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A thermo-degradable hydrogel with light-tunable degradation and drug release

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ABSTRACT

The development of thermo-degradable hydrogels is of great importance in drug delivery. However, it still remains a huge challenge to prepare thermo-degradable hydrogels with inherent degradation, reproducible, repeated and tunable dosing. Here, we reported a thermo-degradable hydrogel that is rapidly degraded above 44 °C by a facile chemistry. Besides thermo-degradability, the hydrogel also undergoes rapid photolysis with ultraviolet light. By embedding photothermal nanoparticles or upconversion nanoparticles into the gel, it can release the entrapped cargoes such as dyes, enzymes and anticancer drugs in an on-demand and dose-tunable fashion upon near-infrared light exposure. The smart hydrogel works well both *in vitro* and *in vivo* without involving sophisticated syntheses, and is well suited for clinical cancer therapy due to the high transparency and non-invasiveness features of near-infrared light.

1. Introduction

Hydrogels have been used for a wide range of biomedical applications, such as vehicles for drug and gene delivery [1], scaffolds for tissue regeneration [2,3], and bioadhesive glues for surgical suture [4]. Hydrogel-based drug delivery systems exhibit remarkable benefits such as local drug delivery, days or even weeks of continuous drug release which together improve drug bioavailability and diminish adverse effects [5]. However, general hydrogels only allow drug release based on passive diffusion or hydrogel degradation, which cannot satisfy the requirements for on demand drug delivery with tunable release kinetics [6]. To address this problem, researchers have developed smart hydrogels that respond to diverse stimuli including pH [7], redox potential [8], enzymes [9], heat [10,11], light [12], magnetic field [13], and ultrasound [14]. Among these smart hydrogels, thermo-sensitive hydrogels are the most studied and preferred ones for controlled drug delivery [15–21].

Generally, hydrogels consisted of thermo-sensitive polymers such as poly(*N*-isopropylacrylamide) (PNIPAM) and poly(ethylene oxide) (PEO) undergo a reversible swell/shrink switch around a lower critical solution temperature (LCST) [15,16,20,22]. This property can be used to control the release of drugs loaded in the hydrogel [23]. However, the LCST-based thermo-sensitive gels suffer from several drawbacks including lack of degradability and difficulty in controlled *in situ* drug release [24,25]. Fabrication of supramolecular hydrogels by hydrogen bond is an alternative strategy to prepare thermo-sensitive hydrogels with improved performance, but the non-covalent bond mediated hydrogels usually possess poor *in vivo* stability and elasticity [7]. Currently, there still remains a significant and urgent need to develop thermo-sensitive hydrogels with excellent *in vivo* stability, inherent degradation, and reproducible, repeated and tunable drug release [23,26]. In this study, we designed a thermo-degradable hydrogel by crosslinking four-arm amine-terminated poly(ethylene glycol) (4-arm-PEG-NH_2) using an azo-containing linker. The gel is degradable above a mild temperature slightly higher than the body temperature. The gel can release encapsulated cargoes such as dyes, anticancer drugs and proteins in an on-demand and dose-tunable fashion upon near-infrared light exposure when it was loaded with photothermal nanoparticles.

2. Materials and methods

2.1. Materials

(*E*)-4,4′-(diazene-1,2-diyl)bis(4-cyanopentanoic acid), hexachloplatinic (IV) acid hexahydrate ($H_2PtCl_6 \cdot 6H_2O$), cupric chloride

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dihydrate ($CuCl_2 \cdot 2H_2O$), poly (vinylpyrrolidone) (PVP, molecular weight ~29000), potassium iodide, ethylene glycol, *N*,*N*′-dicyclohexylcarbodiimide (DCC), 4-(dimethylamino) pyridine (DMAP), rhodamine B, sulfoxide chloride, and doxorubicin (Dox) were obtained from Sigma-Aldrich (St. Louis, MO). 4-Hydroxybenzaldehyde, 4-formylbenzoic acid, methylthionine chloride, lysozyme were obtained from Aladdin (Shanghai, China). (*E*)-1,2-bis(2-(4,5-dihydro-1H-imidazol-2-yl)propan-2-yl) diazene was obtained from Titan Scientific (Shanghai, China). Dichloromethane, methanol, *N*,*N*-dimethyl formamide, triethylamine, dimethyl sulfoxide and sodium sulfate were obtained from Sinopharm Chemical Reagent (Shanghai, China). 4-arm-PEG-NH_2 (Mw: 10000) was purchased from JenKem Technology (Beijing, China). Cy5.5 was obtained from Lumiprobe Corporation (Florida, USA). Upconversion nanoparticles (UCNP) (NaYF4:Yb,Tm, 25 nm) were purchased from FuWo Corp. (Hangzhou, China).

2.2. Instruments

The NMR analysis was performed on a Varian 699.804 MHz NMR spectrometer (Agilent Technologies, USA) at 298.2 ± 0.1 K. Molecular weights of the thermo-degradable linkers (TDLs, TDL1 and TDL2) were measured by mass spectrometry (Bruker micro TOF-Q II, Germany). Rheology characteristics were quantified by monitoring the storage (G′) and loss (G″) moduli with an AR2000ex Rheometer (TA Instruments, USA) under 1.0% strain and 1.0 Hz. Transmission electron microscopy (TEM) images of dendritic platinum-copper nanoparticles (DPCN) and UCNP were taken using a microscope (HT7700, HITACHI, Japan) operated at an accelerating voltage of 100 kV. The efficiency of DPCN to covert NIR excitation to heat was measured by an infrared thermal camera (Magnity Electronics, China). The ultraviolet−visible (UV−Vis) absorption spectra of the nanoparticles were recorded using a Cary 60 UV−Vis spectrophotometer (Agilent Technologies, USA). The ability of UCNP to convert an NIR excitation to localized UV/Vis emission was investigated by transient fluorescence excitation spectroscopy (Steady-state & Time-resolved Fluorescence Spectrofluorometer, QM/TM/IM, PTI Co. Ltd, USA). The released lysozyme and Dox were measured by the UV−Vis spectrophotometer. The luminescence images of mice were taken by an IVIS (Lumina-II, Caliper Life Sciences, USA). UV laser with a wavelength of 365 nm (10 W, Andi Tech Co. Ltd, Zhejiang, China) was used as the UV light source in UV-triggered gel degradation study. An 808 nm NIR laser (New Industries Corp., Changchun, China) was used as the light source for the lysozyme release and *in vivo* Cy5.5 release studies. A 980 nm NIR laser (980 nm, Longxi photoelectric Co. Ltd, Shanghai, China) was applied as the light source for the Dox release and *in vivo* cancer therapy studies.

2.3. Synthesis of TDL1 and TDL2

All the reactions were carried out with the use of standard techniques under an inert atmosphere with clean and dry devices unless otherwise specified. For TDL1, to a stirred solution of 4-formylbenzoic acid (3.0 g, 20 mmoL) in 20 mL toluene, sulfoxide chloride (2.18 mL, 30 mmoL) and *N*,*N*-dimethyl formamide (0.15 mL) were added dropwisely at room temperature. The devices were connected with a spherical drying pipe filled with anhydrous calcium chloride and an anti-siphon bubbler, and the reaction mixture was heated to reflux for 2 h. The reaction mixture gradually turned from suspension into transparent solution. After 1.5 h, the solvent and residual sulfoxide chloride were removed under reduced pressure. The concentrated solution was added into a stirred solution of (*E*)-1,2-bis(2-(4,5-dihydro-1H-imidazol-2-yl) propan-2-yl) diazene (1.25 g, 5 mmoL) and triethylamine (5.57 mL, 40 mmoL) in 50 mL chloroform in an ice bath under argon atmosphere. The reaction solution was then stirred at room temperature for 12 h, followed by addition into methanol (0.43 mL, 10 mmoL), and the final product was dried over anhydrous sodium sulfate, concentrated and purified with column chromatography (chloroform/methanol = 80:1) to obtain light yellow powders (2.10 g). The light yellow powders were added into 4 mL acetone and stirred for 2 h under argon atmosphere, and then filtrated to obtain white powders (TDL1, 1.65 g, 64%).

For TDL2, to a stirred mixture of (*E*)-4,4′-(diazene-1,2-diyl)bis(4-cyanopentanoic acid) (3.39 g, 12.1 mmoL) and 4-hydroxybenzaldehyde (2.68 g, 22 mmoL) in 80 mL dichloromethane in an ice bath, DCC (4.99 mg, 24.2 mmoL) and DMAP (0.27 g, 2.21 mmoL) were added dropwise under argon atmosphere. The reaction solution was stirred at room temperature for 12 h. After filtration, the filtrate was concentrated under reduced pressure. The residual was purified with column chromatography (dichloromethane/methanol = 200:1) and the product was obtained as white powders (TDL2, 4.55 g, 85%).

2.4. Preparation of thermo-degradable TDL1 and TDL2 hydrogels

TDL1 or TDL2 (50 μL, 50 mg/mL) in dimethyl sulfoxide was mixed with 4-arm-PEG-NH_2 (100 μL, 100 mg/mL) in distilled water to form the thermo-degradable hydrogels. 4-arm-PEG-NH_2 cross-linked with glutaraldehyde was prepared as the control hydrogel. For clear observation, TDL1 gel was loaded with methylthionine (blue), and TDL2 gel was loaded with rhodamine B (pink). To evaluate the thermo-degradability, the hydrogels in a glass bottle were incubated at different temperatures (25 °C, 37 °C, 43 °C, 47 °C, 67 °C, 70 °C) in an oil bath for 5 min and the bottles placed upside down were imaged using a camera. For temperature-dependent rheology measurement, the TDL1 gel was formed on the aluminum plate and the system temperature was successively set at 43 °C, 44 °C, 45 °C and 47 °C, and the rheology of TDL2 gel was measured at 67 °C, 68 °C, 69 °C and 70 °C. The system temperatures were maintained for 5 min at each point during the measurement. The weight loss of TDL1 gel was measured at 37 °C and 47 °C, respectively. Generally, the hydrogels (150 μL) formed in the vials were sealed by caps, and then placed upside down in an oven heated to 37 or 47 °C, respectively. At every interval of 5 min, the vials were taken out for observation and the solution generated by degradation was carefully removed by a pipette, and the remaining TDL1 gel was weighted.

2.5. NIR-triggered TDL1 gel degradation

DPCN were synthesized as described in a recent work [27]. 6.2 μg DPCN was loaded in 150 μL TDL1 gel which formed in the bottom of a cap sealed vial, and the gel was irradiated with an 808 nm NIR laser at a density of 5.31 W/cm^2. The solution generated by degradation was carefully removed by a pipette and the weight loss of the remained gel was recorded every 5 min. The time-elapsed temperatures and thermographs on the gel were recorded using an infrared thermal camera (Magnity Electronics, China).

2.6. NIR-triggered lysozyme release

100 μg lysozyme was loaded within 150 μL TDL1 gel for the NIR-triggered release experiment. The gels were incubated in 2 mL phosphate buffer solution (PBS, pH 7.4). A NIR (808 nm, 5.31 W/cm^2) laser was applied on the hydrogel 20 min after the gel was immersed in the buffer. The released lysozyme in the buffer was collected every 5 min and the NIR treatment was continued for