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Well-defined and Biocompatible Hydrogels with Toughening and Reversible Photoresponsive Properties

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Abstract

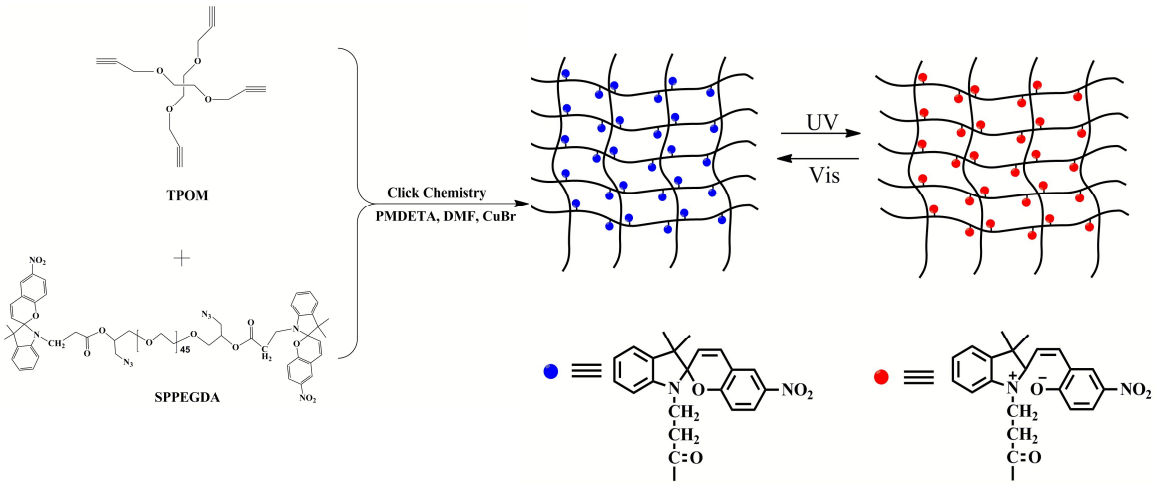
In the present study, novel hydrogels with extremely high strength, reversible photoresponsive and excellent biocompatible properties were prepared. The functional hydrogels were synthesized from a well-defined poly (ethylene glycol) polymer with spiropyran groups at a given position (PEG-SP) via Cu (I)-catalyst Azide-Alkyne Cycloaddition (CuAAC) reaction. The molecular structures of the sequential intermediates for PEG-SP hydrogel preparation were verified by ^{1}HNMR and FT-IR. The mechanical property, swelling ratio, compression strength, surface hydrophilicity, and biocompatibility of the resulting hydrogel were characterized. Since spiropyran is pivotal to the switch in hydrophilicity on the hydrogel surface, the swelling ratio of PEG-SP hydrogel under Vis irradiation has a major decrease (155%). Before and after UV light irradiation, the contact angle of the hydrogel has a change of 13.8°. The photoresponsive property of this hydrogel was thus demonstrated, and such property was also shown to be reversible. The well-defined PEG-SP hydrogel can also sustain a compressive stress of 49.8 MPa without any macro- or micro-damage, indicating its outstanding mechanical performance. Furthermore, it possessed excellent biocompatibility as demonstrated by its performance in an in vivo porcine subcutaneous implantation environment. No inflammation was observed and it got along well with the adjacent tissue. The above features indicate that PEG-SP hydrogels are promising as an implantable matrix for potential applications in biomaterial.

Introduction

Hydrogels have emerged as promising materials in many fields for their special three-dimensional network structure.[1-3] In recent years, "smart" hydrogel has received increasing attention because of its sensitivity to external stimuli, i.e. reactivity actively and reversibly to environment changes.[4] External stimuli could be varied, such as temperature,[5] pH,[6] light,[7,8] electric field,[9] and magnetic force.[10] Smart hydrogels could undergo reversible volume phase transitions or gel–sol phase transitions in response to external stimuli. It has been used extensively in diverse applications, such as artificial muscles,[11,12] immobilization of enzymes and cells,[13,14] and smart drug delivery systems.[15]

Spiropyran (SP) has a reversible structure which consists of two orthogonal aromatic rings connected by a carbon atom of sp^3 hybridization.[16,17] Upon the irradiation of ultraviolet light, the original colorless and hydrophobic SP undergoes the cleavage of C=O bond, forming a colorful and hydrophilic merocyanine (MC).[18,19] Since this process is often reversible and repeatable, the photo-reactive groups are attractive and useful in diverse forms to functionalize hydrogels in a broad range of applications. Moreover, MC can complex with metal cations via the phenolate oxygen atom,[20] which has been attracted more attentions on the design of photochromic sensors for detecting heavy metal ions.[21,22] Taking the above advantages of SP moieties, photo-responsive hydrogels were prepared and their association with peptides or other macromolecules could be controlled by light to construct a photo-responsive hydrogel system.[23]

Copper-catalyzed click chemistry, on the other hand, was shown as a great success in fabricating various functional and well-defined hydrogels because of its fast reaction process, high efficiency, reliability, no by-products, good stability and selectivity.[24–26] Therefore, it has its own superiority in fabricating devices for drug delivery and regenerative medicine.[27-29] Inspired by the advantages of photo-responsive SPs and copper (I)-catalyzed 1, 3-dipolar azide-alkyne cycloaddition (CuAAC), in this work, we designed a well-defined and SP modified PEG hydrogel (PEG-SP) by CuAAC, as shown in Scheme 1. The resulting PEG-SP exhibited specific optical performance, high mechanical performance and good biocompatibility.



Scheme 1 Synthesis of PEG-SP hydrogel by Click Chemistry.

Experimental methods

Materials

2,3,3-Trimethylindolenine (98%), 5-nitrosalicylaldehyde (97%), epichlorohydrin (ECH, 99%) and pentamethyldiethylenetriamine （PMDETA, 99%）were purchased

from Aladdin Industrial Corporation. 3-iodopropanoic acid (99%), 4-Dimethylamino-pyridine (DMAP, 99%), N, N'-dicyclohexylcarboiimide (DCC, 99%) were purchased from J&K Chemical Corporation. Poly(ethylene glycol) (PEG, Mn=2000, Alfa Aesar) was dried at 120^{o}C under high vacuum for at least 4h before use. Propargyl bromide (80%), sodium azide (99%), copper (I) bromide (CuBr, 98%) and pentaerythritol (99%) were purchased from Shanghai Chemical Reagent Plant. Ethyl methyl ketone, piperidine and (ethylenedinitrilo)tetraacetic acid (EDTA) were purchased from Chemical Reagent Company of National Pharmaceutica Group.

Synthesis of carboxyl-containing SP

Carboxyl-containing SP, 1-(β-carboxyethyl)-3', 3'-dimethyl-6-nitrospiro (indoline-2', 2 [2H-1] benzopyran) (SPCOOH) was synthesized according to the method reported by Chen et al..[30] Briefly, during the synthesis, all reaction vessels were wrapped with aluminum foil, so as to ensure that the reaction was performed in dark. Fifteen ml of ethyl methyl ketone solution containing 2, 3, 3-trimethylindolenine (0.02 mol) and 3-iodopropanoic acid (0.02 mol) was refluxed under nitrogen for 3 h. The resulting solid product was collected and then dissolved in water followed by being washed three times with chloroform. The above solid was further subjected to evaporation to give purified 1-(β-carboxyethyl)-2,3,3-trimethylindolenine iodide (72% yield). The above iodide, together with 5-nitrosalicylaldehyde and piperidine, at an equal molar ratio, were dissolved in ethyl methyl ketone. The red solution was refluxed for 3 h and then left overnight. A yellow crystalline solid was precipitated. It was filtered and washed with methanol thoroughly to give the product SPCOOH (72% yield).

Synthesis of Poly(ethylene glycol)-diazide (N_3-PEG-N_3)

Poly(ethylene glycol)-diazide was prepared from PEG according to the method reported previously.[31] Ten gram (2.5 mmol) of PEG_{45} (Mn=2000 g/mol) was dissolved in 50 mL of dry pyridine. The solution was cooled to 0°C, and 0.98 g (12.5 mmol) of methanesulfonyl chloride dissolved in 10 mL of dry dichloromethane was added dropwise over 20 min. The mixture was allowed to return to room temperature and stir for another 12 h. After removal of the solvent in a rotary evaporator, the residue was treated with saturated aqueous $NaHCO_3$ and extracted using CH_2Cl_2. The solution was dried over $MgSO_4$ for 10 h. The organic solvent was removed by rotary evaporation. The product (8.7 g of pale powder) was precipitated by adding excess amount of diethylether. After that, a mixture of 8 g (2 mmol) of the as-prepared PEG and 0.65 g (10 mmol) of sodium azide in 50 mL of dry DMF was allowed to react at 85°C for 24 h. Unreacted sodium azide was removed by passing the reaction mixture through an alumina column, and the filtrate was concentrated by rotary evaporation. The above polymer, at the concentrated DMF solution, was further precipitated by adding diethyl ether followed by filtration (90% yield).

Synthesis of Spiropyran-Containing Poly(ethylene glycol)-diazide (SPPEGDA)

Synthesis of SPPEGDA was shown in Scheme S1. In brief, SPCOOH (0.8 g) and N_3-PEG-N_3 (2.2 g) were reacted in 150 mL of dry methylene dichloride (CH_2Cl_2) in the presence of 0.5 g of N,N'-dicyclohexylcarbodiimide (DCC) and 0.05 g of 4-(dimethylamino)pyridine (DMAP) at room temperature for 12 h. The mixture was then filtrated to get a red filtrate. Residue CH_2Cl_2 was evaporated, and the product

was then dissolved in acetone. Afterward, the petroleum ether (acetone: petroleum ether = 1:6 in volume) was added to precipitate the product (78% yield).

Synthesis of Tetrakis (2-propynyloxymethyl) methane (TPOM)

Tetrakis (2-propynyloxymethyl) methane (TPOM) was synthesized according to the method reported previously.[31,32] About 2.5 g (0.018 mol) of pentaerythritol was added into 30 mL of DMSO containing 15.6 g (0.278 mol) of KOH. After being stirred at 5°C for 30 min, the above solution was added slowly with 20.8 g (0.17 mol) of propargyl bromide over a period of 20 min. The solution then turned brown and the reaction mixture was stirred at 40°C overnight. The reaction mixture was quenched with water and extracted thrice with 50 mL of ethyl ether. The collected organic layers were combined, washed with water and brine, and dried over $MgSO_4$. After removal of the ethyl ether by rotary evaporation, the product was further purified by passing through a silica gel column using mixed ethyl acetate/hexane (2/8 in volume ratio) as an eluent. An orange solid of about 4.18 g was obtained (81% yield).

Synthesis of hydrogels from "Click Chemistry"

Synthesis of PEG-SP hydrogels was based on our previous work with modification.[31] Briefly, SPPEGDA (0.15 g, 0.05 mmol), TPOM (7.2 mg, 0.025 mmol), PMDETA (17.4 mg, 0.1 mmol), and DMF (0.1 mL) were introduced into a plastic cylinder mold (the diameter is 8 mm and the height is 20 mm). After the mixture turned clear, the mold was degassed with argon for 30 min. It was then added quickly with 14.4 mg (0.1 mmol) of CuBr under ultrasonic agitation. The gelation point was reached in 80 s, and the reaction was allowed to continue for another 24 h at 60°C. The obtained

hydrogel was transferred to an EDTA (5%) solution to remove the copper ions and DMF. According to the similar method, PEG hydrogel using poly(ethylene glycol)-diazide (N_3-PEG-N_3) was synthesized.

Measurement of swelling ratio and mechanical properties

Swelling ratio (SR) of hydrogel was measured by a weighing method at room temperature. Fully swollen hydrogels were dried superficially with filter paper and weighed on a microbalance to record the wet weight (m_{wet}). The weighed hydrogels were then lyophilized to a constant weight (m_{dry}). SR is defined as, SR = (m_{wet} - m_{dry})/m_{dry}. The average values of at least three measurements were taken for each sample. Compressive stresses of hydrogels at fracture were measured on a WDW-05 electromechanical tester (Time Group Inc., China) at room temperature. All hydrogel samples were tested after being 70% equilibrated in deionized water. The cylinder shaped hydrogels (12 mm in diameter and 8 mm thick) were used for compression tests. The crosshead speed was set at 5 mm min^{-1}. Three specimens were tested for each kind of hydrogel.

UV–visible characterization

UV–vis adsorption spectrum of the swollen PEG-SP hydrogel was recorded on a UV–visible spectrophotometer (TU-1810, PERSEE, Beijing, China) after visible light irradiation for 1 h. The sample was then scanned by the same apparatus with the same mode after UV irradiation for 0-15 min, respectively.

Measurement of contact angle

Contact angles (CA) of the PEG-SP hydrogels were measured using the sessile drop

method at room temperature. The swollen hydrogel under visible light was dried superficially with filter paper. A 4 μL drop of deionized water was placed carefully on the surface of the hydrogel using a micropipet. The CA was monitored and recorded on a commercial CA meter (OCA 15 plus, Dataphysics, German). The hydrogels were then immersed in water to reach swelling equilibrium again and subsequently illuminated with UV (365 nm) light for 15 min. After removal of the surface water, the CA of hydrogels was measured as mentioned above.

In vivo subcutaneous implantation of PEG-SP Hydrogels in a porcine model

The experimental protocol involving animals was approved by the Animal Care and Experiment Committee of Shanghai Jiao Tong University School of Medicine. PEG-SP hydrogels were used for in vivo subcutaneous implantation in a porcine model for assessing their biocompatibility and their evoked inflammation reaction. PEG-SP hydrogels (9 mm in length, 5 mm width and 5 mm thick) were sterilized in 75% ethanol for 30 min and then washed extensively with sterile 0.9% NaCl. Mini pigs of 8 weeks weighing 10-15 kg were anesthetized through intravenous injection of 0.25% pentobarbital sodium. The abdomen of the pig was then shaved and prepared for surgery. Four lateral incisions (1 cm each) in two rows were made subcutaneously at each side of abdomen in one pig. The subcutaneous tissue was separated from the full skin layer to form a cavity. Each incision was inserted with one disc which was around 2 cm long in distance from the exact site of incision. Moreover, a distance of 8 cm was ensured between two incisions. After that, the incisions were sutured. Postoperatively, normal activities with a regular laboratory diet were resumed after

the pigs were recovered from anesthesia.

Histological examination

The implants with surrounding tissue were harvested after 2 weeks postoperatively. The harvested specimens were fixed in 4% paraformaldehyde in PBS for 2 days at 4^{o}C, embedded in paraffin, and sectioned in 5 mm slices. The slices were stained with hematoxylin and eosin (H&E) and then subjected to optical microscopic observation.

Result and Discussion

Characterization of major compounds

Characterization of N_3-PEG-N_3 and TPOM was already reported in our published work.[31] As for carboxyl-containing spiropyran (SPCOOH) and spiropyran-containing poly(ethylene glycol)-diazide (SPPEGDA), FTIR (Fig. S1) and ^{1}H NMR were used to characterize the chemical structures. ^{1}H NMR spectra of SPCOOH and SPPEGDA were shown in Fig. 1. A proton peak of –COOH was observed obviously in the ^{1}H NMR spectrum of SPCOOH (Fig. 1(a)). However, it disappeared after the esterification of SPCOOH with N_3-PEG-N_3, indicating that SPCOOH was combined with N_3-PEG-N_3 successfully.

The synthesis of PEG-SP hrdrogels: SPPEGDA, TPOM, PMDETA, were dissolved in DMF. After the mixture turned clear, CuBr was quickly added under ultrasonic agitation. The gelation point was reached in 80 s, and the reaction was allowed to continue for another 24 h at 60^{o}C. The hydrogels were obtained and transferred to an EDTA (5%) solution to remove the copper ions and DMF.

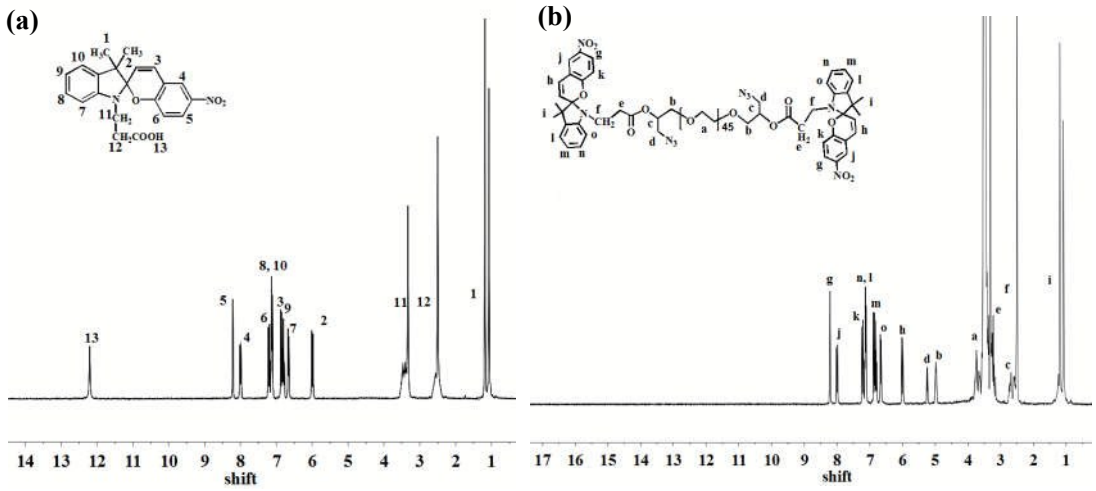


Fig. 1 ^{1}H NMR spectra of (a) SPCOOH and (b) SPPEGDA. (a) ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.0-1.3 (6H, CH_3), 2.6 (2H, CH_2), 3.4-3.5 (2H, CH_2), 5.9-6.0 (1H, olefinic proton), 6.9 (1H, olefinic proton), 6.6-8.2 (7H, aromatic), 12.2 (1H, COOH). (b) ^{1}H NMR (300 MHz, DMSO-d_6, δ): 1.0-1.3 (6H, CH_3), 2.6 (2H, CH_2), 3.4-3.5 (2H, CH_2), 3.5-4.0 (180H, CH_2), 5.9-6.0 (1H, olefinic proton), 6.9 (1H, olefinic proton), 6.6-8.2 (7H, aromatic).

Isomerization of SP in PEG hydrogels

SP could exist in two stable states under a neutral condition: the closed-ring state, known as the SP form, and the open-ring state, MC form. When irradiated by UV light at 365 nm, the colorless, hydrophobic SP chromophore can be isomerized into the colored, hydrophilic MC form.[33] Fig. 2 shows the UV–vis absorbance spectra of the PEG-SP hydrogel before and after UV irradiation. The appearance of an absorbance peak at 526 nm after UV irradiation indicated the formation of MC isomer.[34] The transparent, pale yellow hydrogel turned into a cardinal red hydrogel under UV light illumination (insets in Fig. 2).

The dynamics of the closed-ring and open-ring for the PEG-SP hydrogel were also

investigated and the results were shown in Fig. 3. With the elongation of irradiation time, the absorbance at 526 nm continued to increase, and leveled off at 15 min. With the further increase in irradiation time to 20 min, there was no change in the absorbance, indicating that the isomerization could reach equilibrium in 20 min (Fig. 3a). In addition, upon transferring the PEG-SP hydrogel to the visible light, the peak at 526 nm began to descend and went back to the initial status in 15 min (Fig. 3b). The observed time sequence of color change was similar to spiropyran-based hydrogels reported previously.[7,35,36]

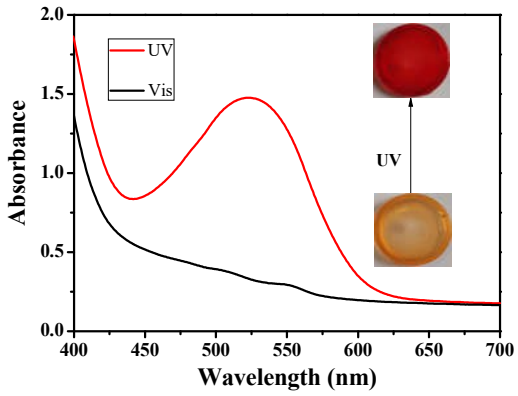


Fig. 2 UV-vis absorbance spectra of PEG-SP hydrogel irradiated by UV light (365 nm) for 15 min. Inset: PEG-SP hydrogel turns from pale yellow to cardinal red after UV lingt illumination.

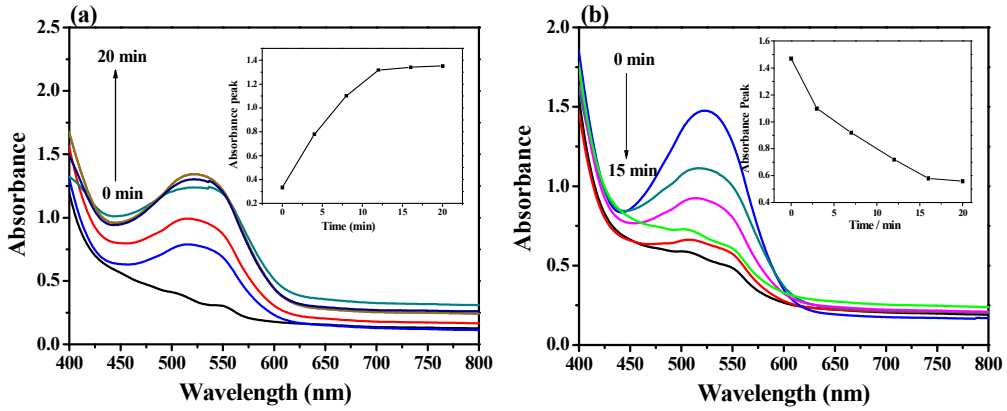


Fig. 3 (a) UV-vis absorbance spectra of hydrogel irradiated by UV light for 0, 2, 5, 10, 15 and 20 min. (b) UV-vis absorbance spectra of hydrogel irradiated by visible light for 0, 2, 5, 10, 12 and 15

min. Inset: time dependence of the UV-vis curve to attain a stable plateau.

Physical and mechanical properties of hydrogels

SP molecules were immobilized on a fixed position, and formed a well-defined hydrogel network with a neat structure. Under light irradiating, the structure of the SP molecule could shift between the hydrophilic MC form and hydrophobic SP form. Properties of PEG-SP hydrogels would thus be influenced, including swelling ratio (SR), mechanical strength and contact angles (CA).

SR is an important physical property of a hydrogel. The effect of SP on the swelling ratio and swelling kinetics of the PEG-based hydrogels was investigated and the results were shown in Fig. 4. The PEG hydrogel exhibited an SR about 660%, which is a little bit higher than that of PEG hydrogel modified with SP at the hydrophilic MC form under UV light (640%). In contrast, the SR of PEG-SP hydrogel under Vis light experienced a major decrease to 505%, owing to the isomerization of hydrophilic MC to hydrophobic SP form in PEG. [29] The decrease in SR of PEG-SP hydrogels was due to the presence of SP and the increase in compactness of the network resulting from the increased density of physical cross-linking.

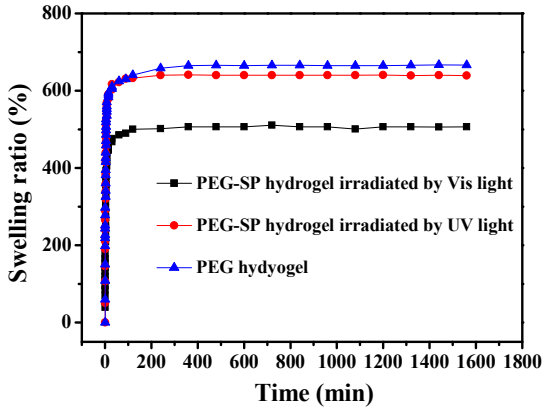


Fig. 4 Swelling ratio of PEG and PEG-SP hydrogels irradiated by Vis light and UV light.

As a potential platform for biomedical applications, a hydrogel must have robust mechanical strength to keep structural integrity under an external force. The typical stress-strain curve of PEG and PEG-SP hydrogels under compression was shown in Fig. 5. Water contents of both samples were kept at about 70 wt%. No visible damage was observed in both hydrogels even at a stress of 49.8 MPa. The PEG hydrogel could sustain a strain up to 82.54%, indicating that the PEG hydrogel prepared by controlled CuAAC methods possessed high compression efficiency.

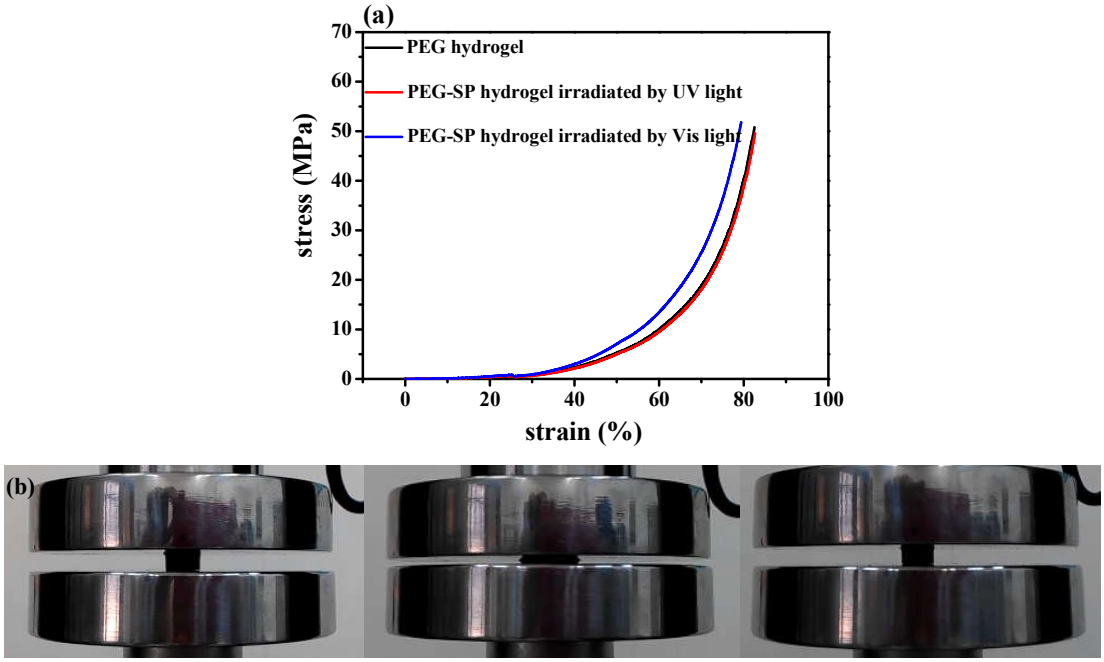


Fig. 5 (a) Stress-strain curves of PEG and PEG-SP hydrogels irradiated by Vis light and UV light. (b) Photographs of a PEG-SP hydrogel experiencing compression and recovery.

Moreover, the PEG-SP hydrogel also exhibited a high mechanical strength, and the strain was 82.34% under the UV light. These results demonstrated that the presence of SP open form in the cross-linked gels would not affect the mechanical properties of hydrogels. However, after the PEG-SP hydrogel was irradiated by Vis light, i.e. the

isomerization of hydrophobic SP (open form) was changed to hydrophilic MC (close form), the strain was decreased to 75.4% of the that of the PEG-SP in its open form. The SP group is closed and formed a chemical bond, the forming bond was hinder the move of the chain in the hydrogel. The photographs of a PEG-SP hydrogel experiencing compression and recovery were shown in Fig. 5b.

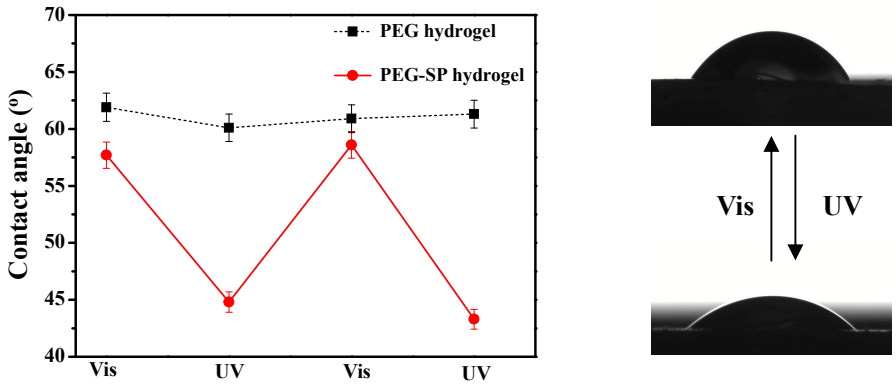


Fig. 6 Light-induced change in water CA on hydrogels. The dotted line stands for the hydrogels containing photosensitive SP moieties (PEG-SP hydrogel), while the solid line is for the hydrogel without SP moieties (PEG hydrogel). The right pictures illustrate the reversible contact angle changes.

Light-induced CA change

To investigate the UV light-induced hydrophobic/hydrophilic switch on the surface of the hydrogels, CA was measured using the sessile drop method before and after UV irradiation. In order to verify the reversibility in CA change, two Vis- UV irradiation cycles by alternating illumination were performed. The results were shown in Fig. 6. After being exposed to UV light for 15 min, the CA of PEG hydrogel remained almost constant under both conditions of illumination. In contrast, the CA of PEG-SP

hydrogel under UV irradiation experienced a minor decrease, indicating that the present of SP was responsible for the change in hydrophilicity on the hydrogel surface. Under the UV irradiation, the isomerization of hydrophobic SP to hydrophilic MC form in PEG occurred. Therefore, the CA of PEG-SP hydrogel is 57.2°, which was decreased to 43.6°, after being placed back under visible light for 30 min, further confirming the transition from MC back to SP form. The second cycle of UV irradiation also exhibited a similar CA shift.

Histological morphology of subcutaneously implanted PEG-SP

To assess the inflammation reaction to PEG-SP hydrogel and also to infer its biocompatibility, in vivo implantation of PEG-SP hydrogel into the subcutaneous area of pigs was carried out. The representative gross observation of the pig with PEG-SP implanted immediately and 2 weeks postoperatively were shown in Fig. 7 (a) and (b), respectively. It can be seen that the wound areas created during implantation were healed after 2 weeks without any signs of tissue swelling or ichors (Fig. 7b). During the sample harvesting process, the hydrogels could be easily located as marked by blue ink (Fig. 7c). No obvious abnormality of the surrounding tissue was observed when cutting off the subcutaneous pocket where the hydrogel was (Fig. 7d).

Histological observation of the PEG-SP hydrogels after being implanted for 2 weeks was shown in Fig. 8. Since the hydrogel remained soft as that of the tissue, the whole sample would fold during histological processing to yield more than one interface as shown in Fig. 8a. Two localized histological images showing the interface between the implanted PEG-SP and tissue were representatively shown in Fig. 8b and

c. No obvious inflammation reaction was observed in the surrounding tissue in consistence with the above gross observation. It seems that tissue grew quite homogeneously with the PEG-SP. Moreover, a well integration between the hydrogel and adjacent tissue was achieved, indicating its excellent biocompatibility.

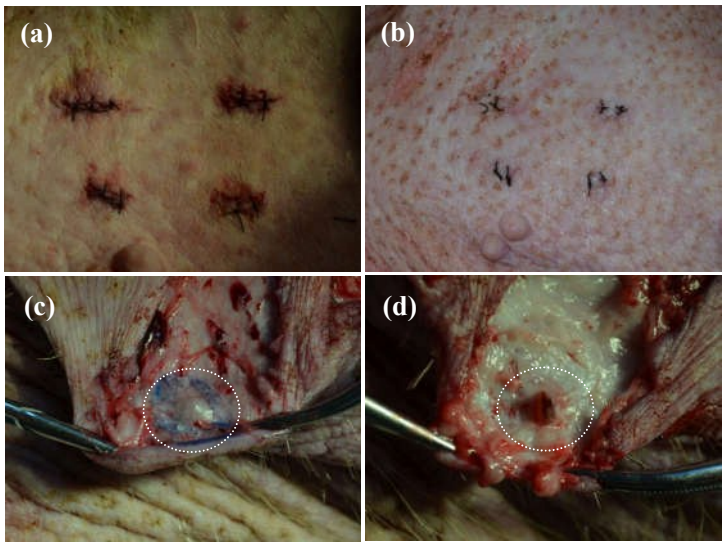


Fig. 7 (a) Gross observation of the pig with PEG-SP implanted immediately and (b) 2 weeks postoperatively. (c) Location of the hydrogel by blue ink during sample harvest. (d) No obvious tissue swelling, ichors, or abnormality of the surrounding tissue was observed when cutting off the subcutaneous pocket.

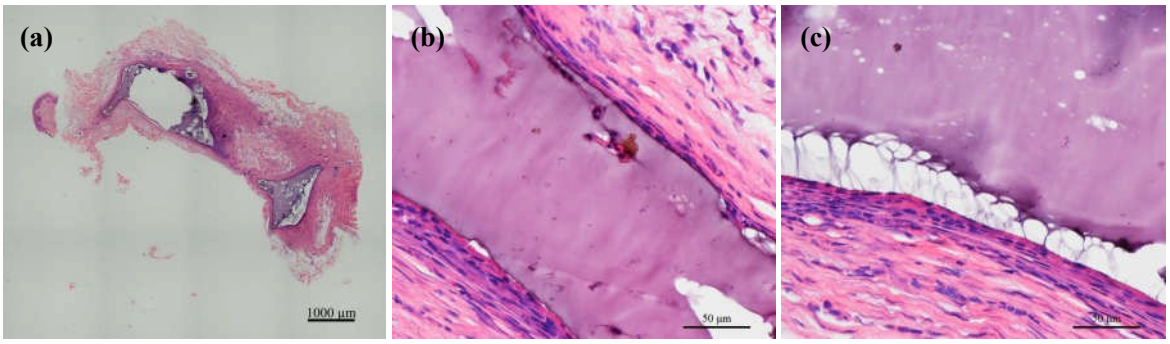


Fig. 8 Histological observation of the PEG-SP hydrogels after being implanted for 2 weeks. Overall (a) and localized (b,c) histological morphology of implanted PEG-SP with surrounding tissues. Scale bars: 1000μm for (a); 50μm for (b) and (c).

Conclusion

A versatile, highly tunable biomaterial artificial carrier based on photo-responsive PEG-SP hydrogel was presented. The well-defined hydrogel exhibited a robust mechanical strength with switchable contact angle and color under UV light illumination. Benefited from the presence of spiropyran, the swelling ratio of PEG-SP hydrogel under Vis light could also be tuned. Before and after UV light irradiation the contact angle of PEG-SP hydrogel has a variation of 13.8°. PEG-SP hydrogels possessed excellent biocompatibility as demonstrated by the in vivo subcutaneous implantation experiments. These studies have demonstrated that the photoresponsive PEG hydrogel has a high potential as a smart biomaterial. However, it is necessary to highlight that the amount of spiropyran groups in the SPPEGDA macromolecules was very important. It could affect the above properties introduced by the presence of spiropyran groups. The quantitative effect of its relative content on properties of hydrogels will be the focus of the following work. It has also to be mentioned that by varying the amount in the precursor, the final amount in the hydrogel could also be tuned. However, detailed determination of the exact concentration of spiropyran has to be developed.

Acknowledgements

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References

1 X. F. Sun, B. Liu, Z. Jing and H. Wang, *Carbohyd. Polym.,* 2015, **118**, 16–23.

2 H. Mittal, A. Maity and S. Sinha Ray, *J. Phys. Chem. B*, 2015, **119**, 2026-2039.

3 A. Döring, W. Birnbaum and D. Kuckling, *Chem. Soc. Rev.,* 2013, **42**, 7391-7420.

4 P. Chakraborty, P. Bairi and B. Roy, *ACS Appl. Mater. Inter.,* 2014, **6**, 3615-3622.

5 D. Li, X. Zhang and J. Yao, *Chem. Commun.,* 2011, **47**, 1710-1712.

6 L. P. Lv, K. Landfester and D. Crespy, *Chem. Mater.,* 2014, **26**, 3351-3353.

7 N. Wang, Y. Li, Y. Zhang, Y. Liao and W. Liu, *Langmuir*, 2014, **30**, 11823−11832.

8 H. Cui, H. Liu, S. Chen and R. Wang, *Dyes and Pigments*, 2015, **115**, 50-57.

9 X. Yang, L. Qiu, C. Cheng, Y. Wu, Z. F. Ma and D. Li, *Angew. Chem. Int. Ed.,* 2011, **50**, 7325-7328.

10 Y. D. Liu and H. J. Choi, *Polym. Int.*, 2013, **62**, 147-151.

11 D. Pattavarakorn, P. Youngta, S. Jaesrichai, S. Thongbor and P. Chaimongkol, *Energy Procedia*, 2013, **34**, 673-681.

12 M. Bassil, J. Davenas and M. E. L. Tahchi, *Sensor. Actuat. B*, 2008, **134**, 496-501.

13 M. Hu, M. Kurisawa, R. Deng, C. Teo, A. Schumacher, Y. Thong, L. Wang, K. M. Schumacher and J. Y. Ying, *Biomaterials*, 2009, **30**, 3523-3531.

14 V. Kuzmenko, D. Hagg, G. Toriz and P. Gatenholm, *Carbohyd. Polym.*, 2014, **102**, 862-868.

15 C. Alvarez-Lorenzo and A. Concheiro, *Curr. Opin. Biotech.*, 2013, **24**, 1167-1173.

16 D. S. Achilleos, T. A. Hatton and M. Vamvakaki, *J. Am. Chem. Soc.*, 2012, **134**,

5726-5729.

17 K. Sakai, Y. Imaizumi, T. Oguchi, H. Sakai and M. Abe, *Langmuir*, 2010, **26**, 9283-9288.

18 H. Lee, W. Wu, J. K. Oh, L. Mueller, G. Sherwood, L. Peteanu, T. Kowalewski and K. Matyjaszewski, *Angew. Chem. Int. Ed.*, 2007, **119**, 2505-2509.

19 L. Florea, A. McKeon, D. Diamond and F. Benito-Lopez, *Langmuir*, 2013, **29**, 2790-2797.

20 K. H. Fries, J. D. Driskell, S. Samanta and J. Locklin, *Anal. Chem.*, 2010, **82**, 3306-3314.

21 H. Cui, H. Liu, S. Chen and R. Wang, *Dyes and Pigments*, 2015, **115**, 50-57.

22 T. Suzuki, Y. Hirahara, K. Bunya and H. Shinozaki, *J. Mater. Chem.*, 2010, **20**, 2773-2779.

23 I. Tomatsu, K. Peng and A. Kros, *Adv. Drug Deliver. Rev.*, 2011, **63**, 1257–1266.

24 H. C. Kolb and K. B. Sharpless, *Drug Discov. Today*, 2003, **8**, 1128–1137.

25 C. A. DeForest, B. D. Polizzotti and K. S. Anseth, *Nat. Mater.*, 2009, **8**, 659–664.

26 A. Huseyin, K. Irem, S. Rana, *Macromolecules*, 2010, **43**, 3801–3808.

27 V. Crescenzi, L. Cornelio, C. Di Meo, *Biomacromolecules*, 2007, **8**, 1844–1850.

28 A. O. Dmitri and H. Jons, *Macromolecules*, 2006, **39**, 1709–1718.

29 S. Q. Liu, P. L. Ee, C. Y. Ke, J. L. Hedrick and Y. Y. Yang, *Biomaterials*, 2009, **30**, 1453–1461.

30 J. Chen, F. Zeng, S. Wu, J. Zhao, Q. Chen and Z. Tong, *Chem. Commun.*, 2008, 5580-5582.

31 L. Q. Xu, F. Yao, G. D. Fu and E. T. Kang, *Biomacromolecules*, 2010, **11**, 1810–1817.

32 M. Touaibia, A. Wellens, T. C. Shiao, Q. Wang, S. Sirois, J. Bouckaert and R. Roy, *ChemMedChem*, 2007, **2**, 1190-1201.

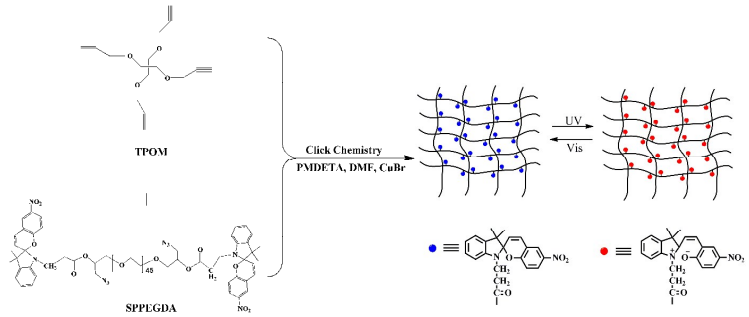
33 N. Wang, J. Zhang, L. Sun, P. Wang and W. Liu, *Acta Biomater.*, 2014, **10**, 2529–2538.

34 J. Edahiro, K. Sumaru, T. Takagi, T. Shinbo, T. Kanamori and M. Sudoh, *Eur. Polym. J.*, 2008, **44**, 300–307.

35 J. E. Stumpel, D. Liu, D. J. Broer and A. P. H. J. Schenning, *Chem. A Eur. J.*, 2013, **19**, 10922-10927.

36 T. Satoh, K. Sumaru, T. Takagi, K. Takai and T. Kanamori, *Phys. Chem. Chem. Phys.*, 2011, **13**, 7322-7329.

Abstract Graphic



Novel hydrogels with extremely high strength, reversible photoresponsive and excellent biocompatible properties were synthesized from a well-defined poly (ethylene glycol) polymer with spiropyran groups at the given position (PEG-SP) via Cu (I)-catalyst Azide-Alkyne Cycloaddition (CuAAC) reation.