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Porphyrin Contained Light-Responsive Capsules for Controlled Drug Release

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Experimental Methods

Materials. L-aspartic acid, phosphoric acid (H_3PO_4),sodium hydroxide (NaOH), hydrochloric acid (HCl), acetone, disodium ethylenediaminetetraacetate (EDTA),potassium carbonate (K_2CO_3) and calcium chloride ($CaCl_2$) were purchased from Shanghai Reagent Chemical Co. (PR China). Chitosan (M_w = 50 KDa, deacetylation degree = 85.3%) was purchased from Haidebei Marine Bioengineering Co. Ltd.(Jinan, PR China).All the reagents were of analytical grade and were used as received. *N, N*-dimethylformamide (DMF) was purchased from Shanghai Reagent Chemical Co. (PR China) and was purified by distillation over P_2O_5 and CaH_2.Dextran (M_w = 60-90 KDa), fluorescein isothiocyanate (FITC) and poly(sodium 4-styrene-sulfonate) (PSS) (M_w = 70 KDa) were purchased from Alfa Aesar China (Tianjin) Co., Ltd. and were used as received.

Synthesis 5-(4-Aminophenyl)-10,15,20-triphenyl-porphyrin (APP). APP was synthesized according to the literature procedure.[1-3]

Synthesis of poly(aspartic-*graft*-APP) (PASP-*g*-APP). PASP-*g*-APP was synthesized by the hydrolysis of poly-L-succinimide (PSI). And the synthesis of PSI was according to our prior work.[4]A total of 15.0 g L-aspartic acid was mixed with 7.5 g phosphoric acid in a 500-mL round-bottomed flask. The flask was placed in a rotary evaporator and heated under reduced pressure in an oil bath at 180 °C for 2.5 h. The mixture was dissolved in 100 mL of DMF. Then the solution was added to water dropwise and white precipitate formed in this course. The precipitate was collected, washed with water until neutrality, and dried in a vacuum oven at 110 °C for 24 h to obtain 9.7 g of PSI. A total of 50 mg APP (0.079 mmol, 0.77% of PSI repeat unit) was dissolved in 10 mL of DMF and then added to 15 mL of DMF solution of 1.0 g PSI (10.3 mmol of repeat unit). The solution was protected in N_2 atmosphere with stirring

at 70 °C for 24 h and then cooled to the room temperature. A 20 mL aqueous solution of 0.40 g of NaOH was added dropwise in an ice bath. After the addition, the suspension was stirred overnight at room temperature. The solution was stirring with the addition of hydrochloric acid until the pH value reaches 7.0, and then dialysized (MWCO: 8000-12000 Da) against de-ionized (DI) water for 6 days and then lyophilized for 3 days to obtain 0.62 g of white solid PASP-*g*-APP (yield: 52.3%).

Characterization of PASP-*g*-APP. ^{1}H NMR spectrum of PASP-*g*-APP was recorded on a Mercury VX-300 spectrometer at 300 MHz (Varian, U.S.A.) by using D_2O as the solvent. The molecular weights and the molecular weight distributions PASP-*g*-APP were evaluated by SEC-MALLS system consisted of a Waters 2690D separations module, a Waters 2414 refractive index detector (RI) and a Wyatt DAWN EOS MALLS detector. Two chromatographic columns (ShodexOHpak SB-803 and SB-802.5, Showa Denko, Japan) with a precolumn (Shodex SB-G) were used in series. 30% acetonitrile aqueous solution was used as the eluent at a flow rate of 0.3 mL/min to evaluate PASP-*g*-APP The eluent was filtrated through a 0.22 μm HPLC filter and degassed prior to use by ultrasonic bath. The data were processed with Astra software (Wyatt Technology).

Fluorescence Measurement. The fluorescence emission spectra and absorption spectra were recorded by a fluorescence spectroscopy. The excitation and emission slit widths were both set at 5 nm. All the measurements were performed within 2 h after preparation of the solutions. To determine the porphyrin substitution degree of PASP-*g*-APP, H_2TPP solution with different concentrations and 0.1 mg/mL PASP-*g*-APP solution in DMSO were prepared and the fluorescence emission spectra were measured respectively. To determine the fluorescence quantum yield of APP-PLL, stock solutions of PASP-*g*-APP (0.10 mg/ mL) and H_2TPP (0.00055

mg/mL) were prepared in DMSO. Each solution contains the same concentration of porphyrin (H_2TPP) moiety. The quantum yield was calculated using a secondary standard method.[5] H_2TPP was used as a secondary standard. The integrated fluorescence intensities of the analyte (FA_u) and standard (FA_s), the absorbencies of the analyte (A_u) and the standard (A_s) at excitation wavelength, the excitation wavelengths of analyte (λ_u) and the standard (λ_s), and the refractive indexes of analyte solution (η_u) and standard solution (η_s) are related to the quantum yield of the analyte (ϕ_u) as follows:

$$\phi_u = \phi_s \frac{FA_u}{FA_s} \frac{A_s}{A_u} \frac{\lambda_{exs}}{\lambda_{exu}} \frac{\eta^2{}_u}{\eta^2{}_s}$$

where ϕ_s is the quantum yield of the reference standard (0.11 for H_2TPP)[6].

Fluorescent labeling of dextran. Dextran was subsequently fluorescently labeled with a dye, fluorescein isothiocyanate (FITC). A total of 0.50 g of dextran was dissolved in 30 mL of dimethylsulfoxide (DMSO). Then 15 mg of FITC (3% weight of dextran) was added. The mixture was stirred for 24 h at room temperature. The solution was dialysized (MWCO: 8000-12000 Da) against DI water for 6 days and then lyophilized for 3 days to obtain FITC labeled dextran (FITC-Dex).

FITC-Dex capture by $CaCO_3$ particles. $CaCO_3$ particles with narrow size distribution were prepared according to the literature.[7,8] FITC-Dex was introduced into the $CaCO_3$ particles as model drug and to label the particles with fluorescence. Briefly, 5 ml 0.33 M K_2CO_3 solution containing 10 mg FITC-Dex was rapidly poured into 5 ml of a 0.33 M solution of $CaCl_2$ containing 10 mg poly(sodium 4-styrene-sulfonate) (PSS) at room temperature. After intense agitation for 30 s, the reaction mixture was left still for about 2 min. Then the precipitate was filtered off, thoroughly washed with DI water and acetone, and dried in air. The whole process

was protected from light wherever possible.

Fabrication of chitosan (CHI)/PASP-*g*-APP capsules. FITC-Dex captured $CaCO_3$ particles were used as colloid template for the fabrication of microcapsules. Briefly, a total of 150 mg of $CaCO_3$ particles were symmetrically dispersed in 1.5 mL of CHI solution (1 mg/mL). And the suspension was shaken constantly for 15 min to establish a CHI layer. After adsorption, the particles were isolated by centrifugation (10,000 rpm for 1 min), followed by washing with 1.5 mL of DI water thrice. For adsorption of the next layer, 1.5 mL of PASP-*g*-APP solution (1 mg/mL) was added, followed by the same washing protocol. The LbL process was repeated to get the microcapsules with a designed numbers of layers. The whole process was protected from light wherever possible.

Hollow capsules were formed by dissolving the $CaCO_3$ core using 0.4 M EDTA solution with pH = 7.4. Three centrifugation (15,000 rpm for 3 min) and water washing steps were applied to remove the EDTA and isolate the microcapsules for analysis.

Characterization of CHI/PASP-*g*-APP capsules. The hollow capsules in water were viewed with confocal laser scanning microscopy (CLSM). (Nikon C1-si, Japan). And dry capsules were characterized with transmission electron microscopy (TEM) and scanning electron microscopy (SEM). For TEM, a drop of a concentrated capsule solution was deposited on a copper grid Formvar film and allowed to dry in air. TEM analysis was carried out with a JEM-100CX II instrument operating at an acceleration voltage of 100 kV. For SEM, a drop of a concentrated capsule solution was deposited onto a glass slide and allowed to dry in air. SEM analysis was carried out with a FEI-QUANTA 200 instrument. Before the SEM observation, the samples were fixed on an aluminum stub and coated with gold for 7 min. The zeta potential of the

capsules before and after core removal was measured using a Nano-ZS ZEN3600 (Malvern Instruments).

Drug encapsulation efficiency (EE) of $CaCO_3$ particles and drug loading content (LC) of (CHI/PASP-*g*-APP)$_{10}$ capsules. The drug encapsulation efficiency (EE) of $CaCO_3$ particles and drug loading content (LC) of (CHI/PASP-*g*-APP)$_{10}$ capsules were measured using a fluorescence spectroscopy. The excitation and emission slit widths were set at 1.5 nm and 3 nm respectively. All the measurements were performed within 2 h after preparation of the solutions. To determine the EE of $CaCO_3$ particles and LC of the capsules, FITC-Dex solution with different concentrations were prepared and the fluorescence emission spectra were measured respectively. All solution in preparing $CaCO_3$ particles was collected to record the fluorescence emission spectra to determine the EE of $CaCO_3$ particles. And the capsules with core removal were lyophilized for quantifying. Then the dry capsules were dipped in 1 mL water and were destroyed with ultrasonic to release the FITC-Dex. The FITC-Dex solution was collected with centrifugation (15,000 rpm for 3 min) to record the fluorescence emission spectra to determine the LC of the capsules. The EE and DL are defined as follows:

$$\text{Entrapment efficiency (\%)} = \frac{\text{Mass of drug loaded in capsules}}{\text{Mass of drug fed initially}} \times 100\%$$

$$\text{Drug loading content (\%)} = \frac{\text{Mass of drug loaded in capsules}}{\text{Mass of drugloaded capsules}} \times 100\%$$

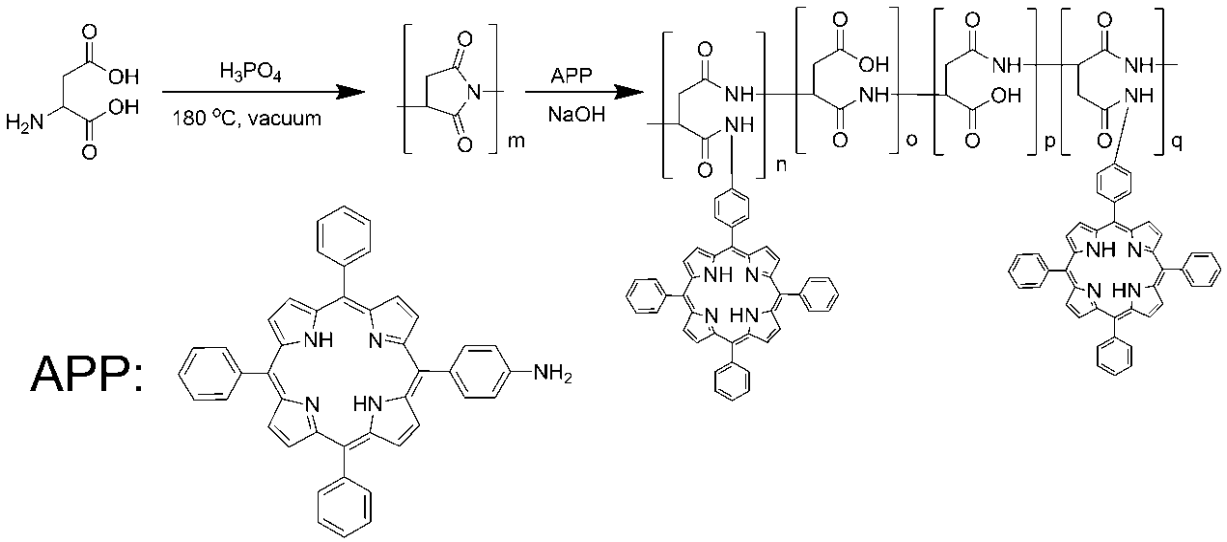
Light illumination of CHI/PASP-*g*-APP microcapsules and drug release. The drug release process of CHI/PASP-*g*-APP microcapsules with light illumination was viewed with CLSM. Appropriate amount of the capsules were dispersed in water and were illuminated by a150 W xenon lamp filtered through a 400-700 nm long-pass filter for 5min, 15min and 30 min respectively. The process was observed with CLSM

continuously.

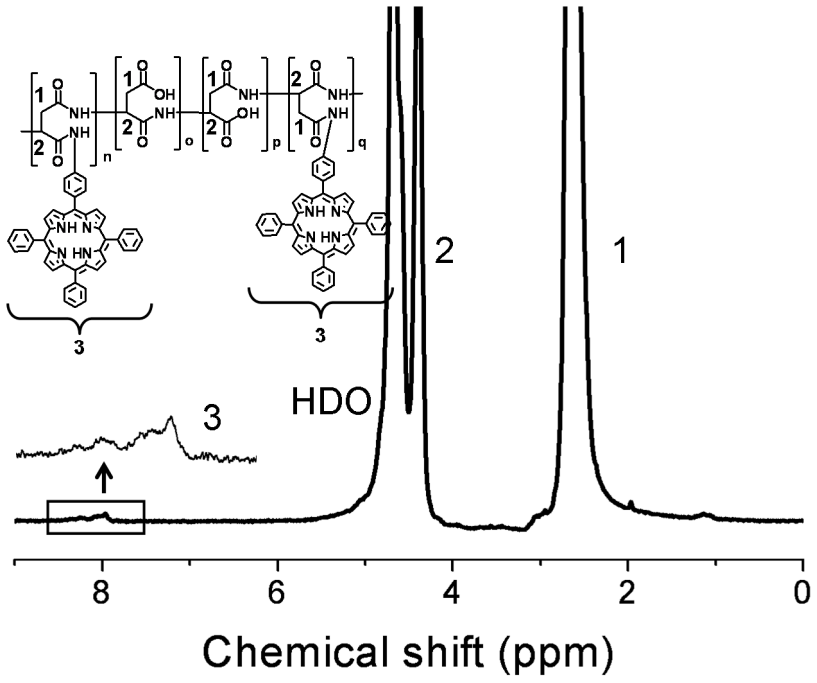
Results and Discussion

Synthesis of PASP-*g*-APP. PASP-*g*-APP was synthesized by hydrolysis of PSI. And the synthesis route is shown in Supplementary Scheme 1.The characteristic signals of PASP chain and porphyrin ring appear simultaneity in the ^{1}H NMR spectrum of PASP-*g*-APP (Supplementary Figure1), showed the successful synthesis of PASP-*g*-APP. Molecular weight of PASP-*g*-APP was measured by SEC-MALLS. The average number molecular weight of PASP-*g*-APP was14900 with PDI = 1.86.

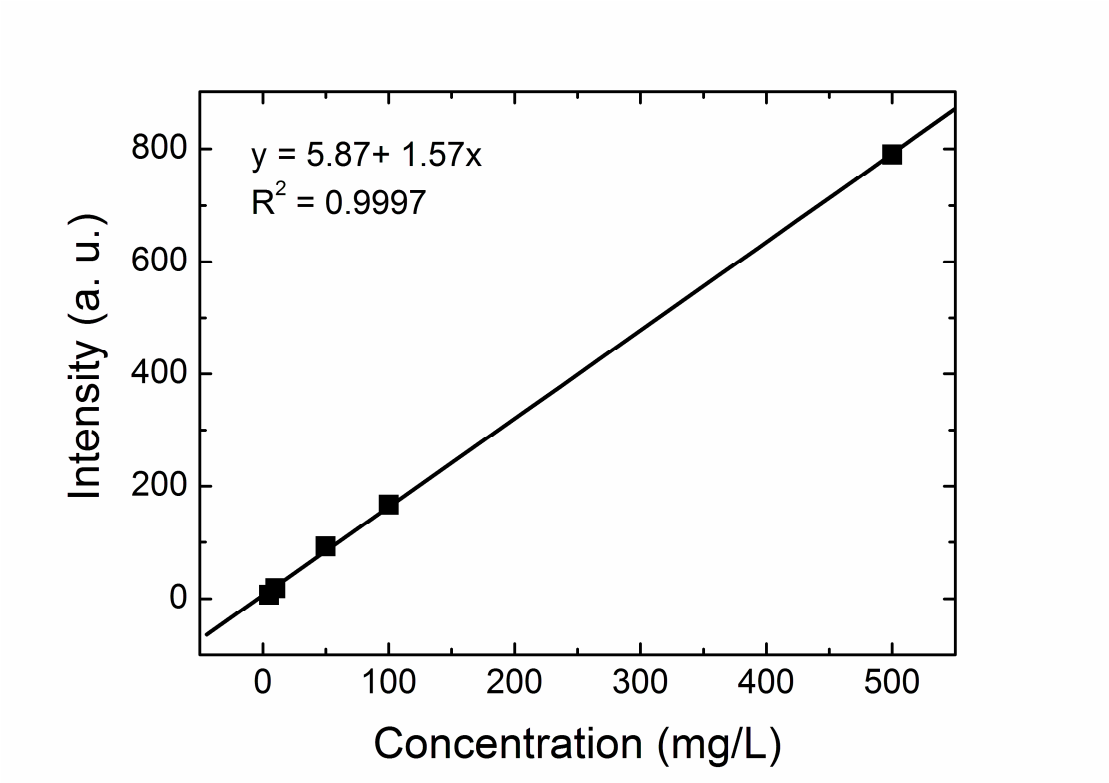
Drug encapsulation efficiency (EE) of $CaCO_3$ particles and drug loading content (LC) of $(CHI/PASP\text{-}g\text{-}APP)_{10}$ capsules. Supplementary Figure 2 shows the standard curve of fluorescence intensity of FITC-Dex at 520 nm with different concentrations. EE of $CaCO_3$ particles was measured as 4.46%. LC of capsules without $CaCO_3$ particles was measured as $CaCO_3$ particles were finally removed by EDTA, and the LC of the capsules is 5.39‰.



Supplementary Scheme 1. Synthesis route of PASP-*g*-APP.



Supplementary Figure 1. ^{1}H NMR spectrum of PASP-*g*-APP.



Supplementary Figure 2. Standard curve of fluorescence intensity of FITC-Dex at 520 nm with different concentrations.

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