Electronic Supplementary Information for

Dynamic Hydrogels via Monoamine Oxidase B Catalyzed Deamination and Aldimine Crosslinking for 3D Printing†

By Qingcong Wei, [a,c] Wei Xu, [b] Qi Zhang,[a] Shuang Zhang,[a] Liming Cheng,[b] and Qigang Wang*[a]*

[a] School of Chemical Science and Engineering, Tongji University, Shanghai 200092 (China). *E-mail: wangqg66@tongji.edu.cn
[b] Spine Division of Orthopaedics Department, Tongji Hospital, Tongji University School of Medicine, Shanghai 200065 (China). *E-mail: chlm.d@163.com
[c] School of Chemistry and Chemical Engineering, Henan Normal University, Xinxiang 453007 (China)

1. Materials

Monoamine Oxidase B (human, recombinant, expressed in baculovirus infected BTI insect cells), catalase (bovine liver), cellulose (from *Trichoderma* sp.), papain (from papaya latex), horseradish peroxidase (EC 1.11.1.7) and glycol chitosan were purchased from Sigma-Aldrich. Tosyl chloride (TsCl) was purchased from 9 Ding Chemistry (Shanghai) Co., Ltd. 4-Hydroxybenzylamine, tyramine, 4-hydroxybenzaldehyde and di-tert-butyl dicarbonate were purchased from Energy-Chemical Co., Ltd (Shanghai). Diamino-PEG (PEG-NH_2) was from Shanghai ToYong Biotech. Co., Ltd. Other reagents were purchased from Sinopharm Chemical Reagent Co., Ltd. (Shanghai). All materials were used as received.

2. Synthesis of di-functionalized-PEG

As shown in Scheme S1, modified procedures were used according to the literature.[1]

Synthesis of Boc-protected amines (1 and 2)

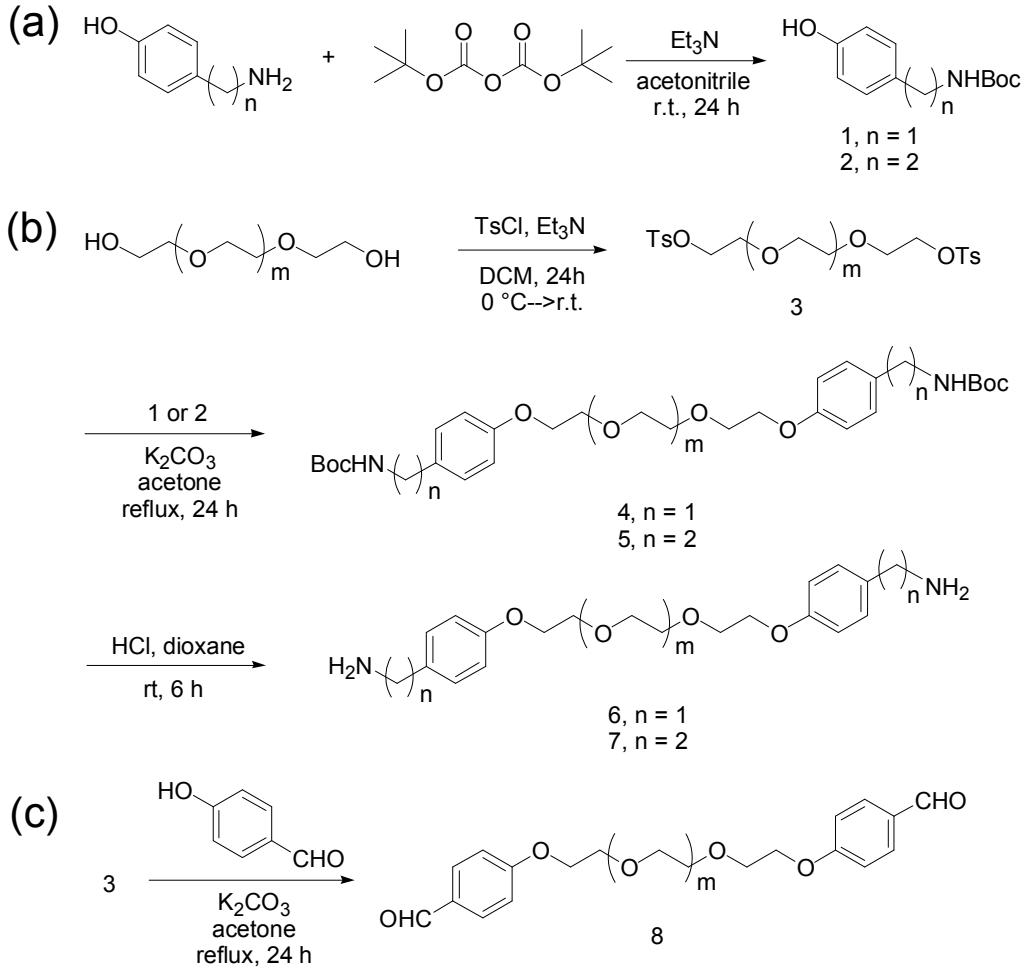
To a solution of 4-Hydroxybenzylamine or tyramine (2.4 mmol) in 30 mL acetonitrile was added triethylamine (7.2 mmol) in one portion and then di-tert-butyl dicarbonate (3 mmol) dropwise. The reaction mixture was stirred for 24 h at room temperature. 30 mL dichloromethane (DCM) was added and the mixture was washed with 2 M HCl (30 mL × 2). Then the organic layer was washed with distilled water (30 mL × 2) and brine (30 mL × 2) respectively. After being dried with anhydrous $NaSO_4$, the solvent was removed in vacuo and dried overnight to yield Boc-protected amines (90 %) as brown thick oil. The products were used directly without further purification.

Synthesis of Ts-functionalized-PEG (3)

Polyethylene glycol 2000 (PEG) (4.5 mmol) and triethylamine (18 mmol) were dissolved in 100 DCM and cooled with ice-water bath. Then TsCl (18 mmol) in 20 mL DCM was added dropwise. The resulting mixture was stirred for 24 h at room temperature. After being washed with 2 M HCl (50 mL × 2), distilled water (100 mL × 2) and brine (100 mL × 2) respectively, the organic layer was dried (anhydrous $NaSO_4$) and concentrated to 1-2 mL. Then it was precipitated in 200 mL cold ethyl ether and white powder was obtained after dried in vacuo (92 %).

Synthesis of PEG-BA (6), PEG-PEA (7) and PEG-AL (8)

3 (1.1 mmol) and 1 (or 2, or 4-hydroxybenzaldehyde, 2.2 mmol) were dissolved in 20 mL acetone, then K_2CO_3 (1.8 mmol) was added resulting a suspension. The reaction mixture was heated to reflux for 24 h, then the solvent was removed in vacuo. DCM (30 mL) and water (30 mL) were added to the light brown viscous solid and stirred thoroughly. The organic layer was washed with distilled water (50 mL × 2) and brine (50 mL × 2) respectively, then dried (anhydrous $NaSO_4$), concentrated (1-2 mL) and precipitated in cold ethyl ether (100 mL). Light brown solid was obtained (70 % for 4, 75 % for 5 and 80 % for 8). Then 4 (or 5, 0.6 mmol) was dissolved in 30 mL dioxane and then 5 mL concentrated HCl was added. The resulting mixture was stirred for 5 h at room temperature. The work-up process is the same as above and light brown solid obtained the product (65 % for 6 and 60 % for 7).



Scheme S1 Synthesis of di-functionalized-PEG: PEG-BA (6), PEG-PEA (7) and PEG-AL (8).

3. Hydrogel preparation

PEG-BA or PEG-PEA or PEG-NH_2 (280 μL, 7 wt%), GC (280 μL, 3 wt%), catalase (20 μL, 200 U) and MAO B (20 μL, 5 mg/mL) were mixed thoroughly in PB solution (7.0) and keeping the obtained solution at 37 °C for 3 h resulted in hydrogel formation (solid, 4.6 wt%).

Another hydrogel was prepared by simply mixing PEG-AL (280 μL, 7 wt%), GC (280 μL, 3 wt%) and PB (40 μL, 7.0). The gelation occurred with about 5 min.

4. SEM characterization

Hydrogel samples were coated on the silicon wafer, then freezed in liquid nitrogen, and further dried 24 h in vacuum. A thin layer of gold was sputter-coated before testing with a field emission scanning electron microscopy (Hitachi S-4800) at 3 KV voltages.

5. Mechanical analysis

The compressive test of hydrogels was taken on a FR-108B testing machine (Shenzhen to think twice Aspect Technology Co., Ltd., China) at a crosshead speed of $1 mm \cdot min^{-1}$. The diameter of the gels is about 19 mm and the thickness is 3-4 mm. The

compressive stress (σ) was approximately calculated as $\sigma = F_{load}/\pi R^2$, where R is the original radius of the sample. The compressive strain (ε) is defined as the change of the thickness relative to the original thickness. The stress and strain between ε = 5 and 15% were used to calculated the Young's modulus with at least 3 parallel tests for each hydrogel. The compressive strain is 30% when subjected to cyclic compression.

6. Rheological tests

The rheological properties of hydrogels were tested using a RS6000 rheometer (Thermo Scientific, Karlsruhe, Germany) with parallel plate geometry (35 mm diameter, 0.3 mm gap) at 37 °C. The frequency-dependent sweep was taken as a function of angular frequency at fixed strain of 0.2 %. The self-healing process of the dynamic gel in response to applied shear forces was performed using continuous step strain sweep test with alternate small oscillation force (γ = 0.2 %) and large one (γ = 200 %). Low viscous silicone oil was added around the edge of parallel plate to prevent the evaporation of H_2O during the test.

7. 3D printing

The pre-gel solution (solid, 1.2 wt%; Ar-NH_2/GC-NH_2, 0.122) was kept at 37 °C for 25 min then loaded into the 3D printer (Ingenovo, Shanghai in-G information Science & Technology Co., Ltd. China). Different arrays and patterns were designed and printed.

8. Multiresponsive properties

Different external stimuli (1 mL) including water (pH = 7), acidic water (pH = 5.0), lysine (20 mg/mL), papain (20 mg/mL) and cellulose (20 mg/mL) were added to the dynamic hydrogel (solid, 4.6 wt%; Ar-NH_2/GC-NH_2, 0.235, 600 μL, containing methylene blue as the model molecule for controlled release) respectively. Then they were incubated at 37 °C for 12 h, and 20 μL of the solution was tested in UV-Vis spectrometer (UV-2700, Shimadzu) each time. The absorbance at 664 nm was recorded.

9. 3D cell culture

NIH-3T3 cells were used as the model cell line and mixed with gel precursor solution (solid, 1.2 wt%; Ar-NH_2/GC-NH_2, 0.122). The 3D encapsulated cells were cultured in serum-free Dulbecco's Modified Eagle's Medium (DMEM) containing 10 % (v/v) of foetal bovine serum and 1 % (v/v) penicillin-streptomycin. 40 μg/mL fluorescein diacetate (FDA) and 10 μg/mL propidium iodide (PI) in DMEM media were used to stain cells. Images were acquired using a Olympus 2000 confocal laser scanning microscope. And the viability was calculated *via* the Imaris Spot Detection function in order to determine the relative proportion of live (green, FDA stained) or dead (red, PI stained) cells.

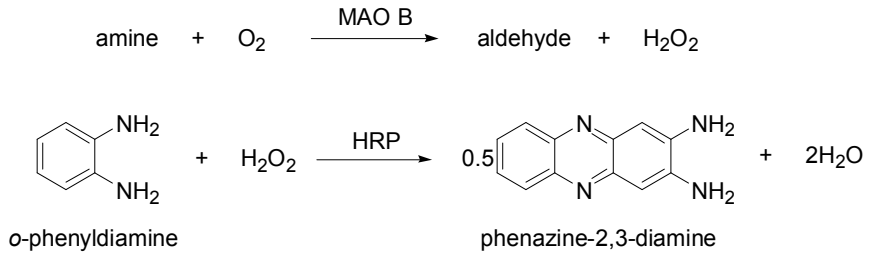
10. PEG-BA conversion test

Oxidative reaction of OPD to phenazine-2,3-diamine (ε_{450} = 16300 $M^{-1}\cdot cm^{-1}$) by

HRP and H_2O_2 was used as the model reaction. The absorbance at 450 nm was measured by a UV-Vis spectrometer (UV-2700, Shimadzu).

Firstly, OPD conversion test was conducted, and there were three groups *viz*: OPD only, GC (containing OPD, MAO B and HRP) and PEG-BA (containing OPD, MAO B and HRP). The typical process is that 1800 μL solution containing 0.08 mmol OPD (5 mg/mL MAO B 60 μL and 0.5 mg/mL HRP 10 μL were added in GC and PEG-BA groups) is added into cuvette and incubated at 37 °C. The absorbance is recorded at different times. And the absorbance (products of completely oxidized OPD) calculated according to Beer-Lambert Law is defined as 100%.

Secondly, PEG-BA conversion test was taken as follows: 1800 μL solution containing 0.08 mmol OPD, 60 μL MAO B (5 mg/mL), 10 μL HRP (0.5 mg/mL), GC (4 wt%) and PEG-BA (4 wt%) is added into cuvette and incubated at 37 °C.



11. Figures

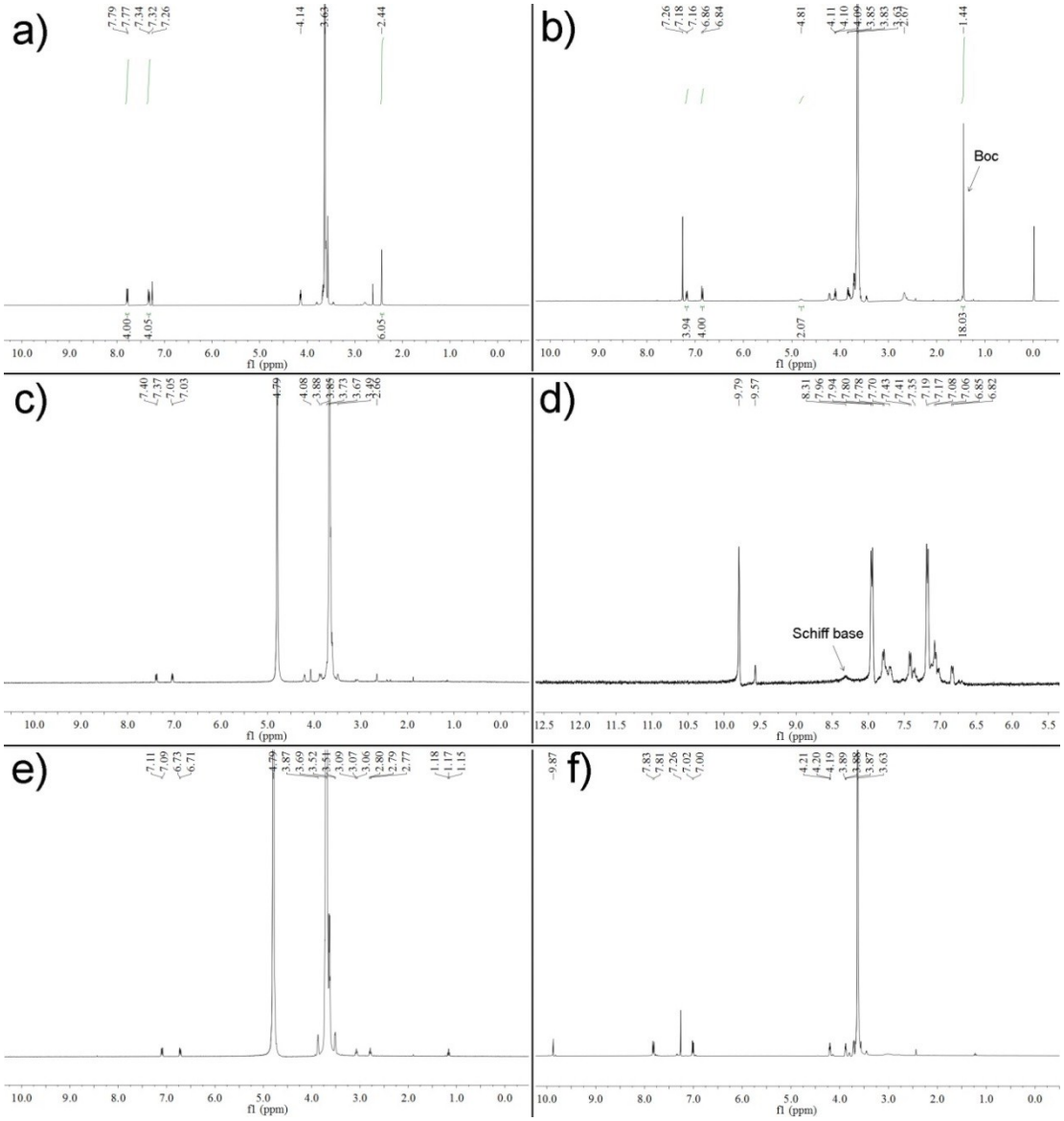


Figure S1 ^{1}H NMR spectra: (a) PEG-Ts in $CDCl_3$. (b) PEG-BABoc in $CDCl_3$, and Boc peak is at 1.44 ppm. (c) PEG-BA in D_2O. (d) Schiff base peak after incubating D_2O-substituted pre-gel solution at 37 °C for 3 h in NMR tube. (e) PEG-PEA in D_2O. (f) PEG-AL in $CDCl_3$.

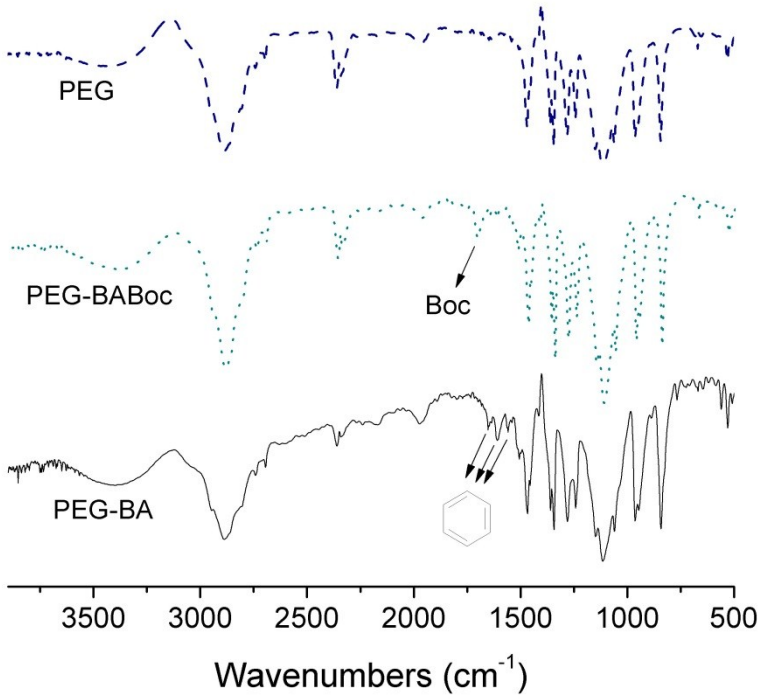


Figure S2 FT-IR spectra of PEG, PEG-BABoc and PEG-BA.

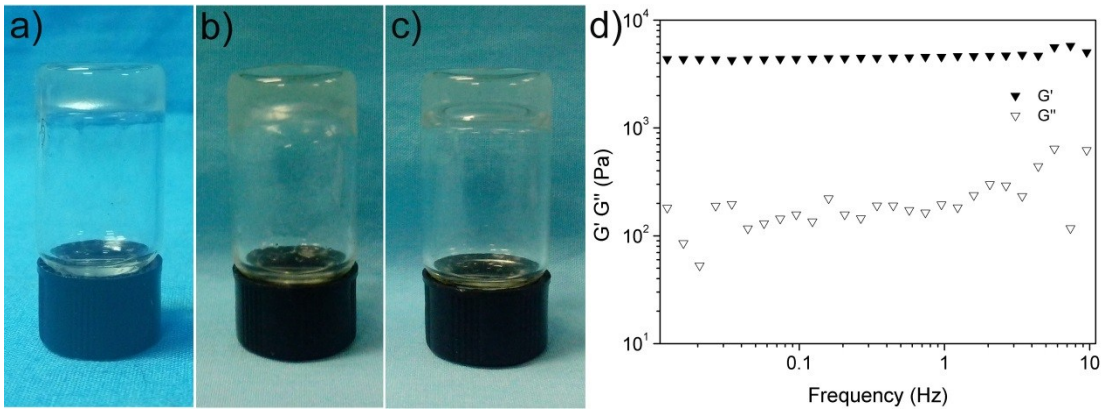


Figure S3 The optical images of hydrogels (0.5 mL in glass vial, (solid 4.6%, PEG-terminal-NH_2/GC-NH_2, 0.235): (a) PEG-PEA + GC + MAO B, (b) PEG-NH_2 + GC + MAO B, (c) PEG-AL+ GC. (d) Frequency sweep test of the hydrogel PEG-AL + GC (solid, 4.6 wt%; CHO/GC-NH_2, 0.235).

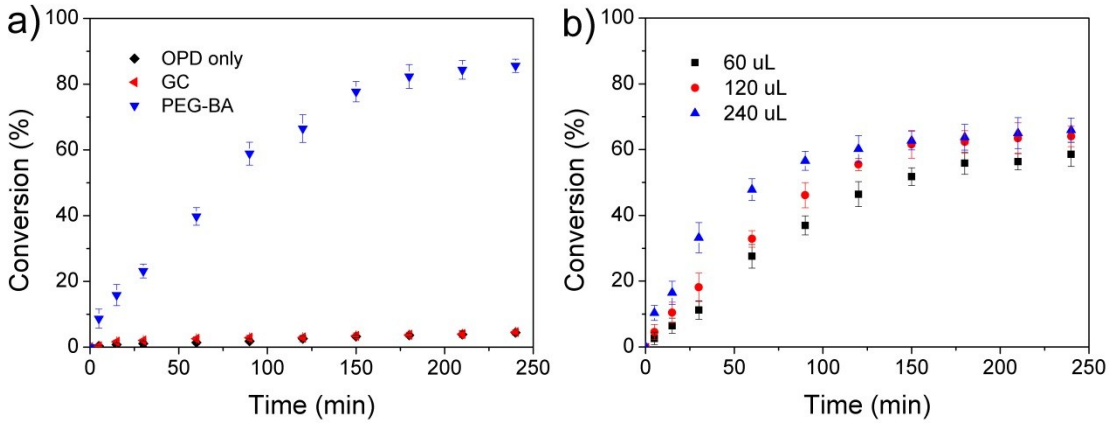


Figure S4 (a) OPD conversion profile of different systems catalyzed by MAO B and HRP: PEG-BA + OPD, GC + OPD, and the control OPD only. (b) PEG-BA conversion profile during the hydrogel formation process with different MAO B concentrations.

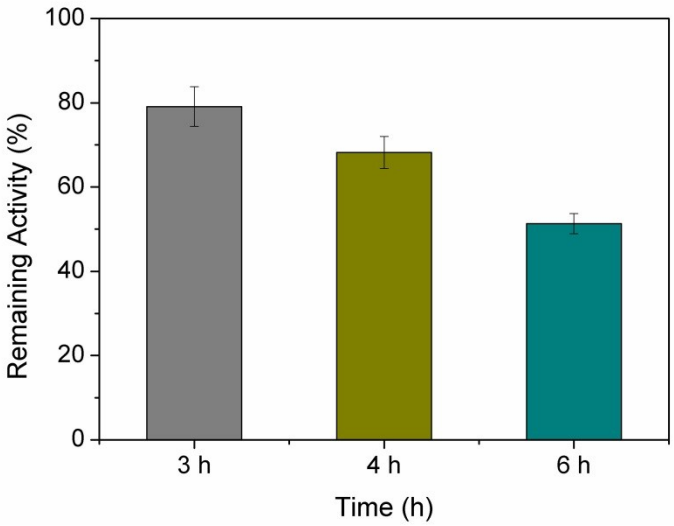


Figure S5 After the PEG-BA/GC gel (without catalase) formation (3 h), remaining activities of the immobilized MAO B were tested.

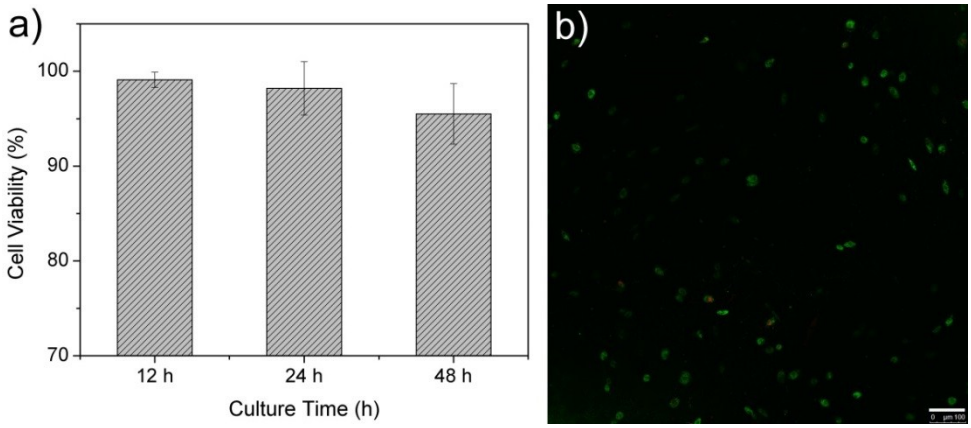


Figure S6 (a) Cell viability at different culture time, (b) Live/dead assay images after 48 h culture from encapsulation (scale bar: 100 μm).

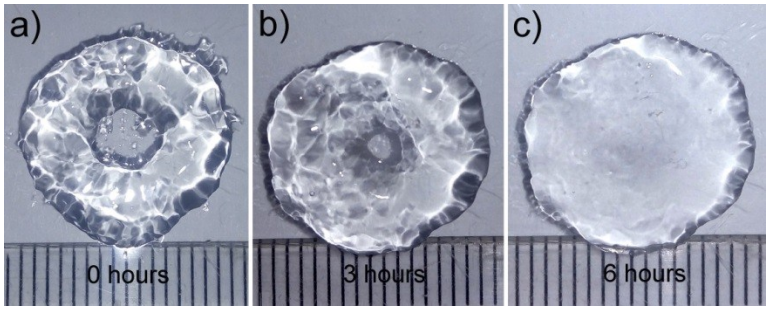


Figure S7 Self-healing test of the dynamic hydrogel (solid, 1.2 wt%; Ar-NH_2/GC-NH_2, 0.122): (a) A man-made hole (diameter 5 mm). (b) Incubation for 3 hours at ambient temperature. (c) The completely mended gel after 6 hours incubation.

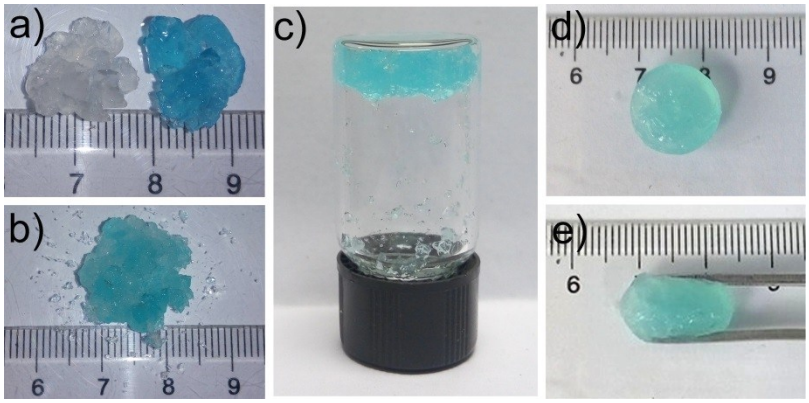


Figure S8 The original gel and gel stained with methylene blue (a); the mashed gel (b) in a vial (c); the self-healed gel after 12 h (d) was squeezed by tweezers (e) without collapse.

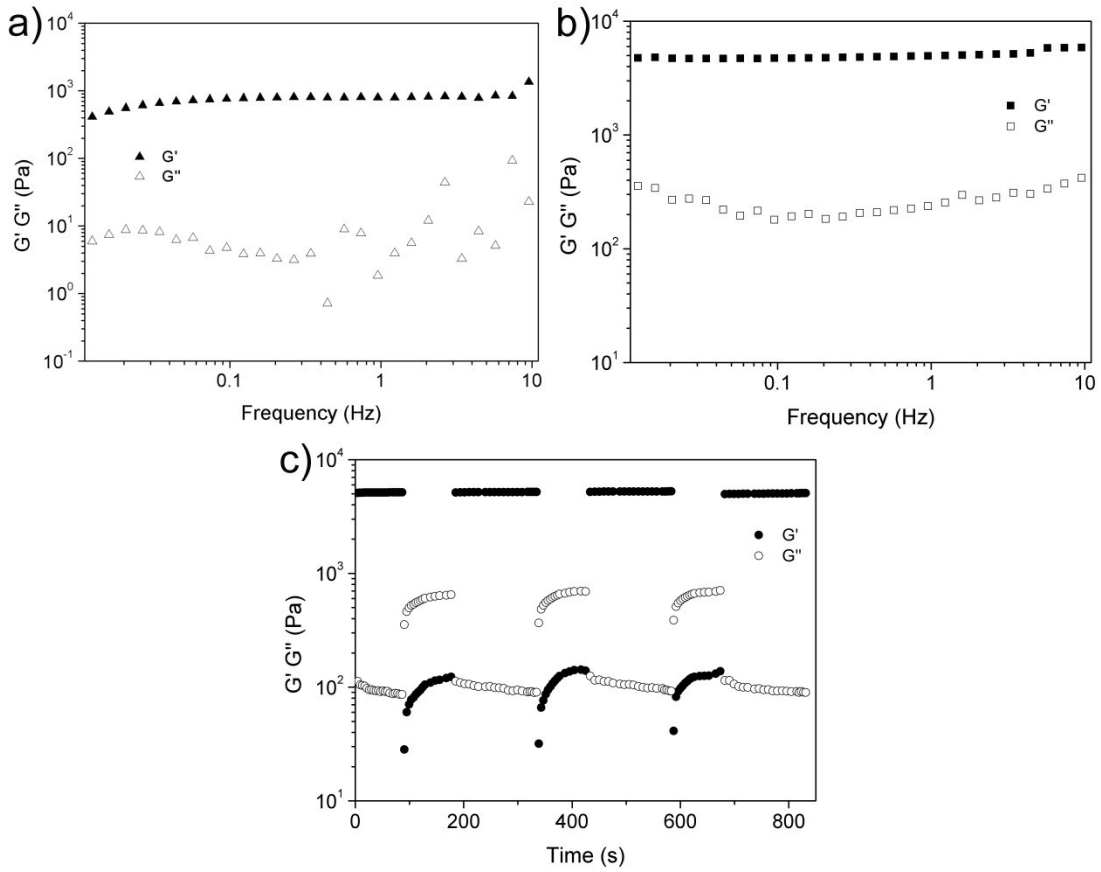


Figure S9 Frequency sweep test of the mashed gel (a) and the merged gel (b). (c) The self-healing property *via* amplitude oscillatory sweep test.

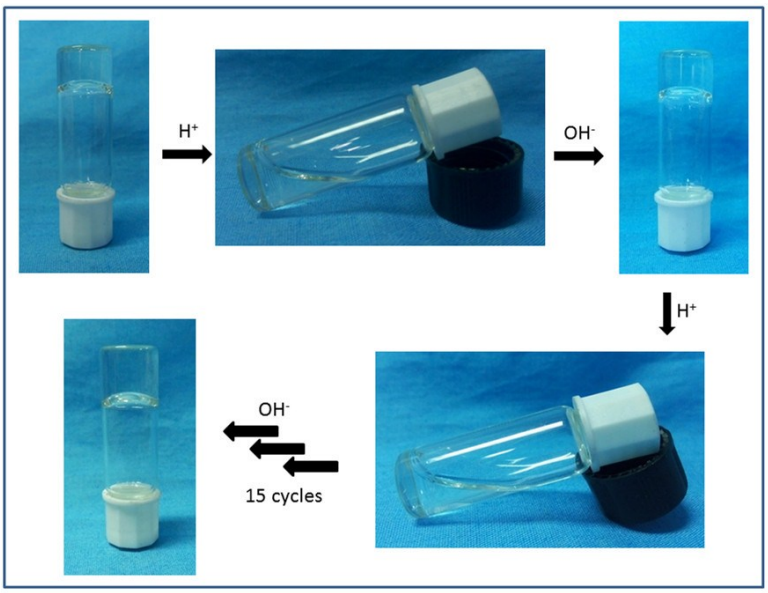


Figure S10 pH responsive test of the dynamic hydrogel.

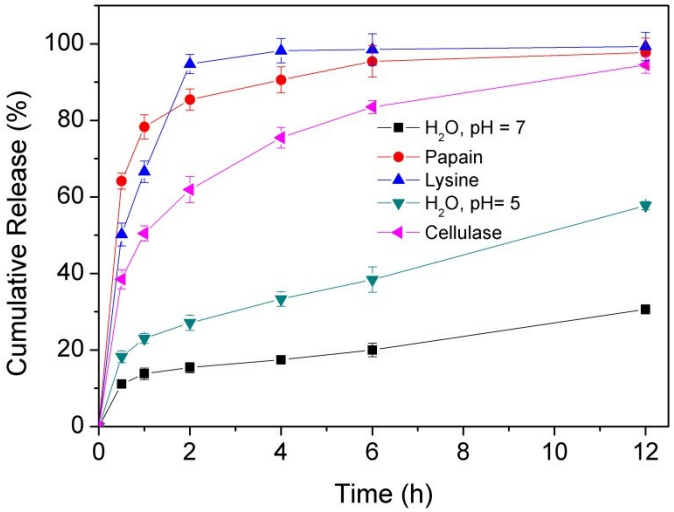


Figure S11 The release of methylene blue from dynamic hydrogel at 37 °C with different stimuli.

12. References

1. (a) Li, Z.; Ke, F.; Deng, H.; Xu, H.; Xiang, H.; Zhou, X., *Org. Biomol. Chem.* **2013,** *11*, 2943-2946. (b) Deng, G.; Tang, C.; Li, F.; Jiang, H.; Chen, Y., *Macromolecules* **2010,** *43*, 1191-1194.