

Hydrogels with Dynamically Controllable Mechanics and Biochemistry for 3D Cell Culture Platforms

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Electronic Supplementary Information

Abstract Many cell-matrix interaction studies have proved that dynamic changes in the extracellular matrix (ECM) are crucial to maintain cellular properties and behaviors. Thus, developing materials that can recapitulate the dynamic attributes of the ECM is highly desired for three-dimensional (3D) cell culture platforms. To this end, we sought to develop a hydrogel system that would enable dynamic and reversible turning of its mechanical and biochemical properties, thus facilitating the control of cell culture to imitate the natural ECM. Herein, a hydrogel with dynamic mechanics and a biochemistry based on an addition-fragmentation chain transfer (AFCT) reaction was constructed. Thiol-modified hyaluronic acid (HA) and allyl sulfide-modified ϵ -poly-L-lysine (EPL) were synthesized to form hydrogels, which were non-swelling and biocompatible. The reversible modulus of the hydrogel was first achieved through the AFCT reaction; the modulus can also be regulated stepwise by changing the dose of UVA irradiation. Dynamic patterning of fluorescent markers in the hydrogel was also realized. Therefore, this dynamically controllable hydrogel has great potential as a 3D cell culture platform for tissue engineering applications.

Keywords Hydrogel; Addition-fragmentation chain transfer; Dynamic mechanics; Controllable biochemistry; 3D cell culture platform

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INTRODUCTION

Traditional 2D cell culture platforms, such as microscope slides, Petri dishes, and well plates, are widely used for cell-based assays *in vitro* due to its simplicity and convenience.^[1] However, 2D platforms fail to mimic key aspects of the *in vivo* cell microenvironment, such as biochemistry, mechanics, and geometry.^[2,3] Recently, many efforts have been made to recapitulate these characteristics mentioned above, like constructing 3D cell culture platforms that imitate dynamic change of the ECM.^[4–13] Although these platforms have static or dynamic single characteristic of ECM to culture cell *in vitro*, they are still quite simple compared to the complex native ECM of tissues.^[14–16] Thus, developing 3D cell culture platform that can dynamically regulate multiple parameters is essential for imitating natural ECM.^[17–19]

The AFCT reaction is a biocompatible radical process and has low radical concentration because the photogenerated

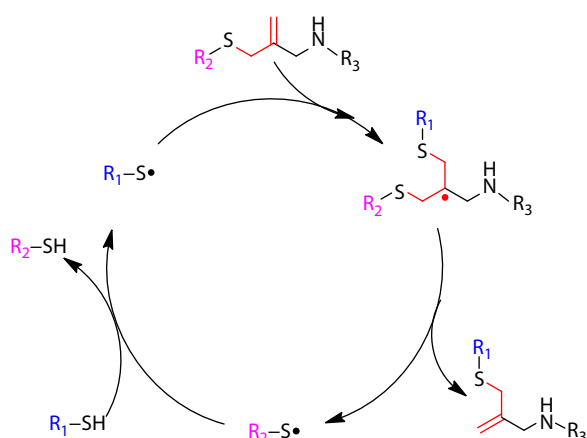
thiyl radicals cleave allyl sulfides, resulting in regenerated new allyl sulfides and new thiyl radicals that can spread through radical transfer (Scheme 1).^[20,21] This characteristic of AFCT provides the potential for dynamic control of bonding formation and breakage.^[21] It has been reported that hydrogels based on AFCT showed reversible bonding of biochemical ligands or amplified degradation.^[22–24] Nevertheless, none of them could regulate both of mechanical and biochemical properties simultaneously. Hence, we expect that a dual function hydrogel with dynamic mechanics and biochemistry can be achieved by employing the AFCT reaction in the gelation, degradation, and functionalization steps.

To demonstrate this, allyl sulfide-modified EPL (EPL-ASPEG) and thiol-modified hyaluronic acid (HA-SH) were synthesized in this study as precursor polymers. Both the gelation of the EPL-ASPEG/HA-SH mixture solution and the degradation of the resulting hydrogel were achieved under UVA irradiation through the AFCT reaction. The elastic modulus of the hydrogel was regulated stepwise during gelation and degradation by controlling the dose of UVA light. Meanwhile, the hydrogels showed fluorescent marker patterning and release, thus providing an opportunity for the temporal and spatial con-

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Scheme 1 Reaction mechanism of allyl sulfide and thiol radical through AFCT.

tol of the local cellular biochemistry (Scheme 2). Moreover, the hydrogel exhibited non-swellable properties and cytocompatibility. Hence hydrogel with dynamically controllable mechanics and biochemistry shows great potential as a 3D cell culture platform for tissue engineering applications.

EXPERIMENTAL

Materials

HA (20–40 kDa), EPL and *N*-acetyl-L-cysteine (NAC) were purchased from Aladdin. Fluorescein-PEG1000-thiol (FITC-PEG-SH) was purchased from Ponsure Biological. Dulbecco's Modified Eagle Medium (DMEM), Trypsin EDTA solution A, Penicillin-Streptomycin solution and fetal bovine serum (FBS) were purchased from Biological Industries. Propidium

iodide, calcein am cell viability assay kit and cell counting kit-8 (CCK-8) were purchased from Beyotime. And other chemicals were purchased from Energy and used as received. Dichloromethane (DCM) and dimethyl sulfoxide (DMSO) were distilled with CaH_2 before use. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP), 3,3'-dithiobis(propanoic dihydrazide) and methoxy-poly(ethylene glycol)-thiol (m-PEG-SH, $M_n=350$ Da) were synthesized according to literature.^[25–27]

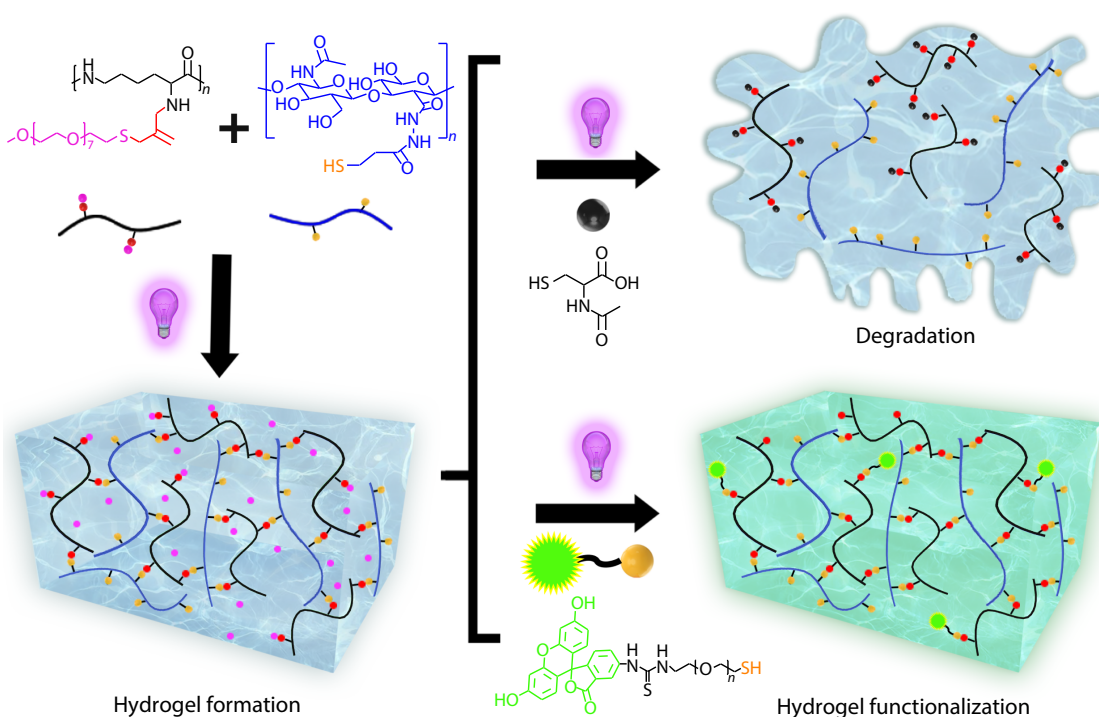
Synthesis of Hydrogel Precursors

Synthesis of EPL-ASPEG

First, m-PEG-SH (0.772 g) and sodium methanolate (0.13 g) were dissolved in methanol (5 mL); 3-chloro-2-chloromethyl-1-propene (0.30 g) dissolved in methanol (10 mL) was added dropwise into the m-PEG-SH and sodium methanolate solution, and then reacted at 60 °C for 20 h. Then, the mixture was diluted with water (20 mL), followed by extraction with DCM (5×20 mL). m-PEG-allyl sulfide was obtained from the dried organic layers (yield: 76.3%). Finally, m-PEG-allyl sulfide (0.476 g), potassium carbonate (0.166 g), and EPL (0.438 g) were dissolved in DMSO (15 mL) and stirred at 70 °C for 24 h. The solution was directly dialyzed (MW cut-off = 2 kDa) against distilled water for 3 days and then lyophilized to obtain EPL-ASPEG (yield: 68.4%).

Synthesis of HA-SH

HA-SH was synthesized as described in a previous literature.^[28] First, 3,3'-dithiobis(propanoic dihydrazide) (1.06 g) and HA (2.0 g) were dissolved in water (100 mL, pH=4.5). 1-(3-Dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDC, 0.25 g) dissolved in water (5 mL, pH=4.5) was added dropwise into the solution, which was stirred at 25 °C for 24 h. Next, D,L-dithiothreitol (DTT, 3.46 g) was added, and the pH was adjusted to 8.5 with HCl (3 mol/L). The mixture was allowed to react at room temperature for 24 h. The solution was adjusted



Scheme 2 Schematic illustration of hydrogel formation, degradation, and biomolecule-functionalization upon UVA irradiation.

to pH=3 and dialyzed (MW cut-off = 2 kDa) against a dilute HCl aqueous solution (pH=3) containing NaCl (0.1 mol/L) for 24 h and diluted with water for another 24 h. Finally, the solution was lyophilized to obtain HA-SH as a white solid (yield: 74.1%).

Synthesis of Hydrogels

A 1:1 molar ratio of thiol group to allyl sulfide group was used for the formation of all hydrogel preparations with different concentrations and fixed concentrations of the photoinitiator LAP (4 mmol/L). As an example, the method for preparing HE-10.5% is as follows: HA-SH (75 mg), EPL-ASPEG (30 mg), and LAP (1 mg) were dissolved in 500, 250, and 250 μ L of Dulbecco's phosphate-buffered saline (DPBS, 0.01 mol/L, pH=7.4), respectively. These solutions were mixed using a vortex oscillator under UVA irradiation (365 nm, 10 mW/cm²) to form a hydrogel. The gelation time of the hydrogels was tested using the tilting vial method.

Characterization

The ¹H nuclear magnetic resonance (¹H-NMR) spectra of the materials were recorded on a Bruker spectrometer (AVANCE III HD 500 MHz), using deuterated chloroform and water as the solvents. Fourier transform infrared (FTIR) spectra of the materials were recorded on a VERTEX 70 FTIR spectrometer in the range of 4500–370 cm⁻¹. Hydrogels and cells were imaged using a ZEISS LSM 700 confocal laser scanning microscope (CLSM). Cryogenic-scanning electron microscopy (cryo-SEM) of HE-7% hydrogels containing NIH3T3 cells and BMSCs were imaged in ZEISS Sigma 300 at 5 kV. The degree of substitution of thiols was determined through Ellman's test using a TECAN Infinite 200 Pro microplate reader according to the literature.^[29]

Swelling Ratio

The as-prepared hydrogels (W_0 , the mass) were incubated in DPBS solution (0.01 mol/L, pH=7.4) at 37 °C. At selected time points, the hydrogels were taken out, and the excess water was gently removed to weigh the hydrogels (W_t). The swelling ratio was determined as $Q = (W_t \times 100\%) / W_0$. The tests were repeated for three times.

Rheological Analysis and Compressive Tests

Rheology studies of the hydrogels were carried out using a stress-controlled rheometer (Anton Paar, MCR 301) with a steel upper plate and a quartz lower plate. In a typical experiment, a hydrogel precursor solution (0.6 mL) was placed on the lower plate, and the upper plate was moved to a gap distance of 1 mm.^[30] For the time sweeps, UVA light, a strain of 1%, and a frequency of 1 Hz were applied. A strain of 1% was applied to the frequency sweeps of the as-prepared hydrogels. All rheological tests were conducted at 37 °C. The compressive properties of the hydrogels were evaluated by using a universal material testing machine (Suns MODUTM 4202) with a 50 N load cell. Hydrogel samples were prepared in a cylindrical mold with a diameter of 9 mm and a length of 4 mm and the compressive rate was 5 mm/min at 25 °C. The compressive modulus was calculated from the average slope in the initial linear region of the stress-strain curves (from 0% to 5%).

Dynamic Patterning of Signaling Molecule in Hydrogel

The as-prepared hydrogels were immersed in FITC-PEG-SH

(100 μ mol/L) and LAP (100 μ mol/L) solution for 12 h, and then irradiated with UVA light (365 nm, 10 mW/cm²) for 180 s through a photomask with a repeating 100 μ m-wide striped pattern. To release FITC-PEG-SH, the patterned hydrogel was immersed in NAC (300 μ mol/L) and LAP (300 μ mol/L) solution for 12 h and then irradiated through the same photomask rotated by 90° with UVA light. Subsequently, the hydrogel was washed with DPBS (3 $\times$ 4 h) to remove any unreacted molecules. Finally, the hydrogel was imaged using CLSM.

In Vitro Cytotoxicity

The cytotoxicity of the materials was evaluated using NIH 3T3 cells and BMSCs received from Chen's group.^[31] Precursor solutions (10 mg/mL) were prepared by dissolving HA-SH and EPL-ASPEG (-SH/-ASPEG, 1/1, mol/mol) in DMEM. The photo-degradation hydrogel solutions (10 mg/mL) were obtained by dissolving the lyophilized photo-degradation hydrogel in DMEM. The cells were seeded in 96-well plates containing DMEM (100 μ L) at a density of 1×10^4 cells per well for NIH 3T3 cells and 2×10^3 cells per well for BMSCs. Then, they were incubated in a humidified atmosphere of 5% CO₂ at 37 °C for 24 h, followed by replacement of the medium with 100 μ L of precursor solution or photo-degradation hydrogel solutions and incubation for 24 h. Next, CCK-8 solution (10 μ L) was added into each well, and the absorbance values at 450 and 630 nm (used for baseline correction) were measured after 3 h of incubation. Negative and positive controls were obtained by incubating the cells in normal DMEM and 0.64% phenol-containing DMEM, respectively.

3D Cell Culture, Reversible Bonding of Signal Molecular in Cell Culture and Release of Cells from Hydrogels

The HE-7% hydrogel was used for the encapsulation of NIH 3T3 cells and BMSCs. First, HA-SH, EPL-ASPEG, and LAP were dissolved in DMEM, which were mixed with the cell solution. The gel was prepared as mentioned above, and 50 μ L of the gel was placed in each well of a 96-well culture plate at a final cell density of 5×10^5 cells/mL for NIH 3T3 cells and 2×10^5 cells/mL for BMSCs. Then, 200 μ L of DMEM solution was added to each well to cover the gels and incubated at 37 °C in 5% CO₂, changing the culture medium every 2 days. The viability of the encapsulated cells was evaluated using fluorescence staining. DPBS (100 μ L) containing calcein am (2 μ mol/L) and propidium iodide (4 μ mol/L) were added to each well and incubated at 37 °C for 30 min, followed by washing with DPBS for three times. The cells were then imaged using CLSM with scans every 10 μ m in the z direction. Live and dead cells were counted manually in 5 slices (spacing at 100 μ m) of z-stack to calculate the percentage viability. Five independent z-stacks were analyzed for each sample. The proliferation of NIH 3T3 cells and BMSCs in the hydrogels was assessed using the CCK-8 method, as mentioned above. The value for 1 day was set to 100%.

To reversibly bond signal molecular in cell culture, HE-7% samples encapsulating NIH 3T3 cells mentioned above were cultivated with DMEM containing 100 μ mol/L LAP and 100 μ mol/L FITC-PEG-SH for 12 h. Then, they were irradiated for 3 min with 10 mW/cm² 365 nm light and washed with media (3 $\times$ 4 h). To release FITC-PEG-SH, half of the samples were

then swollen with DMEM containing 300 $\mu\text{mol/L}$ NAC and 300 $\mu\text{mol/L}$ LAP for 12 h. After same irradiation and washing steps, CCK-8 quantitative tests were taken to all samples as mentioned above. HE-7% encapsulating NIH 3T3 cells incubating in normal DMEM was set as negative control.

To investigate the effect of degradation on the viability of cells in hydrogels, a hydrogel encapsulating NIH 3T3 cells was prepared and cultivated as mentioned above, and the medium was replaced with DMEM containing NAC (50 mmol/L) and LAP (4 mmol/L) for another 12 h of encapsulation, followed by UVA irradiation for 2 min. After degradation, the cell suspension was centrifuged at 1000 r/min for 5 min. The cells were resuspended and centrifuged to remove the degraded hydrogel. These extracted cells were incubated on a new 96-well culture plate at a concentration of 5000 cells per well, and the same concentration of cells grown on tissue culture polystyrene (TCPS) were set as control group. Cells were observed under the brightfield after 12, 24, and 48 h from seeding. Cells viability in 48 h was observed using a live-dead cell staining kit and assessed by the CCK-8 method.

RESULTS AND DISCUSSION

Synthesis and Characterization of Precursors

HA is widely distributed throughout living tissues and has been shown to induce intracellular signal transduction and affect cell proliferation, survival, movement, and differentiation.^[32] However, most cells do not readily adhere to neat HA material because of its negative charge. EPL is a biocompatible cationic polypeptide that has shown promising applications in cell adhesion and tissue regeneration.^[33,34] Previous studies have demonstrated that materials composed of HA and EPL can induce favorable environment for cell adhesion.^[35] Meanwhile, this two polyelectrolytes can form ionic linkages that would benefit mechanical strength and stability of hydrogel. In this study, two hydrogel precursors were designed and synthesized

based on EPL and HA, respectively. EPL decorated with an allyl sulfide group was synthesized through a two-step nucleophilic substitution, as illustrated in Fig. 1(A). Its structure was identified using $^1\text{H-NMR}$ and FTIR spectroscopy (Figs. 1B and 1C). EPL-ASPEG shows characteristic proton peaks of allyl sulfide (k) in the $^1\text{H-NMR}$ spectrum, indicating that allyl sulfide-capped m-PEG was indeed conjugated to EPL. Moreover, symmetrical stretching vibrations of $-\text{C}-\text{O}-\text{C}-$ (1108 cm^{-1}) and wagging vibrations of $=\text{CH}_2$ ($915, 850\text{ cm}^{-1}$) bonds are present in the FTIR spectrum of EPL-ASPEG, which further confirmed the successful modification of allyl sulfide to EPL. The average degree of grafting was then calculated using the peak ratio of allyl sulfide ($-\text{SCH}_2\text{C}=\text{CH}_2\text{CH}_2\text{NH}-$) at 4.92–5.0 ppm to the methylene units of EPL ($-\text{CH}_2\text{CH}_2\text{CH}_2\text{CHNH}_2-$) at 1.09–1.3 ppm, which was approximately 26.5 mol% (Fig. S1 in the electronic supplementary information, ESI). Thiol-modified HA was synthesized through a two-step reaction, according to the literature.^[36] The synthetic route and the corresponding $^1\text{H-NMR}$ and FTIR results are shown in Fig. S2 (in ESI). The characteristic peaks of the 3-mercaptopropion structure ($\text{HS}-\text{CH}_2-\text{CH}_2-\text{CO}-$) in the $^1\text{H-NMR}$ spectrum and the coupling of the stretching vibration of acylhydrazine (1728 cm^{-1}) in the FTIR spectrum indicated that thiol was successfully grafted onto the HA backbone. The degree of thiol substitution was approximately 20.3% as determined using Ellman's test.

Preparation and Characterization of the Hydrogel

In this study, the AFCT reaction was introduced into an *in situ* hydrogel preparation for the first time by mixing HA-SH and EPL-ASPEG at a 1:1 molar ratio of thiol group to allyl sulfide group. Three hydrogels were fabricated with different polymer concentrations, and named as HE-X%, where X is the total concentration of the polymers. The sol-gel translation of hydrogels was evaluated through the tilting vial test (Fig. 2a). It can be found that gelation became faster with polymer

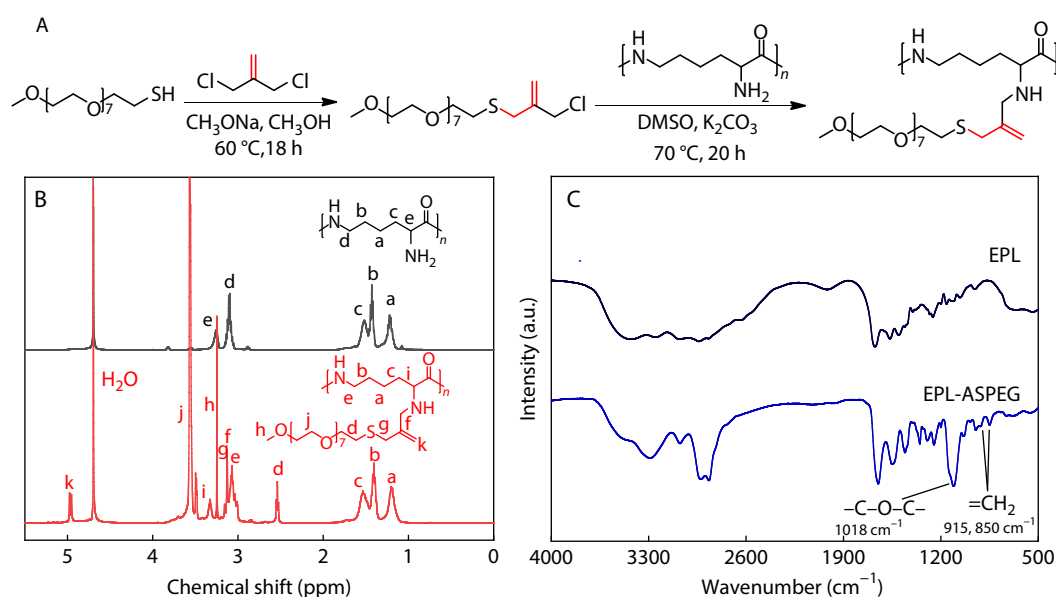


Fig. 1 (A) Synthetic protocol of EPL-ASPEG. (B) $^1\text{H-NMR}$ and (C) FTIR spectra of EPL and EPL-ASPEG.

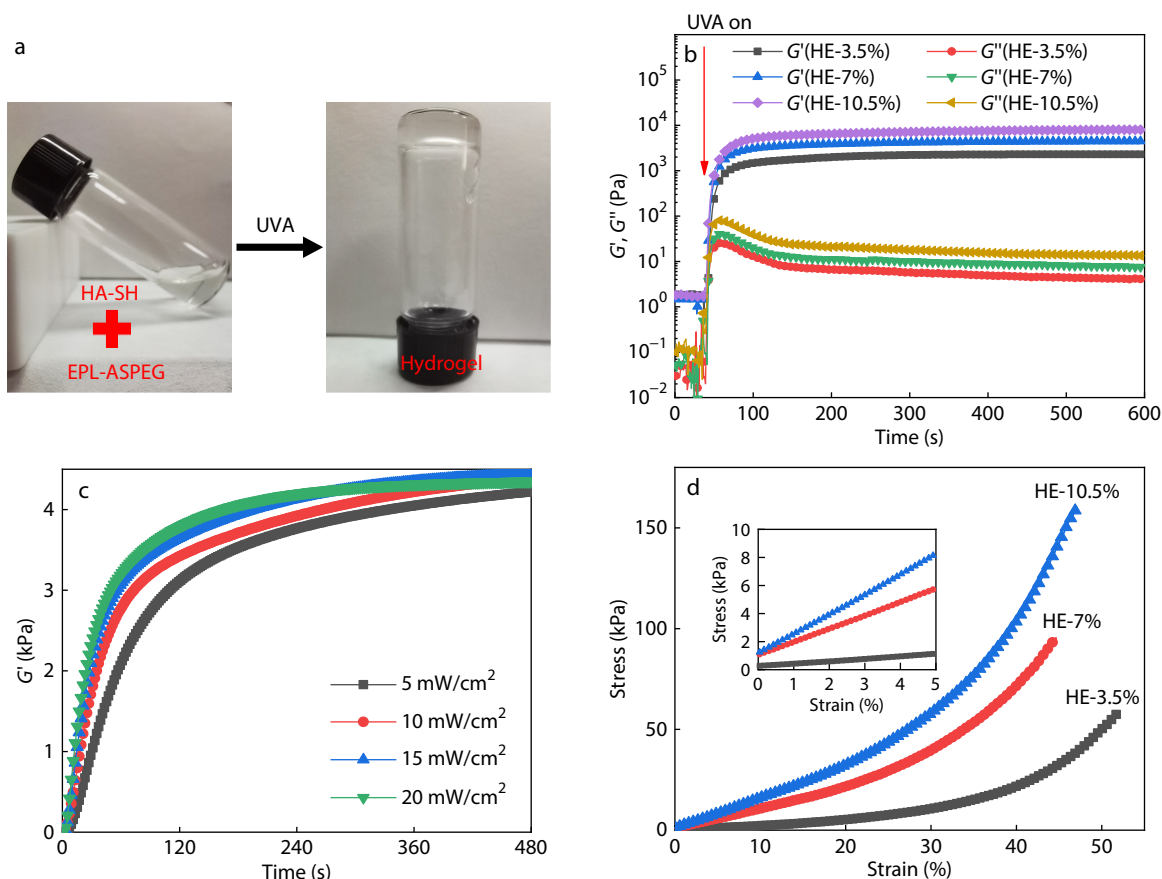


Fig. 2 (a) Photos of hydrogel formation; Storage modulus (G') and loss modulus (G'') during gelation of (b) hydrogels at different concentrations and (c) HE-7% under different UVA light intensities; (d) Compressive stress-strain curves of three hydrogels.

concentration increasing, where HE-3.5% stopped flow within 20 s, HE-7% and HE-10.5% showed instantaneous gelation within 10 s. This gelation time range could avoid cell sedimentation. Gelation was further investigated using a rheometer equipped with an *in situ* UVA source (365 nm, 10 mW/cm²). As shown in Fig. 2(b), G' is higher than G'' before UVA irradiation for all the hydrogels due to the ionic linkages between EPL and HA. Upon UVA irradiating, the G' of all hydrogels increase with time and reached a plateau within 10 min, implying that the network was successfully formed through the AFCT reaction. The G' at plateau is markedly increased from 2000 Pa to 8000 Pa with increasing polymer concentration. The effect of light intensity on the gelation of hydrogel was also studied. As shown in Fig. 2(c), the gelation of hydrogel can be accelerated by increasing light intensity, in which the acceleration was obvious from 5 mW/cm² to 10 mW/cm² and then became slight with the light intensity further increasing. Taking cell incubation into consideration, we employed a light intensity of 10 mW/cm² in the following experiments. Compressive tests were employed to study mechanical properties of the hydrogels (Fig. 2d and Fig. S3 in ESI). With the polymer concentration increasing, compressive strength increases from 58.5 kPa to 159.6 kPa, and compressive modulus increases from 19.1 kPa to 146.8 kPa, which are in the

range of mechanical requirements of cell culture.^[34]

Swelling, Photo-degradation, and Storage Modulus Regulation

Non-swelling property is important for hydrogel upon immersing aqueous solution because the swelling could weaken the mechanical properties by decreasing the crosslinking density. Herein, the swelling property of hydrogels were evaluated by immersing the as-prepared samples in DPBS solution (pH=7.4) at 37 °C for different time (Fig. 3a). After soaking under physiological conditions for 14 days, HE-3.5% remains almost unchanged, HE-7% also shows non-swelling properties with a slight increase in swelling ratio (8%) (Fig. S4 in ESI), while HE-10.5% has a 20% increase in swelling ratio, which may be due to higher concentration of hydrophilic polymers retaining more water. Taking these results mentioned above into consideration, we explored HE-7% in the subsequent tests.

To demonstrate the photo-degradation of the hydrogel, a biocompatible mono-thiol molecule NAC solution containing LAP was prepared.^[37] HE-7% was soaked in NAC solution for 12 h; then, a photo-rheological test was carried out (Fig. 3b). It is found that HE-7% degraded in 2 min, which shows the potential for controlling its mechanics. Herein, the dynamic modulus of the hydrogel was achieved by switching the UVA

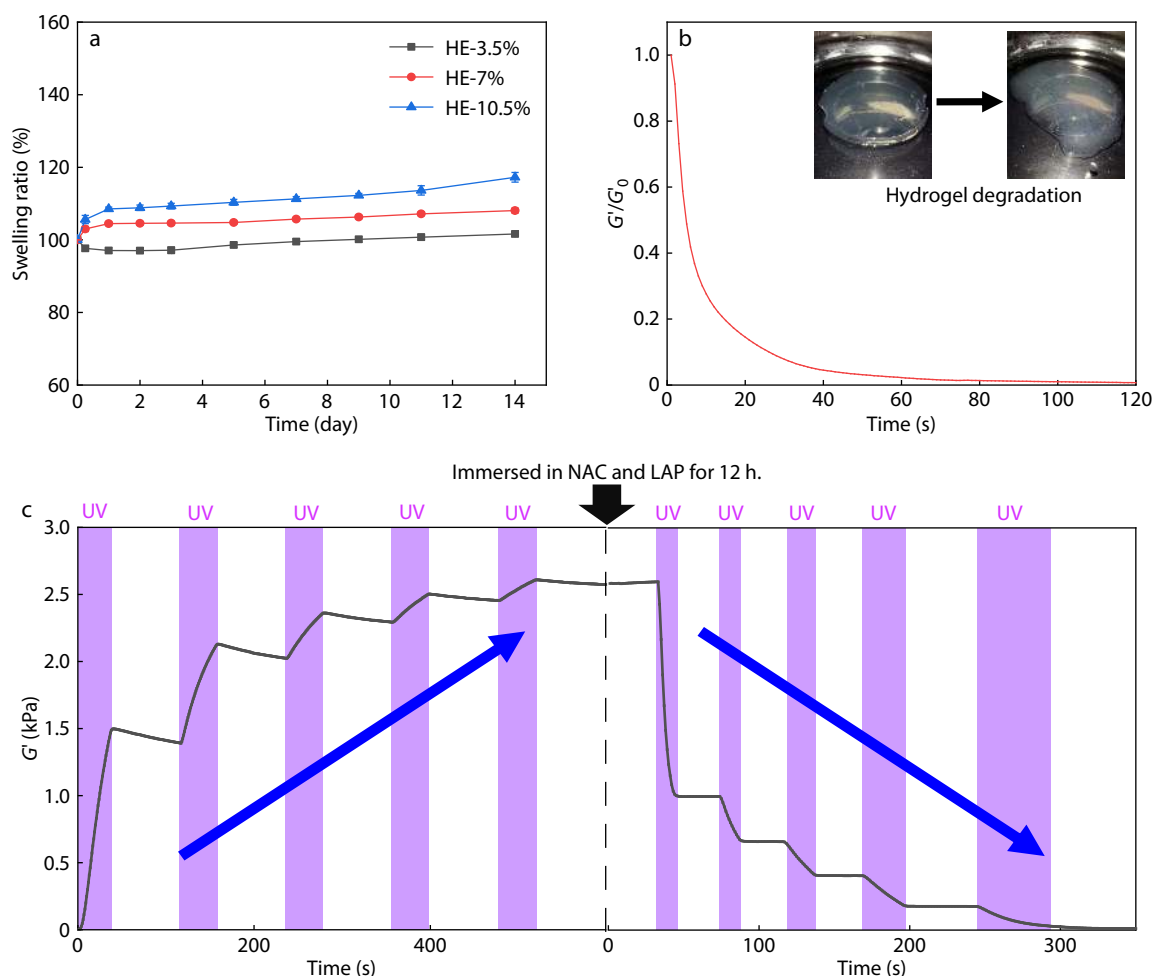


Fig. 3 (a) Swelling ratio of three hydrogels over time in DPBS solution (pH=7.4, 37 °C) (mean±SD, $n=3$); (b) Normalized G' curve of HE-7% during degradation (G'_0 is the storage modulus of HE-7% before test); (c) G' of HE-7% hydrogel elevation and reduction under defined dose of UVA. Purple rectangles represent the period that the UVA light was turned on.

light on and off. As shown in Fig. 3(c), the elevation and reduction of G' are achieved stepwise by controlling the intensity and duration of UVA irradiation because of the characteristic of the AFCT reaction, which can be arrested upon turning off the light. The values of modulus could be precisely tuned from 300 Pa to 2500 Pa, which are within the range of mechanics to support stem cell proliferation.^[38] Therefore, this temporally controllable and dynamic mechanism makes HE-7% a potential platform for exploring the real-time mechanosensitive behaviors between cells and the ECM.

Dynamic Patterning of Signaling Molecules in Hydrogel

During the restoration of natural tissues, the ECM presents dynamic biochemical signals at different stages. A temporal-spatial control of signal molecules is in high demand to imitate the dynamic environment of the ECM. Allyl sulfide has been used as a functional group for the reversible bonding of biochemical molecules in hydrogels.^[22,23] Accordingly, the dynamic patterning of molecules in HE-7% was anticipated in this study. To demonstrate this, FITC-PEG-SH was used as a fluorescent marker to represent signaling protein that was often

employed under low concentration (<100 $\mu\text{mol/L}$). Under this low concentration of FITC-PEG-SH, the radical propagation would be inhibited and the AFCT reaction could be limited by the concentration of LAP.^[22] To increase efficiency of FITC-PEG-SH molecule immobilization, we used a higher concentration of LAP (100 $\mu\text{mol/L}$) in this test. A piece of Cu with a repeating 100- μm -wide striped pattern was used as a photomask, and the fabrication process is illustrated in Fig. 4(a). HE-7% containing FITC-PEG-SH and LAP was irradiated under a mask and the striped pattern was formed on it after being washed with DPBS. Then, the resulting patterned hydrogel containing NAC and LAP was further irradiated with a mask rotated at 90° and followed by washing with DPBS to obtain the final hydrogel with square pattern. Both pattern are observed in CLSM images (Figs. 4b and 4c), where the fluorescent stripe corresponds to the irradiated area due to the bonding of FITC-PEG-SH, whereas the dark squares on the fluorescent stripes were attributed to the replacement of FITC-PEG-SH by non-fluorescent NAC. These results demonstrated that a dynamic patterning of fluorescent markers could be achieved in this hydrogel *via* UVA irradiation. Moreover, although the hydrogel underwent a soak process

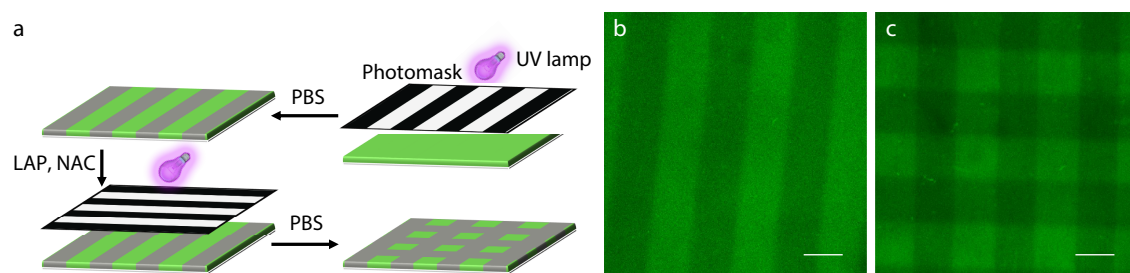


Fig. 4 (a) Schematic illustration of patterning process; Fluorescent patterning of (b) after bonding FITC-PEG-SH and (c) after releasing FITC-PEG-SH. Scale bar: 100 μm .

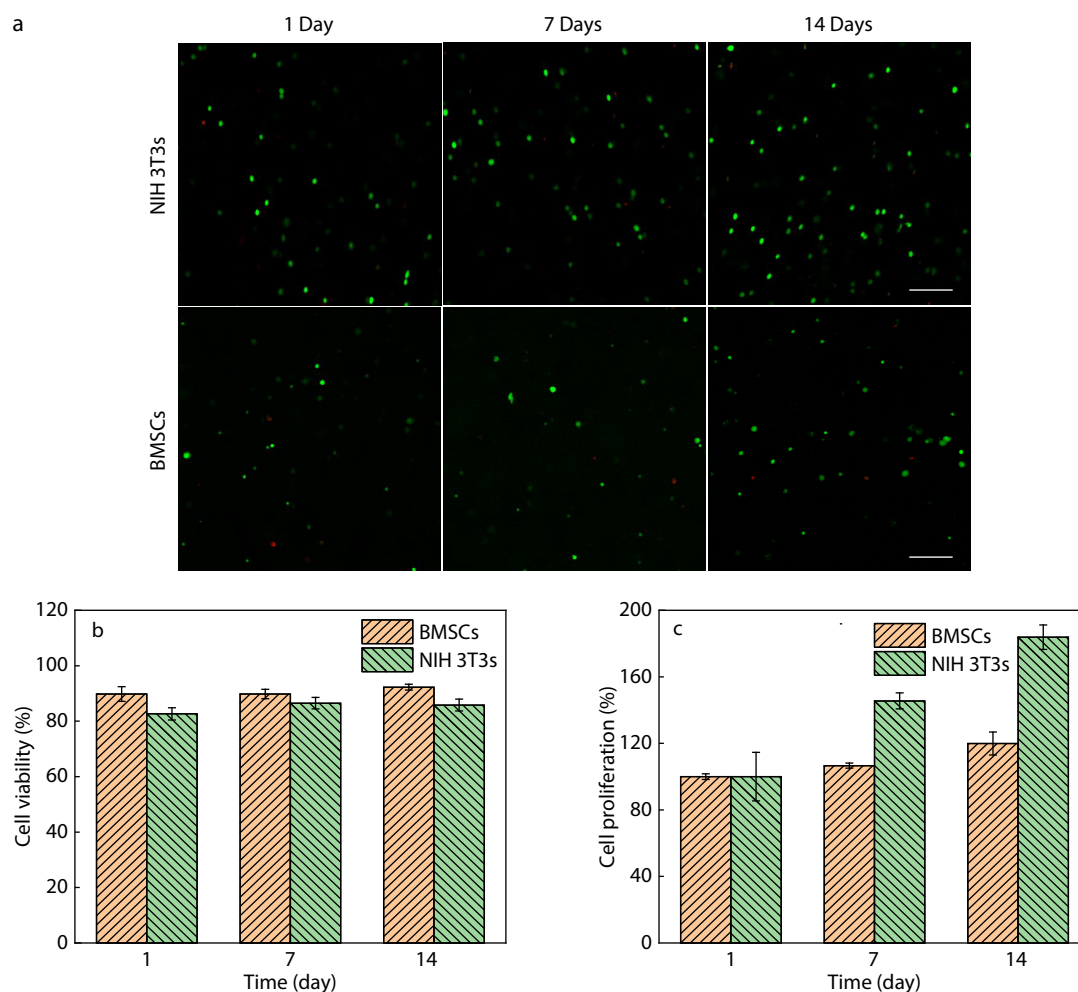


Fig. 5 (a) Fluorescence images, (b) cytocompatibility and (c) quantitative proliferation of NIH 3T3 cells and BMSCs in HE-7% for different time. Scale bar in NIH 3T3 cells image: 100 μm . Scale bar in BMSCs images: 200 μm . Live cell = green, dead cell = red.

for patterning, the G' was stable because the concentration of LAP and monofunctional thiol was lower than what is needed for degradation (Fig. S5 in ESI). This implied that the dynamic patterning of signaling molecules could be performed independently and had no effect on the modulus of the hydrogel.

Cell Compatibility, 3D Cell Encapsulation and Cell Release

Excellent biocompatibility is necessary for 3D cell culture

platform materials. Thus, we chose NIH 3T3 cells and rabbit bone marrow mesenchymal stem cells (BMSCs) as model cells to evaluate the cytocompatibility of the precursor solution and the photo-degradation solution of HE-7%. Both the precursor solution and HE-7% photo-degradation solution exhibited good cytocompatibility with NIH 3T3 cells and BMSCs (Fig. S6 in ESI). The cytocompatibility of the reversible bonding of signal molecular in HE-7% was also examined (Fig. S7 in ESI), and the viability of NIH 3T3 cells in hydrogel which underwent reversible

bonding of signal molecular was comparable with that of negative control.

For the 3D cell culture tests, NIH 3T3 cells and BMSCs were encapsulated in HE-7% by mixing the precursor solutions containing cells. After incubation for 1, 7, and 14 days, live/dead cell staining was performed for the hydrogels. As shown in Figs. 5(a) and 5(b), and Fig. S8 (in ESI), a uniform distribution and a high viability of both cells can be observed in HE-7%, indicating the good cytocompatibility of the photoreaction. It can also be found that both cells encapsulated in hydrogels are rounded, and no spreading of cells is observed during the studied period. This might be attributed to the pore size of hydrogels (about 0.83 μm) which is far smaller than that of cells (NIH 3T3: 12.6 μm , BMSC: 24.4 μm) (Fig. S9 in ESI).^[39] Cell proliferation in HE-7% hydrogels was also demonstrated using the CCK-8 kit method, where NIH 3T3 cells proliferated more than BMSCs after cultivation for 7 and 14 days (Fig. 5c). This might be due to the difference in cells doubling times between NIH 3T3 cells and BMSCs.^[40,41]

Moreover, the NIH 3T3 cells release experiment was carried out. NIH 3T3 cells extracted from the hydrogels were replated on 96-well culture plate, together with cells grown on TCPS as controls to study their morphology and proliferation. As shown in Fig. S10 (in ESI), NIH 3T3 cells released after 12, 24 or 48 h, show the same morphology as control samples. The results of CCK-8 quantitative test and live/dead cell staining test demonstrate that the cells released after encapsulation still had high viability and displayed normal proliferation compared to cells grown on TCPS (Figs. S11 and S12 in ESI). This confirms that the degradation reaction conditions are gentle enough to be used for 3D cell culture. These results mentioned above reveal that HE-7% had excellent material biocompatibility, gelation, and degradation reactions, thus ensuring its potential applications in 3D cell culture platforms.

CONCLUSIONS

We designed a strategy based on the AFCT reaction to develop a hydrogel with dynamically controllable mechanics and biomolecule presentation. Allyl sulfide-modified EPL and thiol-modified HA were synthesized as hydrogel precursors, which can generate hydrogels under UVA irradiation. The resulting hydrogel exhibited elastic, non-swelling, and degradation properties. More importantly, the modulus of the hydrogel can be reversed and stepwise regulated by controlling the dose of UVA light during the gelation and degradation process. Meanwhile, dynamic patterning of fluorescent markers can also be achieved in this hydrogel via UVA irradiation, owing to the characteristics of the AFCT reaction. The hydrogels also showed excellent cytocompatibility, gelation, and degradation, where NIH 3T3 cells and BMSCs in the hydrogel showed good viability for 2 weeks. Further, the NIH 3T3 cells have high viability and display normal proliferation after releasing from these hydrogels. Because the controllable modulus and biochemistry were present in a single system, we believe that this type of hydrogel has great potential as a 3D cell culture platform for tissue engineering applications.

NOTES

The authors declare no competing financial interest.

Electronic Supplementary Information

Electronic supplementary information (ESI) is available free of charge in the online version of this article at <http://doi.org/10.1007/s10118-021-2639-3>.

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