Electronic Supplementary Material

Chemically triggered life control of "Smart" hydrogels through click and declick reactions

Xing Feng[1], Meiqing Du[1] Hongbei Wei[1], Xiaoxiao Ruan[1], Tao Fu[1], Jie Zhang (✉)[2], Xiaolong Sun (✉)[1]

1 Key Laboratory of Biomedical Information Engineering of Ministry of Education, School of Life Science and Technology, Xi'an Jiaotong University, Xi'an 710049, China
2 The Fourth Military Medical University, Xi'an 710032, China

E-mails: x.l.sun86@xjtu.edu.cn (Sun X); zhangjie78@fmmu.edu.cn (Zhang J)

Contents

1. Materials and instruments

1.1 Materials

The Meldrum's Acid derived cross-linker **1** and barbituric acid derived cross-linker **3** were synthesized based on previously reported procedures by Wentrup[1] and Sheikha[2], respectively. In addition, the cross-linker **2** was synthesized based on reported work by Kalow[3]. Chemicals were obtained from TCI Chemicals, Aladdin Chemicals, and other reagent manufacturers.

Meldrum's Acid (>98.0%) was purchased from TCI chemical Industry. 5,5-Dimethyl-1,3-cyclonhexanedione (≥99.0%) was purchased from MACLIN chemical Industry. Barbituric Acid (>98.0%) was purchased from Shanghai Ruida Fine Chemical Co., Ltd. Carbon bisulfide (>99.0%) was purchased from Shanghai Acmec Biochemical Co., Ltd. Iodomethane (>99.5%) was purchased from TCI Chemicals. As solvents, N,N-dimethylformamide (DMF, >99%) and dimethyl sulphoxide (DMSO, >99%) were purchased from Tian in Fuyu Fine Chemical Co., Ltd. Potassium carbonate (>99%) was purchased from Aladdin Chemicals. Triethylamine (TEA) was purchased from Tian in Fuyu Fine Chemical Co., Ltd. Four-arm poly(ethylene glycol) amine (≥95.0%, M_n ca. 10,000 Da) was purchased from Ponsure Biological Co., Ltd, China. Acetonitrile (≥99.0%) were purchased from Tian in Fuyu Fine Chemical Co., Ltd, China. Ethylenediamine (≥99.0%) was purchased from Sinopharm Chemical Reagent Co., Ltd, China. Hydrogel disks with a diameter of 15 mm and thickness of 1 mm were prepared and used at room temperature in all experiments.

1.2 Methods and instruments

1.2.1. Nuclear magnetic resonance (NMR)

^{1}H spectra were recorded on Bruker Avance-400MHz NMR spectrometer. The NMR spectra were referenced to solvent and the spectroscopic solvents ($CDCl_3$, DMSO-d_6, etc.) were purchased from Cambridge Isotope Laboratories.

1.2.2. Hydrogel swelling

Hydrogel were prepared and incubated for 2 h at room temperature to ensure full gelation. The hydrogel mass taken as the non-swollen value, and deionized water (2 ml) was added to the hydrogels, which were incubated at room temperature. Every 1 hour, the water was removed, and hydrogel mass recorded and replaced with deionized water (2 ml) until hydrogel mass no longer changed. Swelling ratio (*ESR*) was recorded as a ratio of gel mass (W_S) at a specific time to non-swollen hydrogel mass (W_d). Calculation formula of swelling rate is as follows:

$$ESR = \frac{W_S - W_d}{W_d} * 100\%$$

1.2.3. Rheometry

Rheological measurements were carried out using a Rheometer (MCR 302, Anton Paar) equipped with a parallel plate geometry (8 mm rotor). The experiments were conducted at constant temperature, i.e. 37 ℃. Storage modulus and loss modulus (G' and G", respectively) were obtained at a constant deformation (1 % strain) with the increasing frequency (from 1 Hz to 100 Hz). Similarly, Young's modulus (G' and G") were obtained at the constant deformation (1 % strain) within the fixed time of 300 s.

1.2.4. Crosslinking density

Crosslinking density (ν_e) was determined using previously described equations[4-5]. ν_e was calculated based on $\nu_e = G'/RT$, where the gad constant R = 8.314 J/K mol and temperature T = 310 K.

1.2.5. Scanning Electron Microscopy (SEM)

SEM images were collected by ultra-high resolution field emission scanning electron microscope (Tescan Maia3 LMH, Czech Republic). The hydrogel samples had a diameter of 10 mm and a thickness of 3 mm; after sufficient swelling, the hydrogel was immersed in liquid nitrogen for freezing. Then the frozen samples were saved in a refrigerator at -80 ℃ overnight. The hydrogel was dried via freeze-drying technique.

The samples were fixed on the objective table and sprayed by the Au (Ted Pella 108A, US).

1.2.6. Tensile tests

All mechanical tests were performed by using an electronic universal testing machine (UTM2203, SUNS, China) equipped with 100 N loading cell and the temperature fluctuation during the test was lower than 2 ^{o}C/h to eliminate the influence of temperature fluctuation on hydrogel testing. For tensile tests, dumbbell-shaped hydrogel specimens were prepared by adding the hydrogel precursor solution to a polytetrafluoroethylene mold (designed according to type 5B of ISO 527-2 standard) and a standing still. Elastic modulus (E) was defined as the initial slope (10%) value of the stress-strain curve. The relationship between the tensile stress-strain curve and the tensile rate was obtained by stretching the hydrogel at a speed of 30 m/s. The tensile strength (R_m) referred to the maximum stress that the material can withstand before it was broken. The tensile strength and the maximum force that can be withstand in this work were directly obtained through the software analysis of the above-mentioned instrument.

1.2.7. Raman spectroscopy and imaging

The hydrogel samples were characterized using Raman spectrometer (Horiba Jobin-Yvon, LabRam Aramis Raman Spectrometer, France) with 532 nm excitation wavelength. To be directly comparable, all spectra were collected in the 500-4000 nm range. Mathematical processing of the spectra, namely baseline correction and smoothing, were carried out with Origin (OriginLab Corporation, US) and LabSpec6 software.

1.2.8. pH meter

The measurement of pH (Potential-of-hydrogen) was performed on an instrument equipped with FE28-Meter (FiveEasy Plus, METTLER TOLEDO). The crosslinking agents (**1, 2, 3**) and four-arm PEG amine were weighed according to the pre-weighted ration and completely dissolved in water (10% MeCN). The electrode of the instrument was inserted into the solution to obtain the pH value.

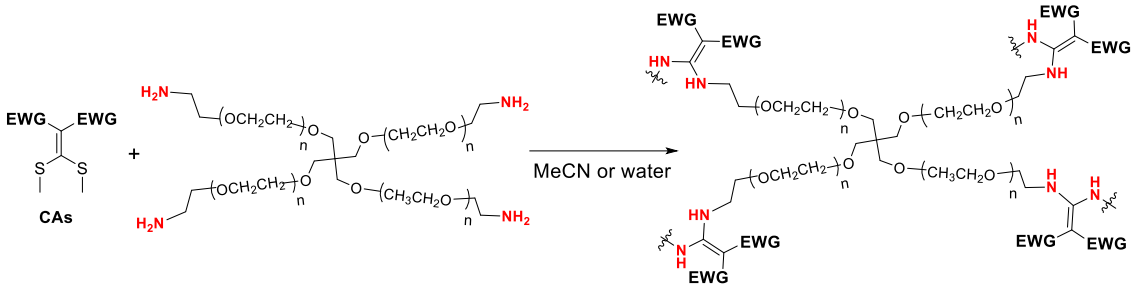
1.2.9. Gel permeation chromatography (GPC)

Gel permeation chromatography (GPC) experiments were performed on a system equipped with an isocratic pump (waters 2414), a DAWN HELEOS 18-angle laser

light scattering detector (also known as multi-angle laser light scattering (MALLS) detector, Wyatt Technology, Santa Barbara, CA), and an Optilab rEX refractive index detector (Wyatt Technology, Santa Barbara, CA). HELEOS' detection wavelength was 658 nm. Separations were achieved using size-exclusion columns connected in series (400, 103, and 104 Å Phenogel columns, 5 μm, 300 × 7.8 mm, Phenomenex, Torrance, CA) at 40 ℃ utilizing DMF containing 0.1 M LiBr as the mobile phase.

2. Synthesis of soft materials

2.1 Synthesis of highly cross-linked polymers A-1, 2, 3



General procedure: Two equivalents of **CAs** and one equivalent of four-arm PEG amines (M_n ~ 10.0 Da) were mixed together in acetonitrile or water. The parallel reactions were operated under vortex or shaker and upon separation of the template to offer uniform hydrogels. The hydrogels were dried to remove acetonitrile. Finally, the hydrogel was allowed to reach maximum water absorbance for storage and test.

Table S1. Weighing instructions of hydrogels in organic system.

Materials	CAs	4-PEG-amine	Acetonitrile
A-1	2.5 mg	50.37 mg	170 μl
A-2	2.5 mg	51.18 mg	170 μl
A-3	2.5 mg	48.04 mg	170 μl

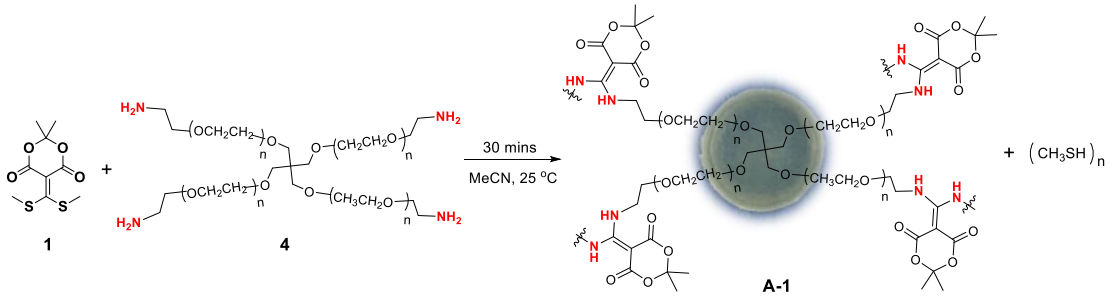


Fig. S1. The synthesis of A-1.

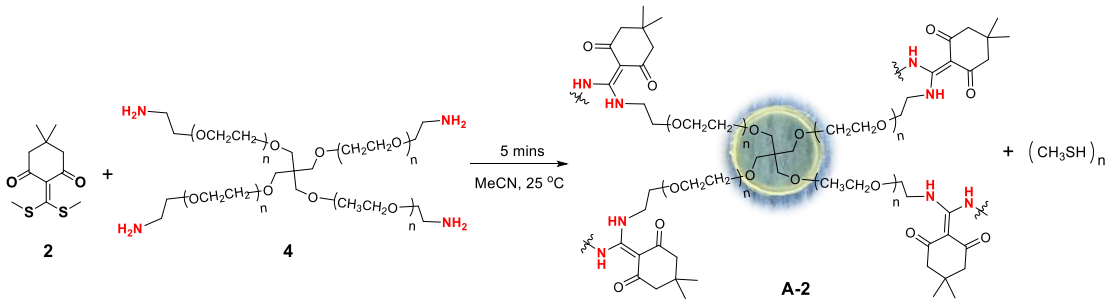


Fig. S2. The synthesis of A-2.

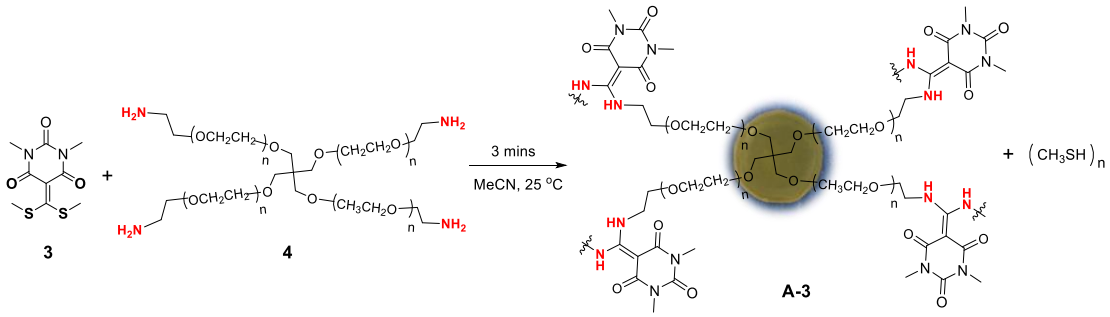


Fig. S3. The synthesis of A-3.

2.2 Stability tests of A-1, 2, 3

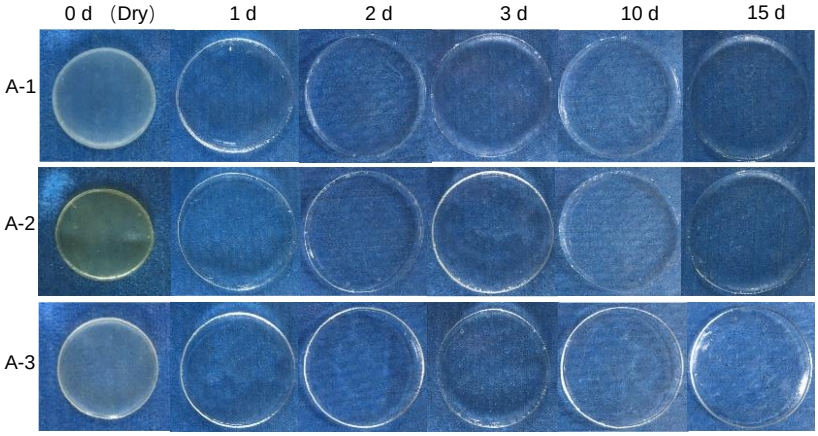


Fig. S4. Photos of hydrogels soaked in water for a long time. The dry hydrogels were immersed in deionized water to verify their stability, and their shapes were observed to be stable.

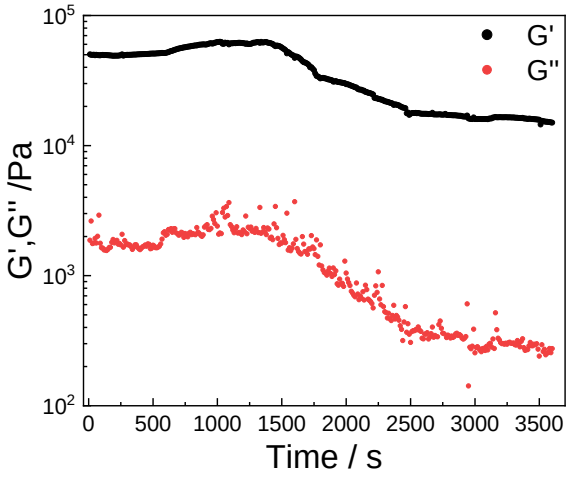


Fig. S5. Storage modulus (G') and loss modulus (G'') for hydrogels A-3 in an hour scan.

2.3 SEM images of A-1, 2, 3

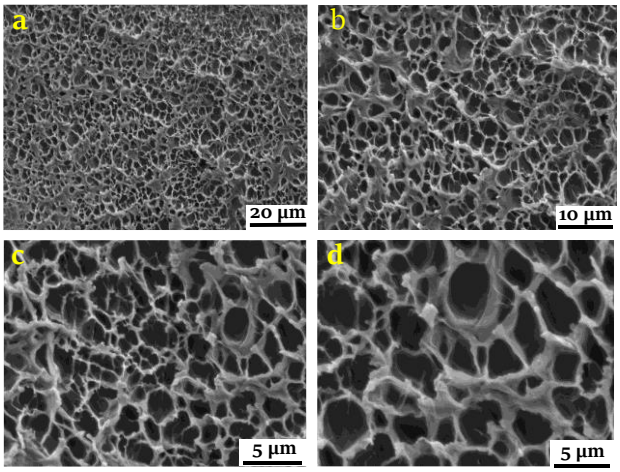


Fig. S6. SEM images of A-1 at different magnification with (a) M = 3 k ; (b) M = 6 k ; (c) M = 9 k ; and (d) M = 15 k. Scale bar in panel a is 20 μm, in panel b is 10 μm, in panels c-d is 5 μm. Aperture of hydrogel A-1 lies between 2.5 μm and 3.5 μm.

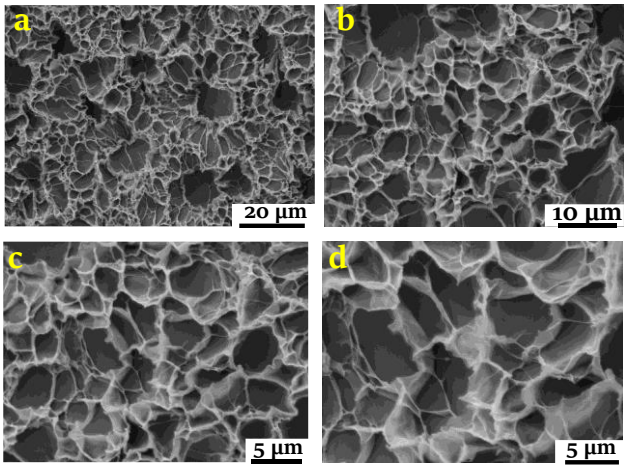


Fig. S7. SEM images of A-2 at different magnification with (a) M = 3 k ; (b) M = 6 k ; (c) M = 9 k ; and (d) M = 15 k. Scale bar in panel a is 20 µm, in panel b is 10 µm, in panels c-d is 5µm. Aperture of hydrogel A-2 lies 5.0 µm.

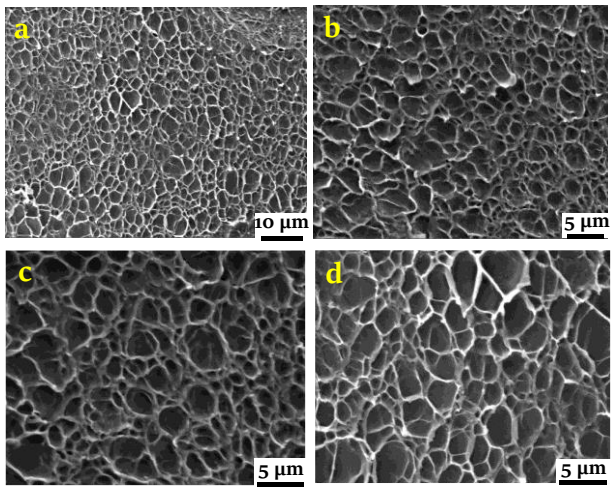


Fig. S8. SEM images of A-3 at different magnification with (a) M = 6 k ; (b) M = 9 k ; and (c, d) M = 15 k. Scale bar in panel a is 10 µm, in panels b-c is 5 µm. Aperture of A-3 lies between 2.0 µm and 3.0 µm.

2.4 Raman spectra of soft materials A-1, 2, 3

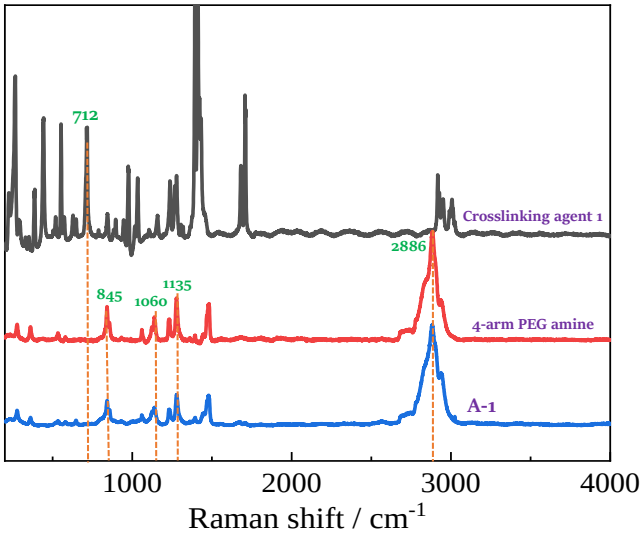


Fig. S9. The Raman spectra (532 nm) confirmed the structure of A-1, the crosslinking agent **1** and 4-arm PEG amine. Peaks for crosslinking agent 1 [v_{C-S}, 712 cm^{-1}], 4-arm PEG amine [ρ_{CH_2}, 845 cm^{-1}] [$v_{CH_2NH_2}$, 1060 cm^{-1}] [v_{OH}, 2886 cm^{-1}] and A-1 [v_{CN}, 1135 cm^{-1}] are labeled on the spectra for **1**, 4-arm PEG amine and A-1, respectively.

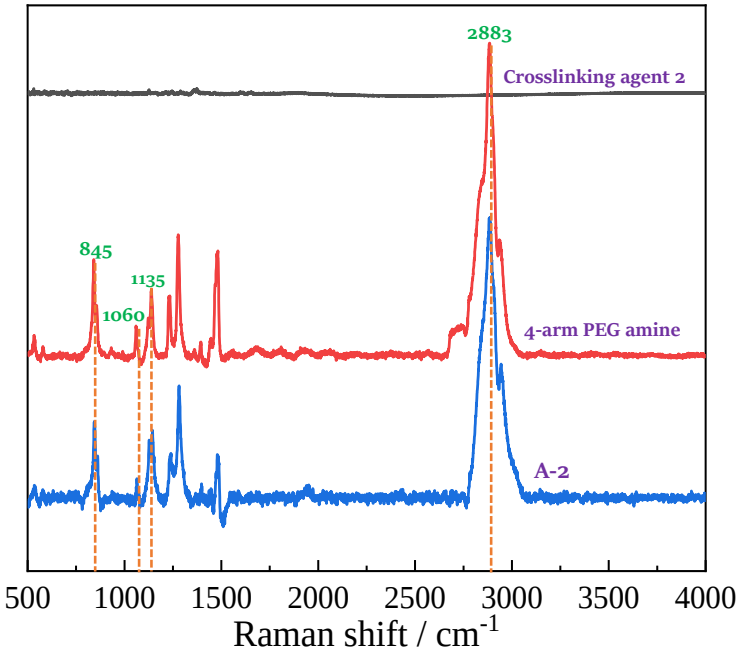


Fig. S10. The Raman spectra (532 nm) confirmed the structure of A-2, the crosslinking agent **2** and 4-arm PEG amine. Peaks for crosslinking agent **2** (no raman signal due to fluorescence), 4-arm PEG amine [ρ_{CH_2}, 845 cm^{-1}] [$v_{CH_2NH_2}$, 1060 cm^{-1}] [v_{OH}, 2883 cm^{-1}] and A-2 [v_{CN}, 1135 cm^{-1}] are labeled on the spectra for **2**, 4-arm PEG amine and A-2, respectively.

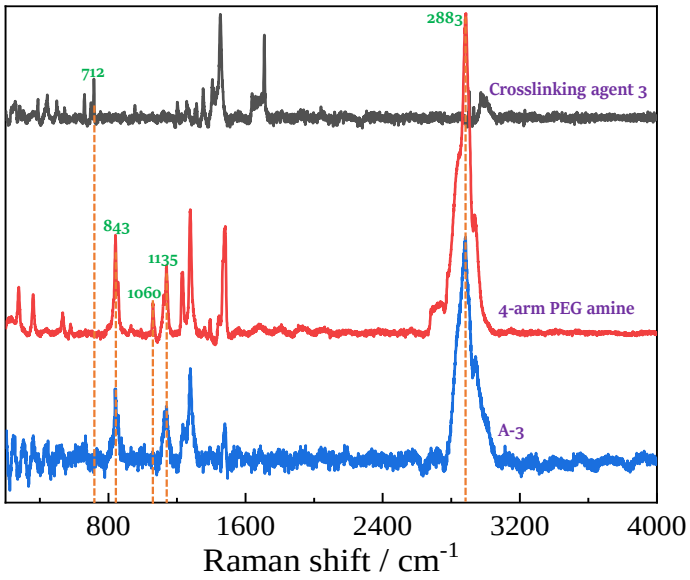


Fig. S11. The Raman spectra (532 nm) confirmed the structure of A-3, the crosslinking agent **3** and 4-arm PEG amine. Peaks for crosslinking agent **3** [v_{C-S}, 712 cm^{-1}], 4-arm PEG amine [ρ_{CH_2}, 843 cm^{-1}] [$v_{CH_2NH_2}$, 1060 cm^{-1}] [v_{OH}, 2883 cm^{-1}] and hydrogel A-3 [v_{CN}, 1135 cm^{-1}] are labeled on the spectra for **3**, 4-arm PEG amine and hydrogel A-3, respectively.

2.5 Rheological analysis of A-1, 2, 3

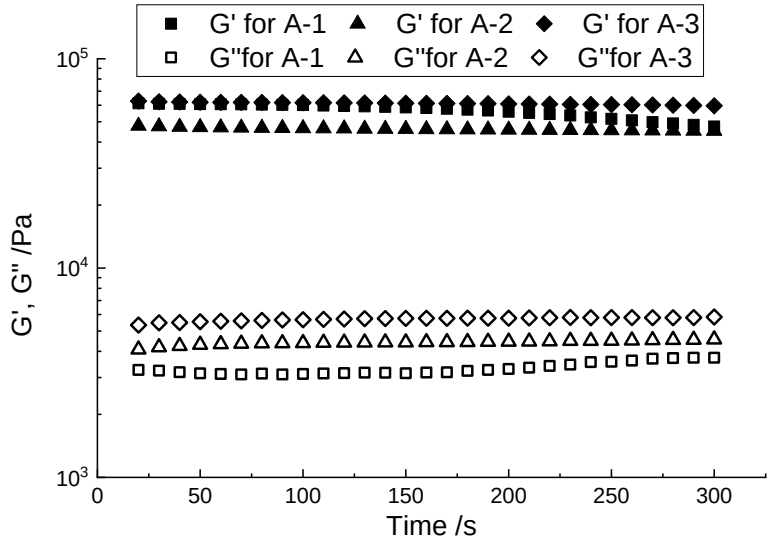


Fig. S12. Storage modulus (G') and loss modulus (G") for hydrogels A-1, -2, -3 after swelling in the mode of time scan.

2.6 Stretching curves under different reaction ratios

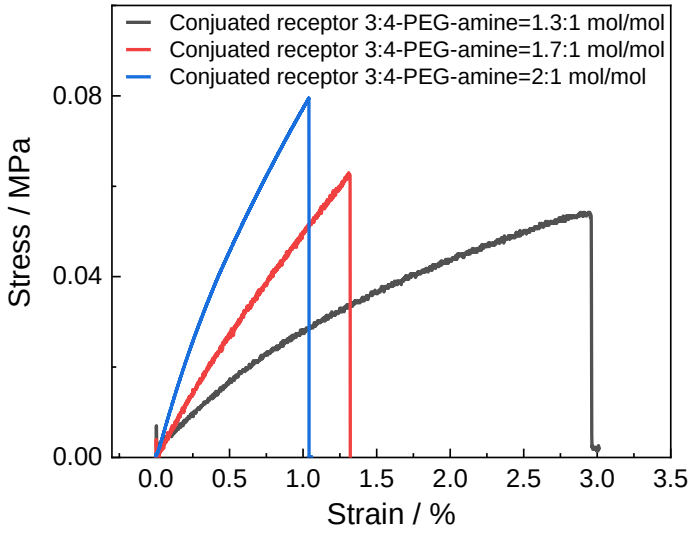


Fig. S13. Stretching curves of hydrogel A-3 with different reaction ratios between conjugated receptor **3** and 4-PEG -amine.

2.7 Synthesis of highly cross-linked polymers W-1, 2, 3

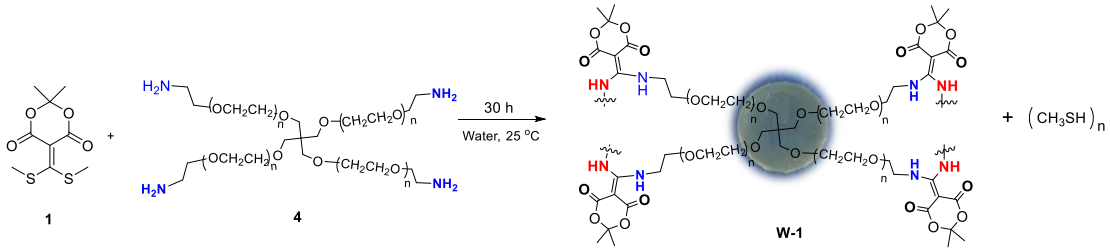


Fig. S14. The synthesis of W-1 in water.

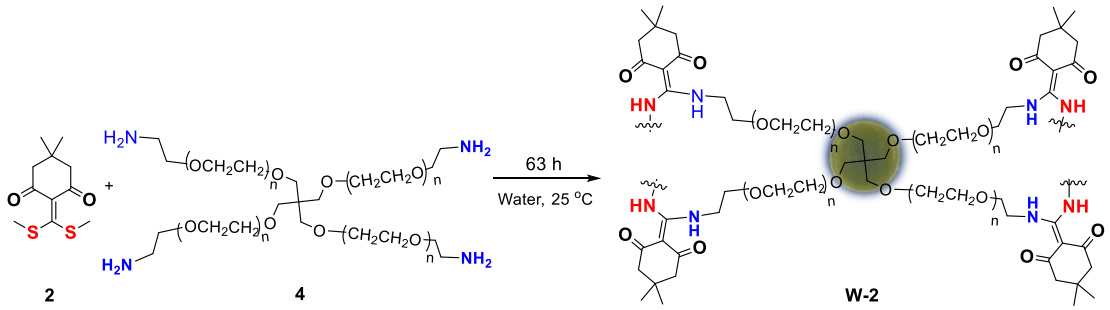


Fig. S15. The synthesis of W-2.

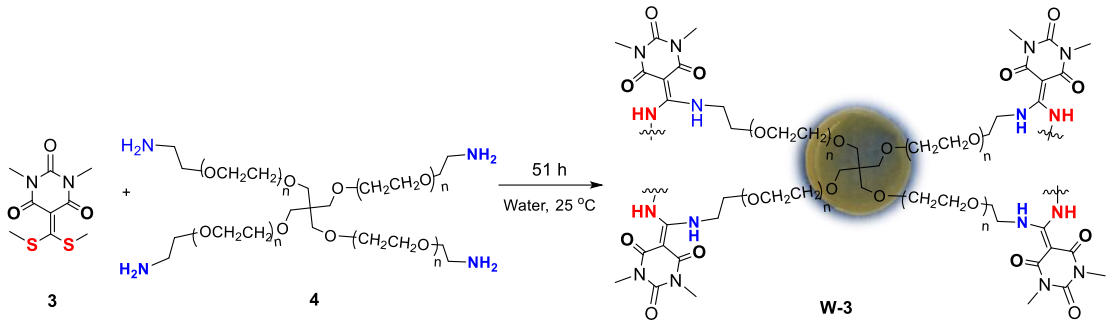


Fig. S16. The synthesis of W-3 in water.

2.8 pH of soft materials synthesized in water

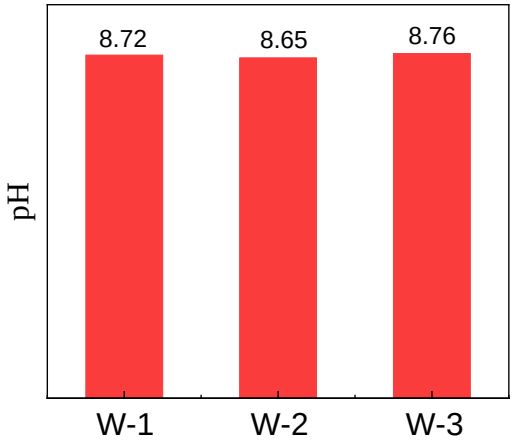


Fig. S17. pH of precursor solution (hydrogel W-1, -2, -3, water system). There is no difference in pH, which is around 8.7.

2.9 Rheological analysis of W-1, 2, 3

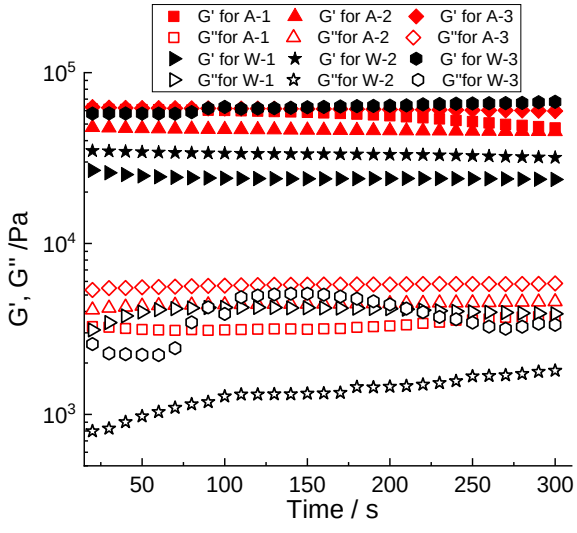


Fig. S18. Comparison of storage modulus (G') and loss modulus (G") for hydrogels W-1, -2, -3 and A-1, 2, 3 after swelling in the mode of time scan.

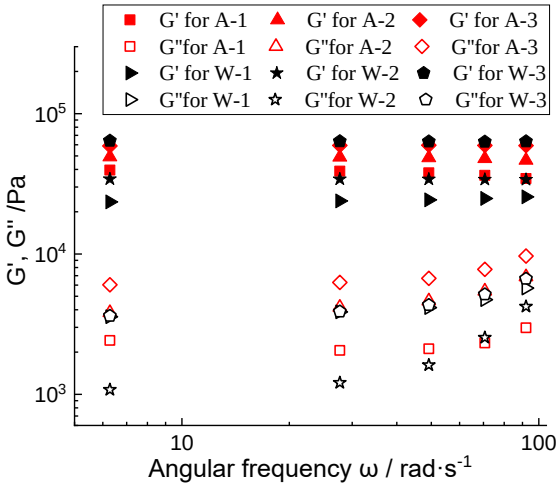


Fig. S19. Comparison of storage modulus (G') and loss modulus (G") for hydrogels W-1, -2, -3 and A-1, 2, 3 after swelling in the mode of frequency scan.

2.10 Swelling ratio of W-1, 2, 3

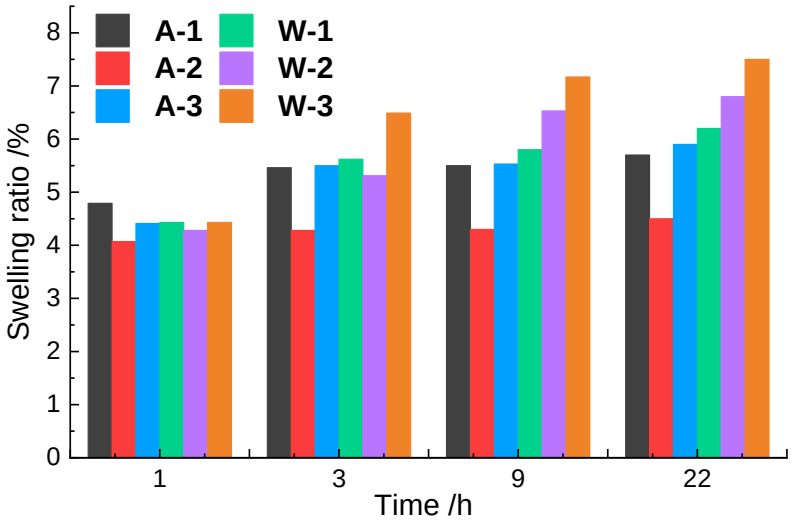


Fig. S20. Swelling ratios of soft materials A-1, A-2, A-3, W-1, W-2, W-3. All dry materials were swelled at deionized water and weighted at predetermined time points.

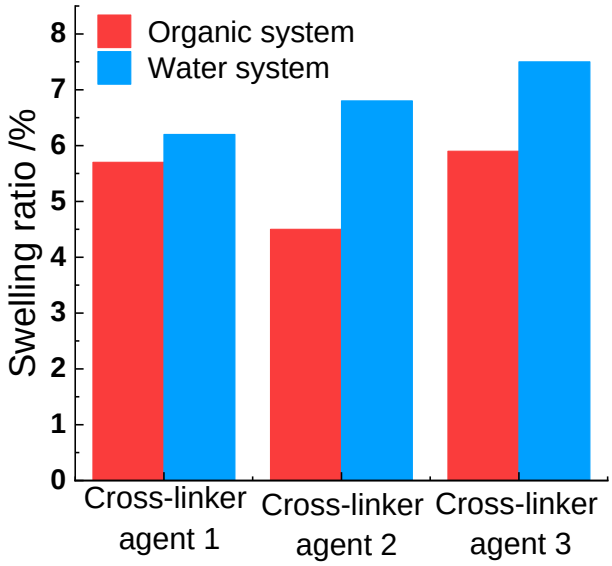


Fig. S21. Comparison of swelling ratios of hydrogels synthesized with cross-linking agent **1**, **2**, **3** in organic and aqueous systems.

2.11 SEM images of W-1, 2, 3

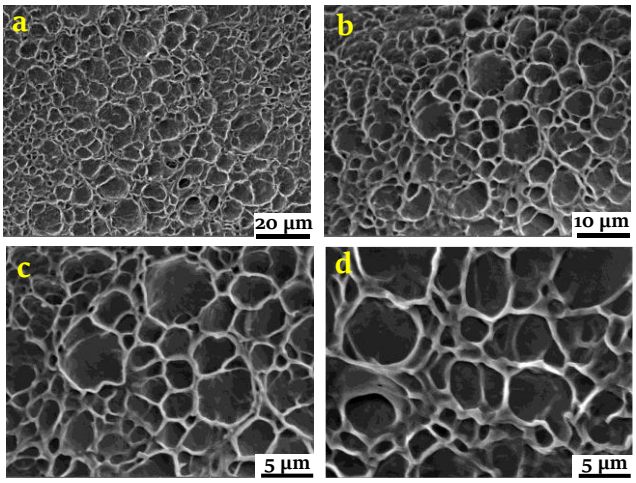


Fig. S22. SEM images of W-1 at different magnification with (a) M = 3 k ; (b) M = 6 k ; (c) M = 9 k ; and (d) M = 15 k. Scale bar in panel a is 20 μm, in panel b is 10 μm, in panels c-d is 5μm. Aperture of W-1 lies between 3.0 μm and 4.0 μm.

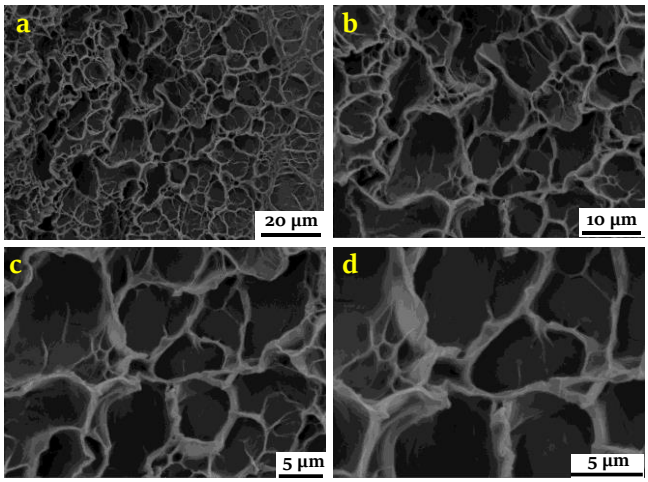


Fig. S23. SEM images of W-2 at different magnification with (a) M = 3 k ; (b) M = 6 k ; (c) M = 9 k ; and (d) M = 15 k. Scale bar in panel a is 20 μm, in panel b is 10 μm, in panels c-d is 5μm. Aperture of W-2 lies 6.0 μm.

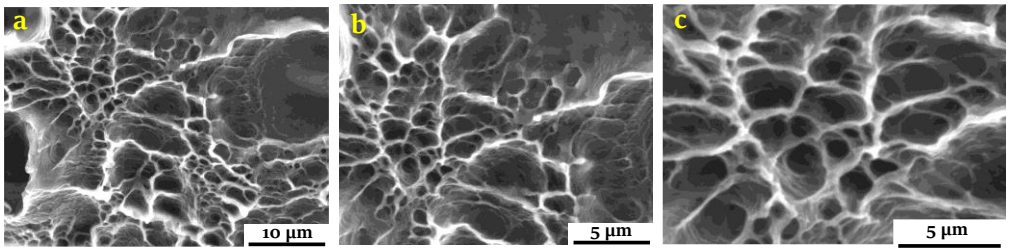
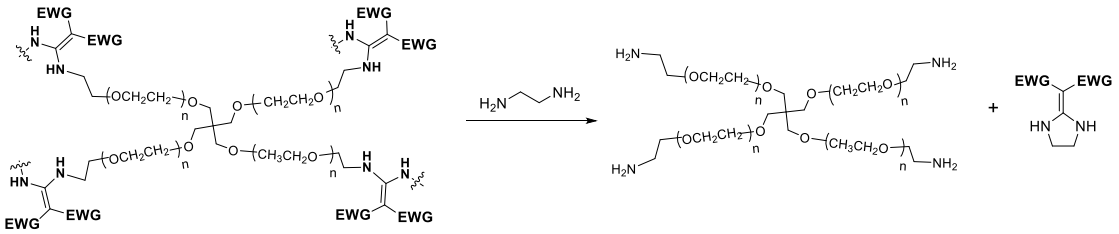


Fig. S24. SEM images of W-3 at different magnification with (a) M = 6 k ; (b) M = 9 k ; and (c) M = 15 k. Scale bar in panel a is 10 μm, in panels b-c is 5μm. Aperture of W-3 lies between 3.0 μm and 4.0 μm.

3. Soft materials' degradation and regeneration

3.1 Soft materials' degradation



A-1, A-2, A-3
W-1, W-2, W-3

4

General procedure. Degradation of polymers A-1, A-2, A-3, W-4, W-5, W-6 in ethylenediamine. The highly cross-linked hydrogel prepared as above (Figure S1, S2, S3) swelled in deionized water. The fully swollen hydrogel is transferred to the

ethylenediamine solution for degradation. After complete degradation, the mixture was collected, and the mixed solution was subjected to rotary evaporation to remove ethylenediamine, and a white solid was obtained and dried in vacuum. Then, the minimum amount of chloroform was used to dissolve the solid matter, and the mixed solution was poured into ice ether for low-temperature centrifugation to obtain a white precipitate, which was then vacuum dried to obtain the recovered compound **4**. Then, it was dried in vacuum to obtain a white powder **4**. Compound **4** was confirmed by NMR spectrum in $CDCl_3$.

Table S2. Water content in degradation solution

Hydrogel	W_d / (g)	ESR	Amount of water / (%)
A-1	0.081	5.7	25.65
A-2	0.1939	4.5	48.47
A-3	0.2408	4.9	65.55
W-1	0.0271	6.2	9.3
W-2	0.0295	6.8	11.14
W-3	0.0141	7.5	17.08

3.2 Rheological analysis of re-gelled soft materials

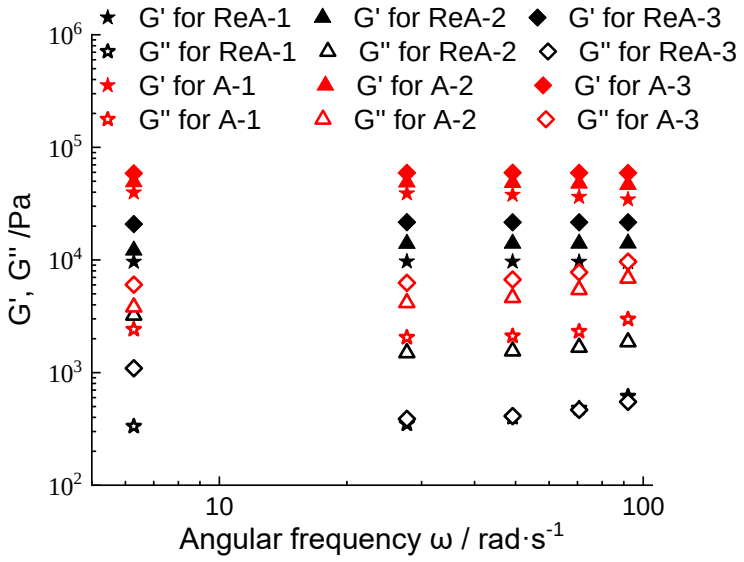


Fig. S25. Storage modulus (G') and loss modulus (G") for comparison of re-gelled hydrogels **ReA-1, 2, 3** and initially synthesized hydrogels **A-1, 2, 3** after swelling in the mode of frequency scan.

3.3 Tensile tests of re-gelled soft materials ReA-1, 2, 3

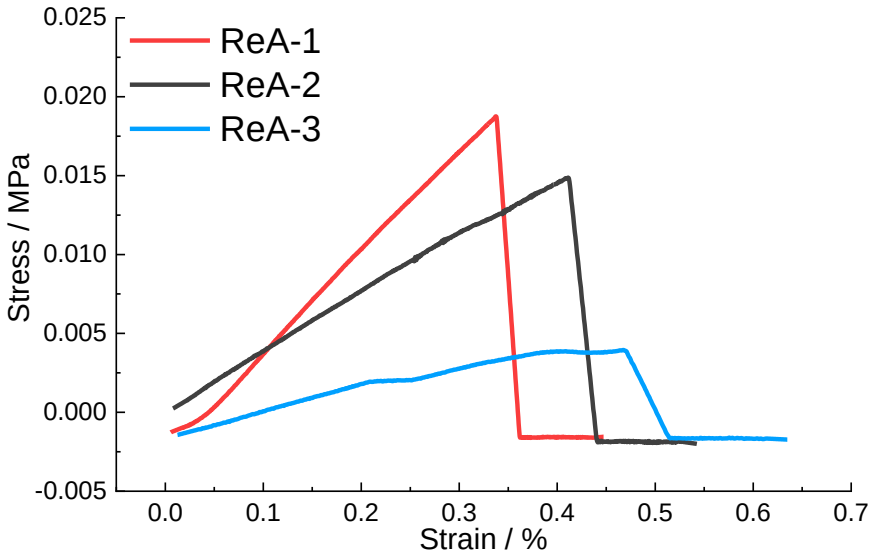


Fig. S26. Strain-Stress curves of the recycled hydrogels in organic system.

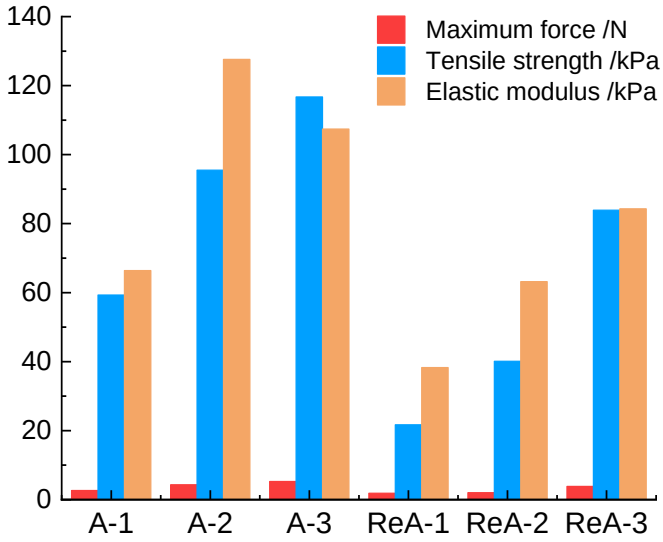


Fig. S27. Comparison of tensile properties of A-1, 2, 3 and ReA-1, 2, 3.

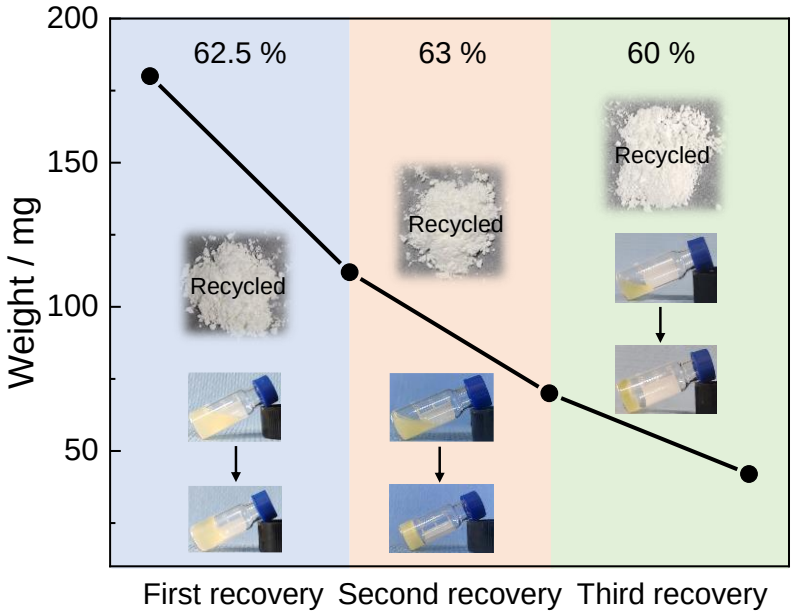


Fig. S28. For the measurement of the weight of four arm PEG-amine recovered three times, taking hydrogel A-3 as an example. Each time the recovery ratio was almost maintained at the level of 60%, and the closed loop recovery of the hydrogel can be completed.

4. Reference

1. Ben Cheikh A, Chuche J, Manisse N, Pommelet J C, Netsch K P, Lorencak P, Wentrup C. Synthesis of α-cyano carbonyl compounds by flash vacuum thermolysis of (alkylamino)methylene derivatives of Meldrum's acid. Evidence for facile 1,3-shifts of alkylamino and alkylthio groups in imidoylketene intermediates. The Journal of Organic Chemistry, 1991, 56(3): 970-975

2. Sweidan K, Abu-Salem Q, Al-Sheikh A, Sheikha G. Novel derivatives of 1,3-dimethyl-5-methylenebarbituric acid. Letters in Organic Chemistry, 2009, 6(8): 669-672

3. El-Zaatari B M, Ishibashi J S A, Kalow J A. Cross-linker control of vitrimer flow.Polymer Chemistry, 2020, 11(33): 5339-5345

4. Tan S, Yamashita A, Gao S J, Kurisawa M, Hyaluronic acid hydrogels with defined crosslink density for the efficient enrichment of breast cancer stem cells. Acta Biomater, 2019, 94 (26): 320-329

5. Piskounova S, Rojas R, Bergman K, Hilborn J. The effect of mixing on the mechanical properties of hyaluronan-based injectable hydrogels. Macromolecular Materials and Engineering, 2011, 296 (10): 944-951