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Supporting information

Near Infrared Light-responsive and Injectable Supramolecular Hydrogels for On-demand Drug Delivery

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Experimental section

Materials

G5-NH_2 PAMAM (molecular weight (Mw) = 28826 Dalton (Da)) dendrimer was purchased from Dendritech, Inc. (Midland, MI). PEG methyl ether tosylate, Rhodamine B and potassium tetrachloroplatinate (K_2PtCl_4) were purchased from Sigma-Aldrich (St. Louis, MO). α-CD and sodium borohydride ($NaBH_4$) were obtained from Aladdin Reagent (Shanghai, China). BTZ was purchased from Yeexin Biochem&Tech (Shanghai, China). Cy5.5 was obtained from Lumiprobe Corporation (Florida, USA).

Synthesis of D-PEG

In a standard synthesis, 100 mg G5-NH_2 PAMAM dendrimers were dissolved in 2 mL of dimethylsulfoxide (DMSO) and then were injected into 10 mL of PEG methyl ether tosylate (86.8 mg/mL) in Dimethyl formamide under magnetic stirring. The reaction continued for 3 days at 65 °C. After that the reaction solution was transferred into a dialysis bag with a molecular weight cut off (MWCO) of 3500 Da and dialyzed extensively against deionized (DI) water for 3 days.

Synthesis of DEPt-PEG

DEPt-PEG were prepared according to the well-developed method by Crooks et al.[1] In a typical synthesis, 3.1 mL of K_2PtCl_4 (10 mM) was mixed with 10 mL of D-PEG (10 mg/mL) in aqueous solution. The reaction solution was stirred for 24 h, and then 3.1 mL of $NaBH_4$ (100 mM) was dropwise added. 2 h after the addition of $NaBH_4$, the reaction solution was transferred in a dialysis bag (MWCO = 3500 Da) and dialyzed against DI water eight times for 8 h. After that, the product was concentrated via centrifugation by using an ultrafiltration device (Millipore).

Characterization

The HRTEM image was taken by using a JEOL microscope (JEM 2100F, Japan) operated at an accelerating voltage of 200 kV. The SEM image was captured by using a Hitachi microscope (S-4800, Hitachi, Japan) operated at 10 kV. The dynamic and steady rheology measurements were carried out by an Anton Paar MCR302 rotational rheometer (Anton Paar GmbH, Austria). The UV-vis spectra were recorded by using a UV-vis spectrometer (Cary60, Agilent Technologies, USA). The NMR analysis was performed on a Varian 699.804 MHz NMR spectrometer (Agilent Technologies, USA) at 298.2 ± 0.1 K. The High Performance Liquid Chromatography (HPLC) analysis was conducted on an HPLC instrument (Agilent 1200, USA) equipped with a C18

column (4.6 mm diameter, 150 mm length, 5 mm particle size, ZORBAX Eclipse XDB, Agilent, USA).

Formation of supramolecular hydrogels

To form supramolecular hydrogel, D-PEG or DEPt-PEG were suspended in aqueous solution at a D-PEG concentration of 50 mg/mL, and α-CDs were dissolved at a concentration of 140 mg/mL. The equal amount of the two solutions were mixed and sonicated to generate a homogenous solution, and then the sol solution was left for 24 h without interruption for gelation. For the formation of drug-laden hydrogel, 33 μL of BTZ (10 mg/mL) in DMSO was primarily mixed with 1 mL of α-CDs (140 mg/mL) in aqueous solution, and then the aforementioned procedure was conducted for gelation.

Thermo-triggered hydrogel degradation

D-PEG/α-CD hydrogel was tested for the thermo-triggered gel degradation. The hydrogels (0.3 cm^3) formed in the bottom vials were sealed by caps, and then were placed upside down in an oven heated to a temperature of 30, 40, 50, and 60 °C, respectively. 30 min later, the vials were taken out for observation. For the rheology measurement, the hydrogel was formed on the aluminum plate, and then a temperature-dependent rheology measurement was carried out while the system temperature increased at a rate of 2 °C/min from 25 to 65°C.

Photothermal properties of the hydrogel

D-PEG/α-CD or DEPt-PEG/α-CD hydrogel (0.4 cm^3) was formed in a plastic cuvette (the cross-section dimension is 1×1 cm), and then was irradiated by a NIR laser (808 nm, New Industries Corp., Changchun, China) at a power density of 0.62 W cm^{-2} for 20 min. The time-elapsed temperatures and thermographs of the hydrogel were recorded by using an infrared thermal camera (Magnity Electronics, China).

NIR-triggered hydrogel degradation

To well evaluate NIR-triggered gel degradation, a fluorescent dye, rhodamine B was embedded in D-PEG/α-CD or DEPt-PEG/α-CD hydrogel. The hydrogels formed in bottom of vials were irradiated by NIR laser at a power density of 0.62 W cm^{-2} for 20 min, and the vials were sloped to a certain angle to observe the liquidity of the hydrogels. To determine the degradation percentage of hydrogels, the as-generated sol solution after NIR irradiation was removed by pipette, and the remaining hydrogel was weighed. The degradation percentage of the hydrogel was evaluated by

the formula, degradation percentage = (1- remaining mass of hydrogel/ original mass of hydrogel)×100%.

***In vitro* hydrogel release kinetics study**

The drug-laden hydrogel (0.4 cm^3) was sealed in a dialysis bag (MWCO = 3500) and dialyzed against 50 mL of phosphate buffer solution (PBS, pH = 7.4). The drug-laden hydrogel was then irradiated by NIR laser at a power density of 0.89 W cm^{-2} for 40 min. While the NIR irradiation, an aliquot of the dialyzing solution (0.15 mL) was collected at a time point of 0, 10, 20, 30, and 40 min, respectively, and then was analyzed by using HPLC to determine the concentration of the released drug. For each collection of the dialyzed solution, the same amount of PBS was fed back to the dialyzing solution to keep the constant solution volume.

Cell Culture

Non-small-cell lung cancer cell line (PC-9) and mouse embryonic fibroblast cell line (NIH3T3) were obtained from American Type Culture Collection. NIH3T3 cells were cultured at 37 °C under a humidified 5% CO_2 in DMEM medium supplemented with 10% fetal bovine serum (Clark Bioscience), 100 units/mL penicillin, and 100 mg/mL streptomycin, and PC-9 cells in RPMI 1640 medium with 10% fetal bovine serum, 100 units/mL penicillin and 100 units/mL streptomycin.

Cell Viability Assay

The cytotoxicity of the released drug was analyzed on PC-9 cells. The cells were seeded in 96-well plate with a density of 10000 cells per well and incubated overnight at 37 °C. The released drug solution was collected via the same method used for the *in vitro* hydrogel release kinetics study. Briefly, D-PEG/α-CD or DEPt-PEG/α-CD hydrogel laden with BTZ was irradiated by NIR laser at power density of 0.89 W cm^{-2} for 20 min, and then 10 μL of the dialyzing solution was collected and added into the wells. The fresh PBS and the dialyzing solution collected from the hydrogel without NIR irradiation were used as control. After incubation for 48 h, the standard MTT assay was carried out to determine the cell viability.

The biocompatibility of DEPt-PEG/α-CD hydrogel was evaluated by AO/EB staining assay. The DEPt-PEG/α-CD hydrogel (0.05 cm^3) was formed in 96 well-plate. NIH3T3 cells were then seeded in the wells and incubated for 24 h. After that, the cells were stained with AO and EB according to the standard protocol, and then were imaged by a fluorescence microscope (Olympus, Japan).

***In vivo* NIR-triggered drug release from DEPt-PEG/α-CD hydrogel**

4-week-old male BALB/c nu/nu mice with average weight of 20 g were purchased from Center for Experimental Animals, East China Normal University. The animal experiments were carried out according to the National Institutes of Health guidelines for care and use of laboratory animals and approved by the ethics committee of East China Normal University. The PC-9 tumor xenograft model was established by injection of PC-9 cells (~10^6 cells) suspended in 20 μL PBS into the right back of mice via a percutaneous approach. The mice with an average tumor volume of 200 mm^3 were ready for the experiment. DEPt-PEG/α-CD hydrogel laden with Cy5.5 (30 mm^3) was injected into the tumors of two groups of mice (three mice in each group). The IVIS images of the mice were recorded 24 h after the injection, and then one group of mice were irradiated by NIR laser at day 1^{th}, 2^{th} and 4^{th} at power density of 1.2 W cm^{-2} for 15 min each time. The final IVIS images of the two groups of mice were captured at day 5^{th} to determine the degradation of the hydrogels and the drug release.

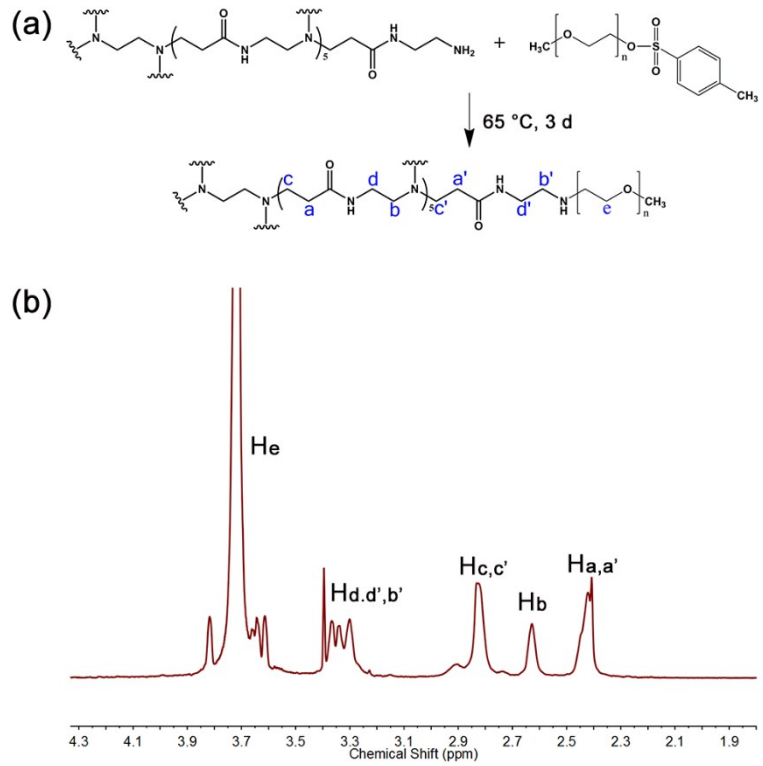

Figure S1. (a) Illustrative synthesis of D-PEG. (b) ^{1}H NMR spectrum of D-PEG.

Reference

1. R. M. Crooks, M. Q. Zhao, L. Sun, V. Chechik and L. K. Yeung, *Acc. Chem. Res.*, 2001, **34**, 181-190.