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ARTICLE

High water-content and high elastic dual-responsive polyurethane hydrogel for drug delivery

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Stimuli-responsive hydrogels are soft, biocompatible and smart biomaterials; however, the poor mechanical properties of the hydrogels limit their application. Herein, we prepared a reductant- and light-responsive polyurethane hydrogel which was made of polyethylene glycol, 1, 6-diisocyanatohexane, azobenzene, cyclodextrin and disulfide. Attenuated Total Reflectance Infrared Spectra and ^{1}H NMR were used to characterize the structure of the hydrogel. The hydrogel has high elasticity (tensile modulus 36.5±0.5 kPa and storage modulus 52.9±1.2 kPa) at high water content (91.2±0.4%). Swelling, mechanical and rheological properties of the hydrogel can be tuned by content of crosslinker, light and reductant. The hydrogel has low cytotoxicity and it can be used for drug delivery. Ultraviolet irradiation helped to load drugs and reductant accelerated drug release. With its high mechanical property and light- and reductant-responsiveness, the hydrogel is hopefully to be used as drug carrier.

INTRODUCTION

Stimuli-responsive hydrogels, which can respond to stimuli such as temperature, electric or magnetic fields, light, pH, ionic factors and chemical agents, have attracted a great deal of attention recently because such hydrogel systems can serve as functional materials with potential applications in the areas of drug/gene delivery[1-5], tissue engineering[6-8], sensors[9, 10], and so on. Because of the large water content, traditional stimuli-responsive hydrogels are quite biocompatible, but they have poor mechanical properties[11-13] at the same time. Therefore, the applications of the traditional stimuli-responsive hydrogels are limited.

Polyurethanes are important classes of polymers that have found many applications as biomaterials, due to their excellent physical properties and good biocompatibility.[14, 15] There are two categories of stimuli-responsive polyurethane hydrogels: one category is prepared by hydrophilic polyhydric alcohols, stimuli-responsive dihydric alcohols and diisocyanates; the other category is a composite which contains polyurethane matrix and stimuli-responsive polymers or particles. The hydrophilic polyhydric alcohols are usually polyethylene glycol (PEG) or PEG derivatives, since these polymers present outstanding physicochemical and biological properties, including hydrophilicity and nontoxicity.[16] The stimuli-responsive dihydric alcohols can be nitrogen compounds or carboxylates, such as bis-1,4-(hydroxylethyl) piperazine[17], 1-(2-hydroxyethyl) piperazine[18], *N*-methyldiethanolamine[19] and 2,2-dimethylol propionic acid[20]. Xiaomeng Li and co-workers[21] use 2, 2'-dithiodiethanol as a reductant-responsive dihydric alcohol. The disulfide bond can be cleavage with glutathione, which give the biodegradability to the polyurethane. However, light-responsive dihydric alcohols are rarely reported. Polyurethanes are excellent composite matrix because of their good moldability and compatibility. Stimuli-responsive polymers, such as polyacrylic acid (PAA)[22] and Poly (N-isopropylacrylamide) (PNIPAM)[23], show good stimuli-responsiveness and enhanced mechanical properties when composited with polyurethanes. Polyurethane hydrogels with fluorescent or luminescent particles and stimuli-responsive polymers can change colour by external stimuli, which can be used as sensor[24], sub-micrometer resolution imaging film[25] or paint for digital colour camera read out[26]. However, as far as we know, no articles reported the preparation of the azobenzene/polyurethane hydrogel.

Light is an ideal stimulus to manipulate the hydrogel, which is a remote stimulus that can be controlled spatially and temporally with great ease and convenience. Moreover, the light irradiation except deep-UV does not have a harmful effect on various bioactive compounds including most of the proteins. Therefore, light-responsive hydrogels are attracting great attention and a variety of systems are being developed.[27, 28] Irradiating azobenzene (Azo) units with light makes them to isomerize from the more stable trans-configuration to the less stable cis-configuration. Photo-controlled molecular recognition of cyclodextrin (CD) with Azo has been widely used for host-guest inclusion compound.[29]

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Electronic Supplementary Information (ESI) available: [^{1}H NMR spectrum of DSO in DMSO and SEM images of the hydrogel after lyophilization.]. See DOI: 10.1039/x0xx00000x

Thus, they have been used as light-responsive units in many hydrogel systems.[30-32] Light can tune the sol-gel phase transition[33], the self-assembly morphology[34, 35] and the macroscopic motion[36] of the CD and Azo hydrogel systems. As a result, the CD and Azo hydrogel systems can be used as controlled drug delivery systems[37, 38] and artificial muscles[39]. The CD and Azo can also combine with temperature or pH responsive segments to form multi-responsive hydrogel systems.[40, 41] Yoshinori Takashima and co-workers[36, 39] reported a light-responsive hydrogel system which consisted of CD, Azo and polyacrylamide, and it could be used as photoresponsive artificial muscle. However, the mechanical strength of the hydrogel was low which might limit its application.

Herein, we successfully prepared a light- and reductant-responsive polyurethane hydrogel. The hydrogel were made of biocompatible and hydrophilic PEG, hydrophobic 1, 6-diisocyanatohexane (HDI), photo-switched supramolecular Azo and CD and disulfide contained 3, 3'-disulfanediyldipropane-1, 2-diol (DSO). With its high mechanical property and light- and reductant- responsiveness, the hydrogel may have meaningful applications.

EXPERIMENTAL SECTION

Materials

Poly ethylene glycol (PEG; M_n = 10000, PDI ≤ 1.1, Alfa Aesar), β-cyclodextrin(β-CD; 98%, Alfa Aesar), 3-mercapto-1,2-propanediol (90%, Alfa Aesar), 4-phenylazophenol (azo-OH; 98%, Alfa Aesar),1,6-diisocyanatohexane (HDI; 99%, J&K Chemical Ltd.), Tin(II) bis(2-ethylhexanoate) (Sn(II), 99%, J&K Chemical Ltd.), coumarin-102 (99%, Aldrich) and Tris(2-carboxyethyl)phosphine hydrochloride (TCP·HCl; 99%, Alfa Aesar) were all used without further purification. Dimethyl Formamide (DMF) was dried over calcium hydride (CaH_2) and distilled under reduced pressure immediately before use. All other solvents were purchased from Beijing Chemical Reagent Factory and used without further purification.

Preparation of 3, 3'-disulfanediyldipropane-1,2-diol (DSO)

The crosslinker with disulfide bond was synthesized via oxidation of 3-mercapto-1, 2-propanediol and the procedure was as followed: In a round bottom flask, the 3-mercapto-1, 2-propanediol (90%, 12.0 g, 100 mmol) was dissolved in 20 mL methanol, hydrogen peroxide (30% aqueous solution, 6.8 g, 60 mmol) was then added dropwise. The reaction mixture was left to stir at room temperature for 6 hours and the solvent was thereafter evaporated. The crude product was recrystallized in hexane/methanol mixed solvent (the ratio of hexane to methanol is 3:1) to obtain a white solid (9.9 g, yield: 93.1%). ^{1}H NMR (DMSO-D_6, ppm; Figure S1): 4.83-4.98 (d, 2H, CH_2-O-*H*), 4.55-4.70 (t, 2H, CH-O-*H*), 3.60-3.74 (m, 2H, C-*H*), 3.28-3.46 (m, 4H, CH_2), 2.65-3.00 (m, 4H, S-CH_2).

Preparation of hydrogel

All hydrogels were prepared by similar procedure and a representative procedure for preparing H2.5/2.5 was as followed: In a three neck flask protected by N_2 atmosphere, the PEG (8.370g, 0.837mmol) was dissolved in 30 mL DMF. The solution was heated to 90 °C, and HDI (0.988 g, 5.874mmol) and Sn(II) (0.023 g, 0.057 mmol) were added to the solution. The reaction mixture was magnetically stirred for 3 hours at 90 °C and the solution was cooled to room temperature. Azo-OH (0.044 g, 0.222 mmol), β-CD (0.250 g, 0.222 mmol) and DSO (0.250 g, 1.167 mmol) were dissolved in 10 mL DMF and the solution was mixed with the reaction mixture for 30 minutes. The mixture was then poured into the mould and the mould was heated to 85 °C. The reaction mixture was cured for 24 hours to form gel. The gel was immerged into THF and methanol to remove unreacted molecules and high boiling point DMF. After immerging three times of both THF and methanol, the gel was dried in vacuum to remove the solvent. The dry gel was then immerged in water for 18 hours to form the hydrogel.

Characterization

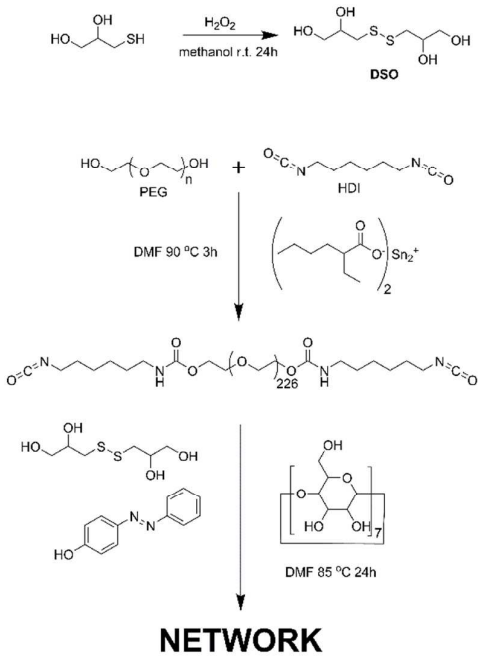
Infrared spectra were collected on dried samples by attenuated total reflectance (ATR) FTIR using a Nicolet 5700 FTIR spectrometer (Thermo Electron Corp, Madison, WI) equipped with a Nicolet Smart Orbit ATR accessory incorporating a diamond internal reflection element. Spectra were collected at a resolution of 4 cm^{-1} in the range 4000-600 cm^{-1} for a total of 32 scans for all the samples and are not corrected for penetration depth variation.

^{1}H NMR measurements were carried out on a Bruker AV400 spectrometer at room temperature with $CDCl_3$ as a solvent.

In order to investigate photoresponse, UV irradiation was applied by a UV lamp (8 W) with an emission maximum at 365 nm for the samples.

Swelling Measurements

The water-swelling behaviour of the polyurethane hydrogel was studied by a gravimetric procedure. Each dry sample was immersed in deionized water at room temperature for 24 hours. The water-swollen samples were removed from the



Scheme 1. Synthetic Route of DSO and hydrogel.

bath at prescribed time points, gently pressed between two pieces of filter paper to remove any excess water on the surface, and weighed by an electronic analytical balance (AL204, Mettler Toledo Inc.) with an accuracy of (10^{-4} g. The water absorption ratio (WAR) and the water content (WC) were quantified using the equation:

$$WAR = \frac{m_t - m_0}{m_0}, \quad (1)$$

$$WC = \frac{m_t - m_0}{m_t} \times 100\%, \quad (2)$$

where m_t and m_0 are the mass of swollen and dried samples, respectively.

All mechanical tests were performed in air and at room temperature using a universal mechanical tester (INSTRON5567) with a 10 N load cell at a constant strain rate of 0.25 min^{-1}. The water loss was measured to be less than 5% during the course of the experiments. Rheological characterization of the hydrogels was done with a DHR-1 rheometer (TA Instruments) equipped with a steel plate geometry of 25 mm diameter. Rheological gel characteristics were monitored by frequency sweep experiments. During frequency sweep experiments the storage modulus (G') and the loss modulus (G") were measured at 25°C for a period of 5 min.

Cytotoxicity evaluation

Cytotoxicity evaluation of the hydrogel was done in its extract medium using fibroblast cells (L-929). Toward this end, the hydrogel sample was freeze-dried, and lixiviated in sterile Dulbecco's modified Eagle medium (DMEM) for 24 h at 37 °C. Next, the supernatant was passed through a 0.4 μm sterile syringe filter, and mixed with fetal bovine serum (FBS) in a 9:1 volume ratio. The mixture is denoted as extract medium. L-929 cells were seeded in 96-well plates (6500 cells/well) and pre-cultured for 24 h in 100 μL DMEM containing 10% FBS under a humidified 37 °C/5% CO_2 environment. Then, the medium was replaced with isometrical extract medium and the cells were cultured for 1, 3, 5, 7 days at 37 °C/5% CO_2 environment. 100 μL DMEM containing 10% FBS served as the negative control group and 100 μL DMEM containing 10% FBS and 0.64% phenol served as the positive control group. To determine the cell viability, 10 μL CCK8 solutions were added for further 2h incubation at 37 °C. The optical density (O.D.) of each well was read at 490 nm using microplate reader (Bio-rad Model680). Each viability test was performed in triplicate. The relative cell count ratio can be calculated from the follow formula:

$$\text{Cell viability} = \frac{\text{O.D.}_{\text{sample}} - \text{O.D.}_{\text{positive control}}}{\text{O.D.}_{\text{negative control}} - \text{O.D.}_{\text{positive control}}} \times 100\%, \quad (3)$$

Where O.D. sample, O.D. positive control and O.D. negative control are optical density of the sample, optical density of the positive control and optical density of the negative control, respectively.

Fluorescence spectra of the coumarin-102 aqueous solution were recorded on a Varian Cary Eclipse fluorescence spectrofluorometer. Excitation was carried out at 335 nm, and emission spectra were recorded ranging from 350 to 500 nm. The excitation and emission band widths were set at 5 and 10 nm, respectively. The fluorescence spectra of the coumarin-102 aqueous solution at different concentration were measured and a calibration curve between concentration and fluorescence intensity was drawn.

Drug load and release measurements

Excess amount of coumarin-102 was dispersed in aqueous solution under stirring at room temperature for 48 hours and the coumarin-102 saturated solution (0.047 mg/mL) was prepared. The swollen hydrogels (which were cut to form rectangle with similar volume and weight) were immerged into the 6 mL coumarin-102 saturated solution. 3 mL of the coumarin-102 solution were taken out and the same volume of the coumarin-102 saturated solution was added every 30 minutes. The drug load process was carried out under both visible and ultraviolet light. The fluorescence spectra of the taken out solution (I_l) and the saturated solution (I_s) were measured. The relative drug load amount was determined using the calibration curve:

$$RDLA = \frac{DLA_l}{DLA_f} = \frac{\sum_{i=1}^{l}(3I_s + 3I_{i-1} - 6I_i)}{\sum_{i=1}^{n}(3I_s + 3I_{i-1} - 6I_i)} \times 100\%, \quad (4)$$

where RDLA is relative drug load amount, DLA_l and DLA_f are drug load amount of the sample at certain time and drug load amount of the sample at final time, respectively, I_l and I_s are the fluorescence spectra of the taken out solution and the saturated solution, respectively, and l and n are drug load time and final drug load time, respectively.

The swollen hydrogels were immerged into the coumarin-102 saturated solution for 2 days at room temperature and final drug load amount were measured. The samples were then taken out and put into 6 mL deionized water. 3 mL of the aqueous solution were taken out and the same volume of

deionized water was added every 30 minutes. The drug release process was carried out under control, reductive treatment and UV irradiation. The fluorescence spectra of the taken out solution (I_r) were measured. The relative drug release amount was determined using the calibration curve:

$$RDRA = \frac{DRA_r}{DLA_f} = \frac{\sum_{i=1}^{r}(6I_i - 3I_{i-1})}{\sum_{i=1}^{n}(3I_s + 3I_{i-1} - 6I_i)} \times 100\% , \qquad (5)$$

where RDRA is relative drug release amount, DRA_r and DLA_f are drug release amount of the sample at certain time and drug load amount of the sample at final time, respectively, I_r and I_s are the fluorescence spectra of the taken out solution and the saturated solution, respectively, and r and n are drug release time and final drug load time, respectively.

Result and discussion

Preparation and structure characterization of the hydrogel

We prepared a polyurethane hydrogel by the reaction between hydroxyl and isocyanate. PEG was used as soft and hydrophilic segment, β-CD, azo-OH and DSO were used as crosslinker, and HDI was used to link the hydroxyl compound. The dry gel (Figure 1a) was translucent, yellow and tough. When the dry gel swelled with water (Figure 1c), it became transparent, light yellow and soft. The dry gel was characterized by ATR-FTIR (Figure 2). The peak at 3338 cm^{-1} was the stretching vibration of N-H bond and 1716 cm^{-1} was the stretching vibration of C=O bond, which indicated the existence of urethane group in the gel. The peak at 1107 cm^{-1} was the stretching vibration of C-O bond, which indicated the existence of PEG segment in the gel. The peak around 1900~2000 cm^{-1} was the overtone band of benzene derivatives, which indicated the existence of azobenzene group in the gel. The ATR-FTIR result proves that the gel is a hydrophilic polyurethane contained azo group.

The structure of the PEG prepolymer before crosslinking was characterized by ^{1}H NMR (Figure 3). The sample was prepared by the reaction between excess HDI and PEG (the mole ratio of HDI to PEG was 7:1): the mixture was magnetically stirred for 3 hours at 90 °C and ethanol was added to terminate the reaction; the mixture was precipitated in ether and dried in vacuum; the obtained white solid was dissolved in $CDCl_3$ for ^{1}H NMR characterization. The integral area ratio of the peaks of PEG (peak a), the peaks of hexamethyl (peak b, c or d) and the peaks of methyl ends

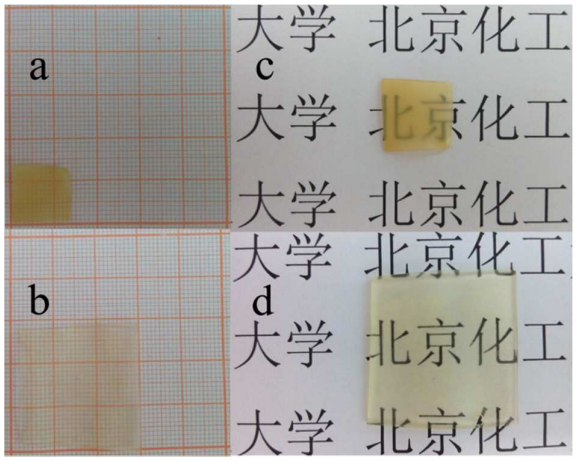


Figure 1. Digital photograph of the dry (a, c) and swollen (b, d) gel.

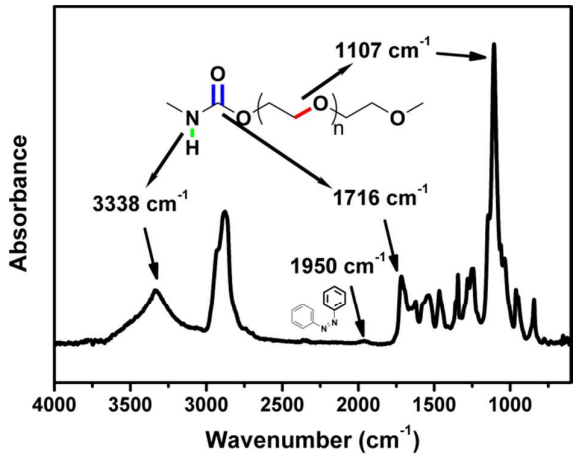


Figure 2. ATR-FTIR of the dry gel.

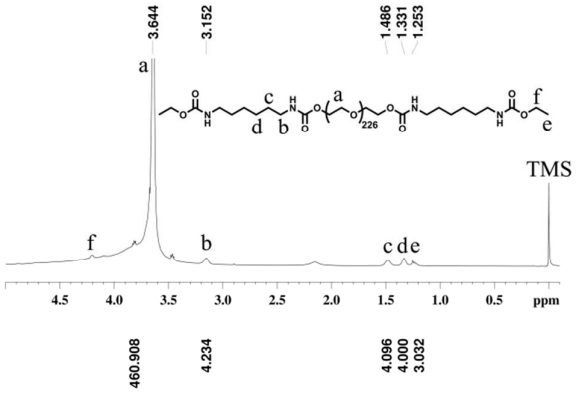


Figure 3. ^{1}H NMR of isocyanate-terminated PEG.

(peak e) was 460:4:3, which was close to the theoretical ratio (454.5:4:3). This indicated that isocyanate-terminated PEG was prepared when the mole ratio of PEG to HDI was 7:1 and little chain extension products were obtained. The precise structure of the isocyanate-terminated PEG was good for preparation and was able to improve mechanical properties. Other samples with different mole ratios of HDI to PEG were also prepared.

The unique isocyanate-terminated PEG was obtained when the ratio was larger than 7:1. However, the ratio smaller than 7:1 caused chain extension and the mixture had high viscosities and the products were hard to dissolve in solvents.

Formula design and high elasticity of the hydrogel

The procedure of hydrogel preparation was as followed: the end-groups of PEG were modified by excess HDI; the hydroxyl containing crosslinkers (CD, AZO and DSO) were linked by the residual HDI and isocyanate-terminated PEG chains to form gel network.

The formulas are shown in Table 1: the mole ratio of HDI to PEG was more than 7:1 to avoid chain extension of PEG; the mole ratio of the CD to the azo-OH was 1: 1 and the mole ratio of isocyanate to hydroxyl was 1.05: 1. The swelling and rheological properties of the hydrogels were measured and each test was performed in triplicate (Table 2). The storage modulus (G') increased and the water absorption ratio, water content and loss modulus (G") decreased as the amount of the crosslinkers increased. The crosslinked part was hydrophobic and inhibited the water absorption. The increase of crosslinking density enhanced the elasticity of the hydrogel. The hydrogels with only light-responsive or reductant-responsive crosslinkers were prepared. The hydrogels with sample crosslinker amounts had similar properties because the hydroxyl amounts per gram of CD (0.0185 mol/g) were close to that of DSO (0.0186 mol/g). As a result, the properties of the hydrogel can be optimized by adjusting the crosslinker amount.

The swelling, tensile and rheological properties of sample H2.5/2.5 were studied, because it had high water content with appropriate mechanical properties. The water absorption ratio was 10.4±0.2, the water content was 91.2±0.4% and the volume swelling ratio was 12.8±0.4. With the high water content, the hydrogel was able to stretch and warp. The tensile strength was 37.7±1.1 kPa, the elongation at break was

Table 1. The formula of the hydrogels at different crosslinker content.

Sample name[a]	Mole ratio of HDI to PEG	PEG (g)	CD (g)	AZO[b] (g)	DSO (g)	HDI (g)[c]
H2.5/2.5	7.0:1	8.370	0.250	0.044	0.250	0.988
H3.75/3.75	10.8:1	7.640	0.375	0.065	0.375	1.395
H5.0/5.0	15.5:1	6.912	0.500	0.087	0.500	1.802
H5.0/0	7.2:1	8.312	0.500	0.087	0	1.003
H0/5.0	6.9:1	8.428	0	0	0.500	0.973

a: the samples are named by the content of the crosslinkers. The first number is the mass percentage content of CD and the second number is the mass percentage content of DSO. b: the mole ratio of CD to AZO is 1:1. c: the mole ratio of isocyanate to hydroxyl is 1.05: 1 and the dosage of Sn(II) is 2 wt% of HDI.

Table 2. Swelling and rheological properties of the hydrogel at different crosslinker content.

	H2.5/2.5[a]	H3.75/3.75	H5.0/5.0	H5.0/0	H0/5.0
Water Absorption Ratio	10.4±0.2	7.3±0.3	5.3±0.3	9.7±0.3	9.8±0.1
Water Content (%)	91.2±0.4	88.2±0.5	84.2±0.6	90.4±0.5	90.8±0.3
G' (kPa)[b]	52.9±1.2	56.4±0.7	59.5±1.1	52.2±1.3	50.5±0.9
G" (kPa)[b]	6.8±0.2	2.2±0.2	0.5±0.2	7.0±0.3	6.5±0.3

a: the error are calculated by three-time preparation (same conditions and formulas).b: G' and G" are measured by frequency sweep and the data at 10 rad/s are shown in the table.

110±3.2% and the tensile modulus (G_T) was 36.5±0.5 kPa. The G' was 52.9±1.2 kPa and the G" was 6.8±0.2 kPa. G_T or G' of normal stimuli-responsive hydrogels were usually less than 10 kPa.[42] Even though the strength of the hydrogel was smaller than that of double network (DN) gel[43], it was similar to that of slide ring (SR) gel and nanocomposite (NC) gel.[44] These indicated that we obtained a high elastic hydrogel with high water content.

Xuanhe Zhao[45] reported that high elastic hydrogels should have interpenetration of long-chain networks, hybrid physical and chemical crosslinkers, high-functionality crosslinkers, networks with long monodisperse polymer chains or meso-/macro-scale composites. The hydrogel we obtained is a high elastic hydrogel possibly because the hydrogel have hybrid physical and chemical crosslinkers, high-functionality crosslinkers and networks with long monodisperse polymer chains. We designed such a formula: the hydrogel contained both hydrophobic azobenzene and CD cavity and the mole ratio of azo to CD was 1: 1; the crosslinkers contained more hydroxyl than PEG and the mole ratio of HDI to PEG was larger than 7: 1. The excess HDI first reacted with the monodispersed high molecular weight PEG to form isocyanate-terminated PEG chains rather than chain-extended product; the rest of the HDI linked CD, azo-OH and DSO to form multi-hydroxyl crosslinkers and the isocyanate-terminated PEG chains were crosslinked by the multi-hydroxyl crosslinkers at the same time. When the gel was swollen with water, the azo group at the end of the PEG chains or on the crosslinkers trended to go into the CD cavity and the urethane group formed hydrogen bond. The supramolecular interaction between azo and CD, hydrogen bond and urethane chemical bond connected hydrophilic PEG chains to crosslinkers indicating that the hydrogel had hybrid physical and chemical crosslinkers. The hydrogel also had had high-functionality crosslinkers (multi-hydroxyl crosslinkers) and long monodisperse polymer chains (isocyanate-terminated PEG chains), which resulted in a high elastic hydrogel. We were interested in the high elasticity of the hydrogel and also hoped the hydrogel have good properties for application. Light-controlled supramolecular assembly of CD and AZO and reductant-responsive disulfide bond were used in the hydrogel in order to endow environment responsiveness to the high elastic hydrogel. As expected, the swelling, mechanical and rheological properties of the hydrogel could be tuned by light and reductant.

Swelling properties

Water content, water absorption ratio and volume swelling ratio were measured to characterize the swelling properties of the hydrogel. The hydrogel was immerged into reductive solution or irradiated by ultraviolet light and the swelling properties were tuned by reductant or UV irradiation.

To observe the reductant-responsiveness, the hydrogel was immerged into 0.1 mg/mL Tris(2-carboxyethyl)phosphine hydrochloride (TCP·HCl) aqueous solution. The weight of the hydrogel at different time was measured. The ratio of the weight at different time to the original weight was calculated. The relationship between the weight ratio and time was drawn in Figure 4. We can see from the figure that the weight of the hydrogel increases over time, which results in the increase of the water content and the water absorption ratio. The water content, the water absorption ratio and the volume swelling ratio of the control sample were 91.2±0.4%, 10.4±0.2 and 12.8±0.3, respectively. After 24 hours treatment under TCP·HCl aqueous solution, the water content, the water absorption ratio and the volume swelling ratio of the hydrogel were 95.5±0.2%, 21.3±0.2 and 25.0±0.4, respectively. After 48 hours treatment

under TCP·HCl aqueous solution, the water content, the water absorption ratio and the volume swelling ratio of the hydrogel were 95.8±0.2%, 22.7±0.3 and 26.7±0.4, respectively. The reductant continuously and effectively increased the swelling properties of the hydrogel. The reason why the reductant increased the swelling properties of the hydrogel was that the reductant could break the disulfide bond at crosslinking points, which resulted in the reduction of crosslinking density and the hydrogel was looser to accommodate more water.

To observe the light responsiveness, the hydrogel was immerged into aqueous solution and irradiated by UV light. The weight of the hydrogel at different time was measured. The weight ratio of the weight at different time to the original weight was calculated. The relationship between the weight ratio and time was drawn in Figure 5. We can see from the figure that the weight of the hydrogel decreases over time, which results in the decrease of the water content and the water absorption ratio. The water content, the water absorption ratio and the volume swelling ratio of the control sample were 91.2±0.4%, 10.4±0.2 and 12.8±0.3, respectively. After 24 hours treatment under UV irradiation, the water content, the water absorption ratio and the volume swelling ratio of the hydrogel were 90.0±0.6%, 9.0±0.3 and 11.2±0.3,

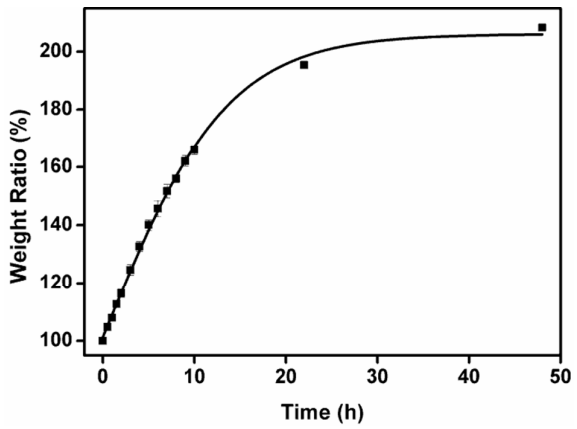


Figure 4. Weight ratio changes of the hydrogel under the treatment of 0.1 mg/mL TCP·HCl aqueous solution.

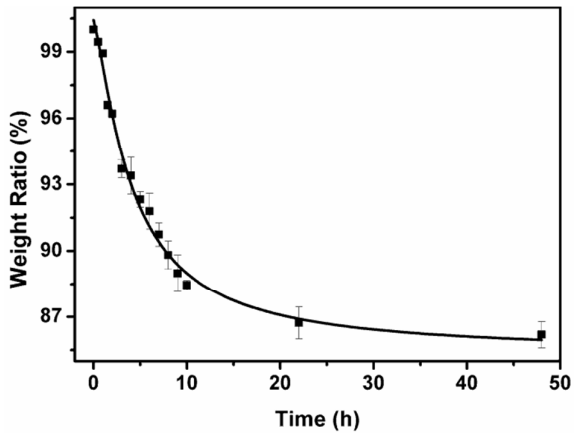


Figure 5. Weight ratio changes of the hydrogel under the treatment of UV irradiation.

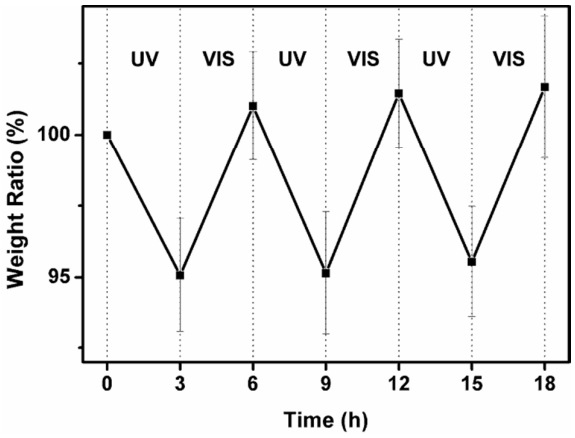


Figure 6.Reversible changes of the weight ratio under the irradiation of ultraviolet and visible light.

respectively. After 48 hours treatment under UV irradiation, the water content, the water absorption ratio and the volume swelling ratio of the hydrogel were 89.8±0.8%, 8.9±0.3 and 11.2±0.4, respectively. The reason why UV irradiation decreased the swelling properties of the hydrogel was that UV irradiation could isomerize the trans-azo group to cis-azo group, which resulted in the exclusion of the hydrophobic azo group from CD cavity. The free cis-azo group made the hydrogel more hydrophobic, so water was discharged. UV irradiation decreased the water absorption properties of the hydrogel and visible light irradiation led to the recovery of the swelling properties. As shown in Figure 6, the weight ratio present reversible change when visible and ultraviolet light are used alternately. This indicated that the swelling properties of the hydrogel could be tuned by ultraviolet and visible light and the light responsiveness of the hydrogel was reversible.

Mechanical and rheological properties

The reductant- and light- responsiveness influence not only the swelling properties but also mechanical and rheological properties of the hydrogel. The sample for tensile test and rheological test were prepared on the same film of the hydrogel. Some of the sample were immerged into 0.1 mg/mL TCP·HCl aqueous solution, some of the sample were irradiated by UV light and others were left for comparison. Tensile strength, elongation at break, tensile modulus (G_T),storage modulus (G') and loss modulus (G") of the control sample, the sample treated with TCP·HCl and the sample under UV irradiation were measured (Figure 7 and 8) and each test was performed in triplicate. Tensile strength, elongation at break, G_T, G' and G" of the control sample, the reductant-treated sample and the UV irradiated sample were shown in table 3.The viscoelasticity of the hydrogel was influenced by light and reductant.

When the hydrogel was treated with reductant, tensile strength, G_T and G' decreased and G" and elongation at break increased. These indicated that the elasticity of the hydrogel decreased and the viscosity of the hydrogel increased. The hydrogel became more flexible because

Table3. Tensile and rheological properties of the hydrogel under different conditions.

Sample	Tensile strength (kPa)[a]	Enlongation at break (%)[a]	Tensile modulus G_T (kPa)[a]	Storage modulus G' (kPa)[a,b]	Loss modulus G" (kPa)[a,b]
Control	37.7±1.1	110.0±3.2	36.5±0.5	52.9±1.2	6.8±0.2
Reductant	18.8±0.8	146.6±3.0	13.5±0.2	29.5±0.8	13.3±0.1
UV Irradiation	26.6±0.7	72.3±2.3	39.5±0.5	59.1±1.1	1.0±0.1

a: the error are calculated by three-time preparation (same conditions and formulas). b: G' and G" are measured by frequency sweep and the data at 10 rad/s are shown in the table.

the reductant broke the disulfide bond at crosslinking points, which resulted in the reduction of crosslinking density and the hydrogel was looser to accommodate more water. The free polymer chains entangled which increased the viscosity and decreased the elasticity of the hydrogel.

When the hydrogel was irradiated by UV light, tensile strength, G_T and G' increased and G" and elongation at break decreased. These indicated that the elasticity of the hydrogel increased and the viscosity of the hydrogel decreased. The hydrogel became stiffer because UV irradiation isomerized the trans-azo group to cis-azo group, which resulted in the exclusion of the hydrophobic azo group from CD cavity. The hydrophobic cis-azo caused

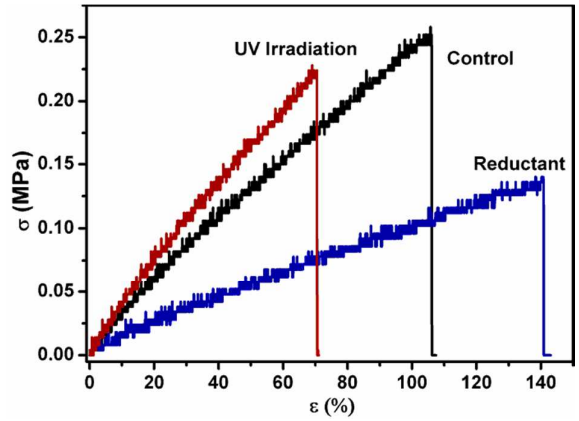


Figure 7. Stress-strain curves of the hydrogel samples under control, reductive treatment and UV irradiation.

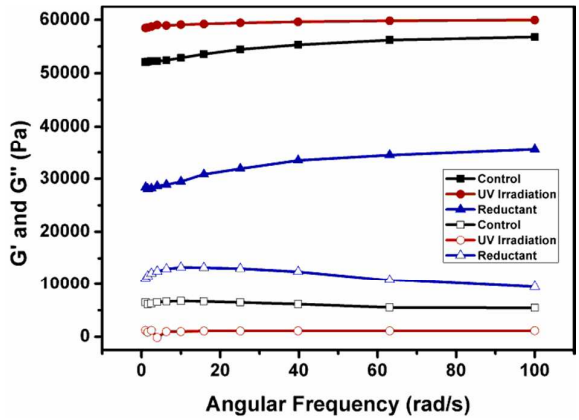


Figure 8. Storage modulus (G', solid) and loss modulus (G", hollow) of the hydrogel samples under control (square), reductive treatment (triangle) and UV irradiation (circle).

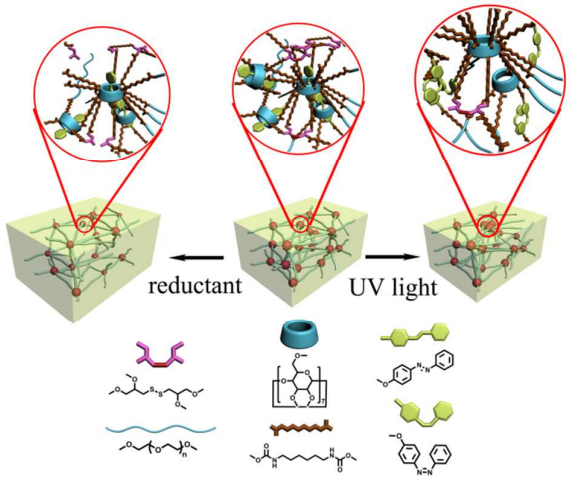


Figure 9. Possible structures of the hydrogel ball-and-stick models (ball for crosslinkers and stick for polymer chains). UV light irradiation make azo dislocate from CD cavity and the crosslinkers aggregate by hydrophobic interactions. Reductant breaks the disulfide bond and the crosslinkers dissociated.

the aggregation of the crosslinkers and limited the movement of the polymer chains. The bound polymer chains increased the elasticity and decreased the viscosity of the hydrogel.

Cell viability

The cytotoxicity of the hydrogel was evaluated by the CCK8 cell viability assay. DMEM containing extract medium of hydrogel (hydrogel sample), absolute DMEM (negative control) and DMEM containing 10% FBS (positive control) were used for cell culture. The results presented in Figure 10 reveal that the viability of the proliferated cells on the hydrogel increases with increasing cell culture time. L929 cells on the hydrogel sample and on the negative control were increasing in viability and L929 cells on the positive control were keeping invariable. The cell viability for day 1, 3, 5 and 7 were 92.7%, 87.9%, 79.5% and 73.9%, respectively. These result suggested that the hydrogel had good cytocompatibility in five days, but long time contact might cause cell toxicity. Long-time immersion in water caused the hydrolysis of the urethane bond in hydrogel and made the hydrogel toxic. However, the hydrogel had good cytocompatibility for short time usage.

Drug load and release test

The nano-sized porous structure of the gel was observed by scanning electron microscope (SEM) (Figure S2). The

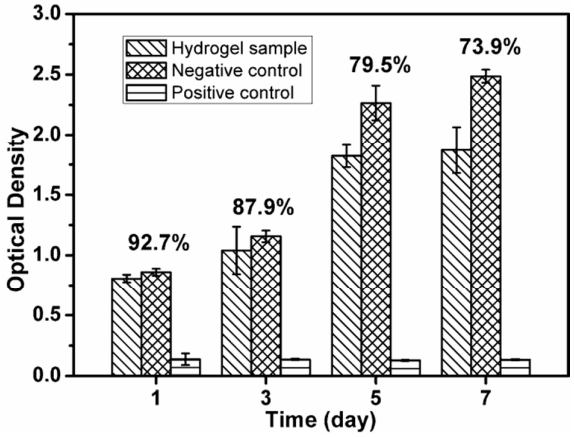


Figure 10. CCK8 results of L929 cells in the extract medium of the hydrogel, negative control and positive control sample. The cell viability for each day was marked on top of the bar graph.

porous structure may be beneficial to load drugs. The reductant- and light- responsive hydrogel can be used for drug delivery. The coumarin-102 was used as a hydrophobic model drug and we hoped that light and reductant would tune the drug load and release process. Light irradiation was a reversible and effective way to tune the drug load process. Short-time irradiation did not break the chemical structure of the hydrogel and could improve the drug load amount. We can see from Figure 11 that the relative drug load amount of the sample under UV irradiation is larger than that of the control sample. The increase of drug load rate could be explained as follows: UV light changed the hydrophilicity of the hydrogel; UV irradiation isomerized the trans-azo group to cis-azo group and the hydrophobic azo group excluded from CD cavity; the free cis-azo group made the hydrogel more hydrophobic, so the hydrophobic drug trended to load on the irradiated hydrogel. UV irradiation could contribute to the drug load process, while it influenced little on the drug release process (Figure 12).

The hydrogel was immerged in saturated coumarin-102 for 12 h under UV light irradiation. The drug load amount was calculated and the sample was used for drug

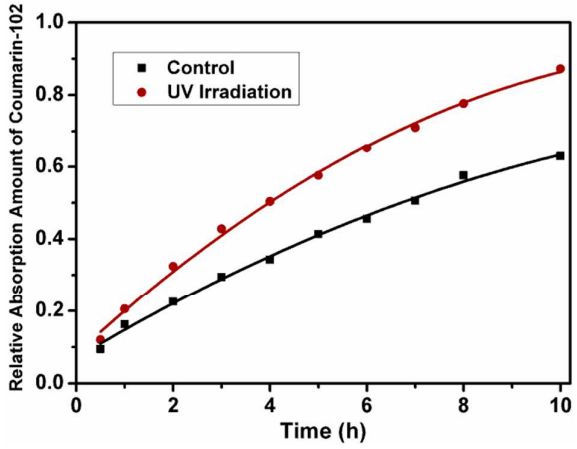


Figure 11.Relative drug load amount of the control sample and the sample under UV irradiation.

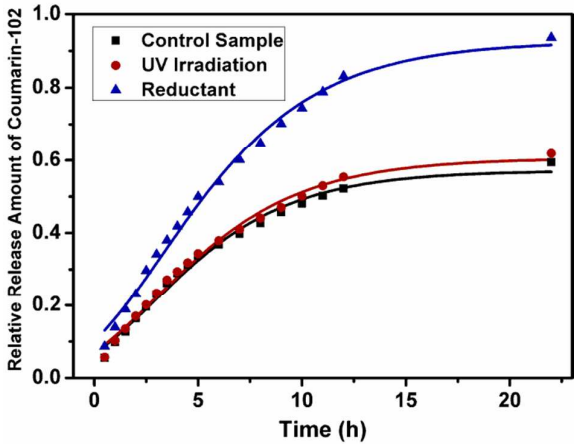


Figure 12.Relative drug release amount of the sample under control, reductive treatment and UV irradiation.

release study. Reductant can contribute to the drug release process. The hydrogel immerged in TCP·HCl solution release the drug faster than the hydrogel immerged in aqueous solution (Figure 12). The increase of drug release rate was because reductant made the hydrogel looser and more hydrophilic than the control sample. Reductant could break the disulfide bond at crosslinking points and decreased the crosslinking density. The hydrogel were looser to accommodate more water. The hydrogel was more hydrophilic and the drug trended to diffuse to the low concentration aqueous media.

Our drug delivery systems tuned drug load and release process individually. Light tuned drug load amount within limits and did not damage the structure of the hydrogel. Reductant accelerated drug release but UV light influenced the drug release process little. As a result, we can use light to tune drug load process and use reductant to tune drug release process individually. The individual control may be good for application.

Conclusions

We prepared a light- and reductant- responsive polyurethane hydrogel which were made of low cytotoxic and hydrophilic PEG, hydrophobic HDI, photo-switched supramolecular Azo and CD and disulfide contained DSO. High content of crosslinker can improve the elasticity of the hydrogel but goes against water absorption. The hydrogel H2.5/2.5 had high water content (91.2±0.4%). The hydrogel also had high elasticity (tensile modulus 36.5±0.5 kPa and storage modulus 52.9±1.2 kPa) and the swelling, mechanical and rheological properties of the hydrogel could be tuned by reductant and light. Reductant increased the water content and viscosity and decreased the elasticity, while UV irradiation decreased the water content and viscosity and increased the elasticity. The hydrogel has low cytotoxicity and the drug delivery process could be tuned by light and reductant. UV irradiation could contribute to drug load process and reductant could accelerate drug release process. With its

high mechanical property and light- and reductant-responsiveness, the hydrogel is hopefully to be used as drug carrier.

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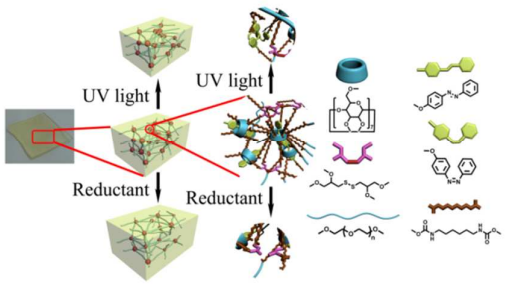

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The light- and reductant- responsive hydrogel with high elasticity can be used for drug delivery and wound dressing.