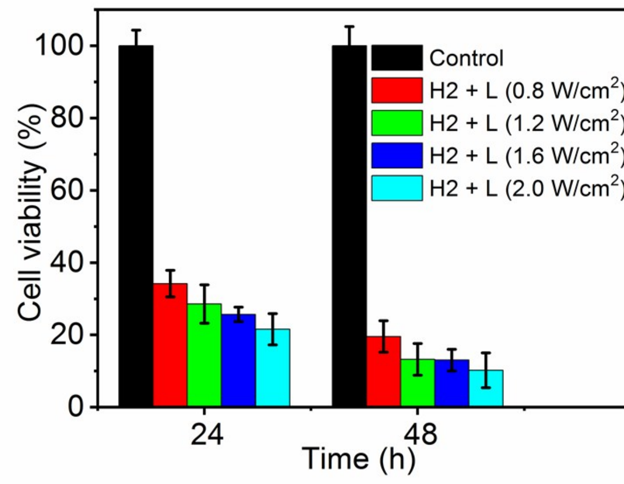



**Figure S8**. Cell viability after treatment with H2 hydrogels under laser irradiation at different power density.