

Supporting information

Highly stretchable, self-healing elastomer hydrogel with universal adhesion driven by reversible cross-links and protein enhancement

Kun Lei^{a*}, Meijun Chen^a, Xinling Wang^c, Jingpi Gao^a, Jianbo Zhang^a, Guangda Li^a,
Jianfeng Bao^a, Zhao Li^{b*} and Jinghua Li^{a*}

a. School of Medical Technology and Engineering, Henan University of Science and Technology, 263 Kaiyuan Road, Luolong District, Luoyang 471023, China;

b. Institute of Engineering Medicine, Beijing Key Laboratory for Separation and Analysis in Biomedicine and Pharmaceuticals, Beijing Institute of Technology, 5 South Zhongguancun Street, Haidian District, Beijing 100081, China;

c. School of Chemistry and Chemical Engineering, Shanghai Jiao Tong University, 800 Dongchuan Road, Minhang District, Shanghai 200240, China.

* Corresponding authors:

Kun Lei: leikun@haust.edu.cn

Zhao Li: lizhao@bit.edu.cn

Jinghua Li: anubiss1860@163.com

1.1. Materials and Chemicals. Reagents were used in the experiments including N-(2-Hydroxyethyl)acrylamide (HEMAA, stabilized with MEHQ, 98%, TCI), 2,2-Diethoxyacetophenone (DEAP, 98%, Adamas), N,N'-Methylenebisacrylamide (MBA, 99%, Adamas), Casein (BR, Adamas), Sodium Tetraborate Decahydrate (Borax, $\text{Na}_2\text{B}_4\text{O}_7 \cdot 10\text{H}_2\text{O}$, $\geq 99.5\%$, Greagent), Rhodamine B (RhB, $\geq 98\%$, Adamas), Methylene Blue Trihydrate (MBT, 90%, Adamas), Methyl Orange (MO, 90% Adamas), Deuterium Oxide (D_2O , 99.9 atom% D, Sigma-Aldrich, contains 0.05 wt.% 3-(trimethylsilyl)propionic-2,2,3,3-d4 acid), Deionized water (18.2 Ω cm resistivity at 25 °C) was prepared in the lab. All chemicals were used as received without any purification.

1.2. Synthesis of Highly Stretchable, Self-Healing Elastomer Hydrogel. N-(2-Hydroxyethyl)acrylamide (HEMAA), 2,2-Diethoxyacetophenone (DEAP), borax, and casein were used as a monomer, photo-initiator, reversible cross-linker, and protein model (forming a physical network by the micelle aggregation) to fabricate the highly stretchable, self-healing elastomer hydrogel, respectively. Specifically, a casein solution with different concentrations was obtained by dispersing and dissolving casein in deionized water of pH 7.0 with a stir violently using a digital Ultra-Turrax (IKA T18), and stored in a 4 °C refrigerator. Different amounts of HEMA, borax and 1mol% photo-initiator DEAP (relative to the amount of HEMA) were dissolved in prepared casein solution under vigorous stirring to obtain a uniform precursor solution, and the pH of solutions were gradually adjusted to 6.5 by adding HCl solution. To eliminate dissolved gas, the precursor was sonicated under 35 °C for 5 minutes. Subsequently, the resulting precursor was poured into a glass mold consisting of two slides with a 5 mm-thick silicone spacer (using for compressive assay) or a 2 mm-thick silicone spacer (using for tensile assay). Then, the glass molds were exposed to UV light (16 W, an intensity of 1.5 mW/cm²) for 40 min and kept for 24 h at 2-8 °C for casein gelation. For the experiments, the contents of HEMA are 25 wt% accounting for the total amount of the HEMA and casein solution; the casein solution concentrations are 2.5, 5, 10, 15, 20 w/v%; the borax is 5, 10, 15 wt% of HEMA mass, respectively. For comparison, several PHEMA hydrogels were

constructed under the same condition without any casein or borax.

1.3. Morphology observaiton of casein micelles. The TEM test was performed to evaluate the morphology of casein micelle. 25 μL casein solution was dropped on carbon-coated copper TEM grids and the sample was stained with 1.5% uranyl oxalate for 25 s. The micelle images were observed using a TEM (Thermo T200F) with an 200 kV acceleration voltage.

1.4. Mechanical Properties Experiments. The compression properties of the hydrogels are tested by a universal testing instrument (Model 43 MTS Criterion). Hydrogels were obtained in cylindroid molds with a size of 12 mm in diameter and 5 mm in height, determined by a digital caliper. The testing rate is 2 mm/min until the sample fractured. The experiment was performed five times, and the average with standard deviations was reported. Tensile experiments of the as-prepared hydrogels were performed using the same mechanical testing instrument as the compression tests with tensile speeds of 40 mm/min, and the hydrogels were cut into dumbbell shapes with size of $20 \times 5 \times 2 \text{ mm}^3$. The toughness of the hydrogels stretched to rupture was determined from the area under the tension stress-strain curve of a hydrogel sample. The cyclic loading and unloading compressive assays of hydrogels were performed at a constant compression rate of 5 mm min^{-1} with strains of 80%, 85%, 90% for hydrogels of different casein contents (5%, 10% and 15%), respectively. The ratio of dissipated energy is defined and calculated by the following equation (1):

$$\text{Ratio of dissipated energy (\%)} = \frac{A_{ci}}{A_{li}} \times 100\% \quad (1)$$

Where, A_{ci} is the area between the cyclic loading and unloading curves, and A_{li} is the area under the loading curves, $i=1, 2, 3, 4, 5, 6, 7, 8, 9, 10$.

The dissipated energy recovery is calculated using the following equation (2):

$$\text{Dissipated energy recovery (\%)} = \frac{A_{ci}}{A_{c1}} \times 100\% \quad (2)$$

Where, A_{ci} is the area between the cyclic loading and unloading curves, and A_{c1} is the area under the first cyclic loading and unloading curve, $i=2, 3, 4, 5, 6, 7, 8, 9, 10$.

The elastic modulus recovery is calculated using the following equation (3):

$$\text{Elastic modulus recovery (\%)} = \frac{\sigma_{li}}{\sigma_{l1}} \times 100\% \quad (3)$$

Where, σ_{li} is the stress of the cyclic loading curves at $\varepsilon=75\%$, and σ_{l1} is the stress of the first loading curve, $i=2, 3, 4, 5, 6, 7, 8, 9, 10$.

1.5. Conversion Determination of Hydrogels. The hydrogel was immersed in deionized water for one week with the continually changed water daily at room temperature to eliminate any probable unreacted monomer and photo-initiator. Then, the gels were dried at $65\text{ }^{\circ}\text{C}$ in an oven for 3 days to a constant weight, and the weight was determined and named as $m_{\text{dried gel}}$ (m_d). The m_d denotes the total weight, including cross-linked HEMAA, casein, and borax in the formed networks. θ was defined as the ratio of m_d to the total mass of HEMAA, casein, and borax, m_t , in the feed mixture represents the monomer conversion after gelation, as shown in equation (4):

$$\theta (\%) = \frac{m_d}{m_t} \times 100\% \quad (4)$$

1.6. Volume Fraction of Polymer within a Hydrogel. The volume fraction of polymer within a as-prepared discs gel, ϕ , is calculated by equation (5):

$$\phi = (D_0/D)^3 \quad (5)$$

Where, D_0 and D are the diameters of dried and as-prepared disc hydrogels, respectively.

1.7. Measurement of the Cross-Linked Density of Hydrogels. The theoretical cross-linked density, v_t , is displayed by equation (6), including the concentration C (mol/L) of cross-linker and functional parameter f , according to the literature¹:

$$v_t = Cf/2 \quad (6)$$

In equation (6), for cross-linked agent borax ($M_n=381\text{ g/mol}$), the value of f is 2; C was obtained from the mass (m_c) of borax in the feed mixture, the mass of dried gel m_d , and the density of dried gel ρ (g/mL). The ρ as the mean value of densities is calculated from the weights and measured sizes of the dried disc hydrogels. C is calculated by equation (7):

$$C = (m_c/M_n)/(m_d/\rho) \quad (7)$$

Put $f=2$ and equation (7) into equation (6), the v_t is expressed in the following

equation (8):

$$v_t = (2m_c/M_n)/(m_d/\rho) = \frac{\rho m_c}{381m_d} \quad (8)$$

The effective cross-linked density v_e is calculated from the compressive tests, using equation (9) and (10). At low compression strains, a elastic modulus (G) of hydrogels is obtained as the slope of plot of σ vs. $(\alpha - \alpha^2)$, shown in equation (9). The v_e is obtained by equation (10):

$$\sigma = G(\alpha - \alpha^2) \quad (9)$$

$$G = RTv_e\phi^{1/3}(V_d/V_f)^{2/3} \quad (10)$$

Where, α is a ratio of the thickness of the gel before and after compression; T is a absolute temperature and R is the gas constant; ϕ is the volume fraction of polymer within a hydrogel in equation (5); V_d and V_f refer to the volumes of the dried gel and formed gel, respectively. V_d is obtained as $V_d = m_d/\rho$. V_f can be obtained by a cylindrical hydrogel. Take equation (9) and $V_d = m_d/\rho$ into equation (10), the v_e is calculated by the equation (11):

$$v_e = (\sigma V_f^{2/3} \rho^{2/3}) / [(\alpha - \alpha^2) RT \phi^{1/3} m^{2/3} d] \quad (11)$$

1.8. Rheological Behaviors of Hydrogels. The rheological behaviors of the tough and stretchable hydrogels are measured at an AR-G2 Rheometer from TA Instruments of USA) in the angular frequency-dependent oscillatory mode. The gel samples of 20 mm diameter and 2 mm height were fabricated according to the section ‘‘Synthesis of Highly Stretchable, Self-Healing Elastomer Hydrogel’’ with different contents of borax, and performed with angular frequency from 0.001 to 1000 rad/s, the constant strain of 1% at 25 °C. In order to prevent the dehydration of samples during tests, the surrounding of samples was sealed a thin layer of silicone oil.

1.9. Lap Shear Experiments. The lap shear experiments were performed to evaluate the adhesion abilities of the hydrogels using a mechanical testing machine. A piece of hydrogel with the dimension of 25 mm × 25 mm × 2 mm was sandwiched between two pieces of Ti panels, silicone rubbers, Al panels, Quartzs, or PMMA boards (35mm × 50mm × 2mm) to obtain a area of 25 mm × 25 mm. Then, a touch pressure of 150 g was imposed for 15 min to obtain a tight contact between the

sample and panels. All the assays were conducted at a constant shear rate of 25 mm/min. Tests for every sample were repeated at four times and the results were recorded as average \pm standard deviation.

1.10. ^{11}B NMR Characterizations. ^{11}B NMR spectra were recorded on a Varian MERCURY plus-400 spectrometer at 25 $^{\circ}\text{C}$ in D_2O , using boron trifluoride as an external standard. For the test samples, the reaction solution including HEMAA, borax and photo-initiator with a total amount of 15 mg in 0.5 mL D_2O was mixed into a thin-walled quartz NMR tube. After shaking for 5 min, UV light ($\lambda=365$ nm) was used to irradiate for 30 min.

1.11. Self-Healing Experiments. The disc hydrogels were cut into two pieces from top to bottom, one of which was stained faint red with a trace of rhodamine, and then keep the two pieces of hydrogels in contact for 24 h.

Two hydrogels were fabricated in glass tubes, one of which was dyed red with a trace of rhodamine, and cut into small pieces respectively. Then, keep the two different kinds of pieces (dyed and undyed) in contact in sequence.

In addition, the prepared dumbbell-shaped hydrogels was cut into two pieces, one of which was stained faint red with a trace of rhodamine, and then keep the two pieces of hydrogels in contact for 24 h. The mechanical performances of the healed hydrogel were measured in accordance with the above section “Mechanical Properties Experiments”.

1.12. Cell Viability Experiment. The cell viability of the hydrogels was measured in accordance to MTT assay using Human Umbilical Vein Endothelial Cells (HUVE Cells).² Typically, the HUVE cell was seeded into 48-well plates with a density of 1×10^5 cells/well and incubated alone for 24 h to obtain a monolayer of cells before the sample was added. The freeze-dried as-designed hydrogel samples were added, and after being cultured for 24 h, 48 h, 72 h, the medium was refreshed. Then, 50 μL of 1 mg/mL of MTT solution was added and remained for 4 h. Finally, the culture medium was replaced with DMSO (150 μL) to dissolve the formazan and the absorbance of the DMSO solution at 570 nm was measured by a microplate reader (Synergy HT). Relative cell viability was measured as the ratio between the average absorbance

value of the sample and that of cells cultivated in pure DMEM. The assay was performed four times for each culture. Values were shown as average \pm standard deviation ($n = 4$).

1.13. Cell Adhesion Test. The gel sample was cultured utilizing 2 mL of 1×10^5 cells/well suspension of HUVE cells in 12 well plates at 37 °C and 5% CO₂. After 24, 48, 72 hours, substrates were washed using fresh medium to remove un-attached cells. Then, cells were digested, and counted by a cell counter. Subsequently, calcein-AM and PI solutions were used to stain the cells for 12 and 2 min on the gel surface, followed by immobilization with 2% glutaraldehyde. The cells were then observed and photographed by a fluorescence microscope. The assay was repeated four times and the averages with standard deviations are reported.

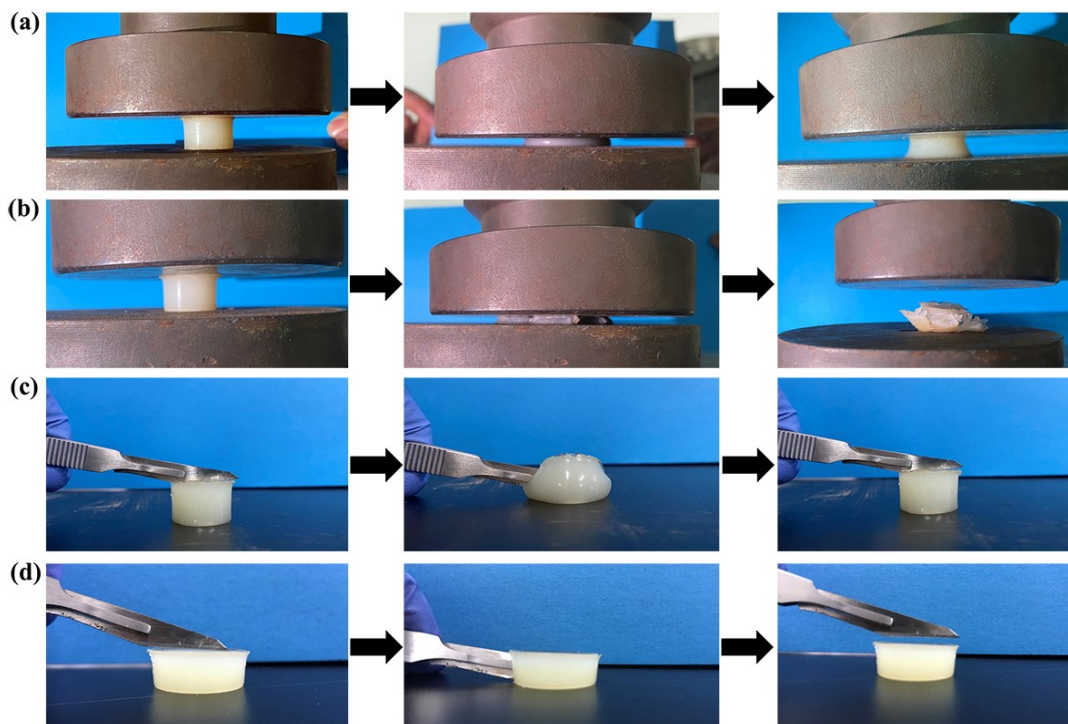


Figure S1. Schematic images of compression and cutting of designed hydrogels. Images of hydrogels with 25 wt% HEMAA, (a) 15 w/v% casein, 10 wt% borax; (b) 15 w/v% casein, 15 wt% borax before and after compression. Images of hydrogels with 25 wt% HEMAA, (c) 15 w/v% casein, 10 wt% borax; (d) 15 w/v% casein, 15 wt% borax before and after cutting.

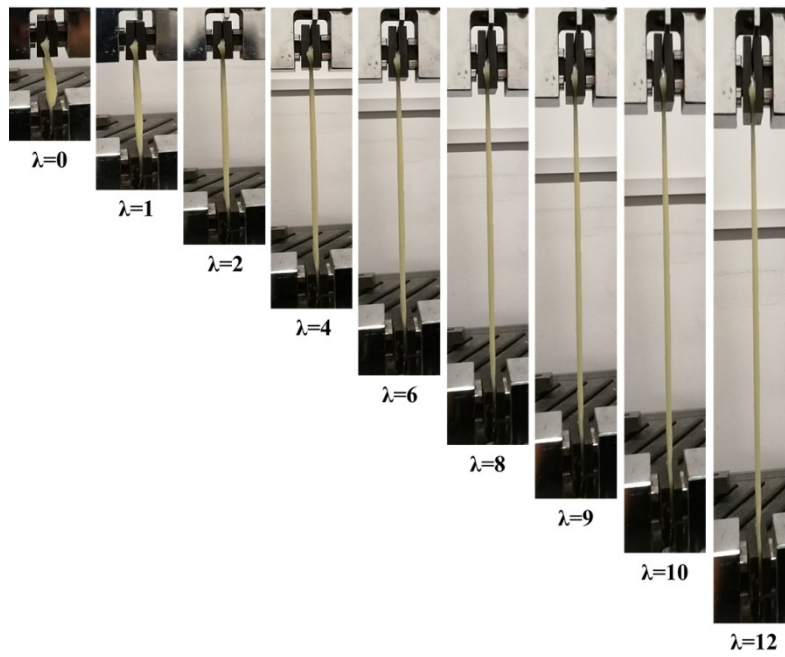


Figure S2. Tensile images of hydrogels with 25 wt% HEMAA, 15 w/v% casin and 10 wt% borax at various strains.

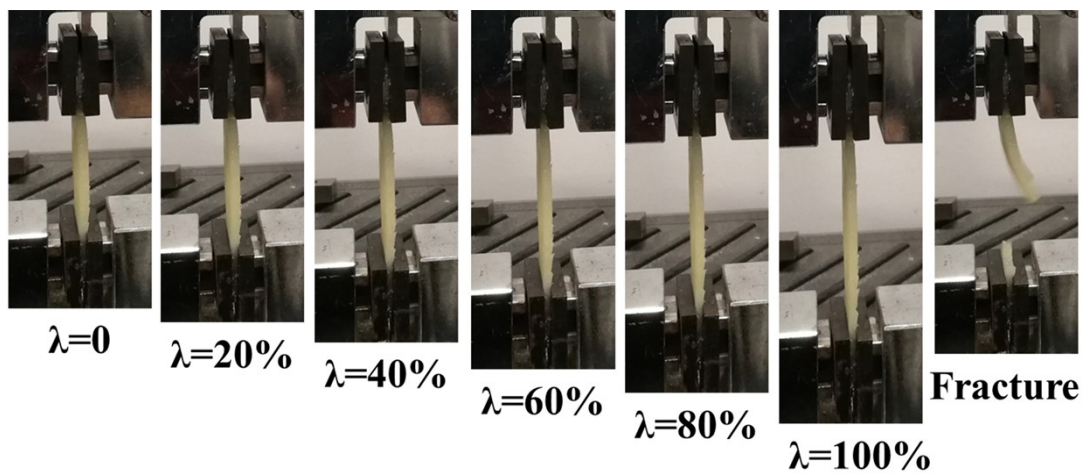


Figure S3. Tensile images of hydrogels with 25 wt% HEMAA, 15 w/v% casein, 15 wt% borax at various strains.

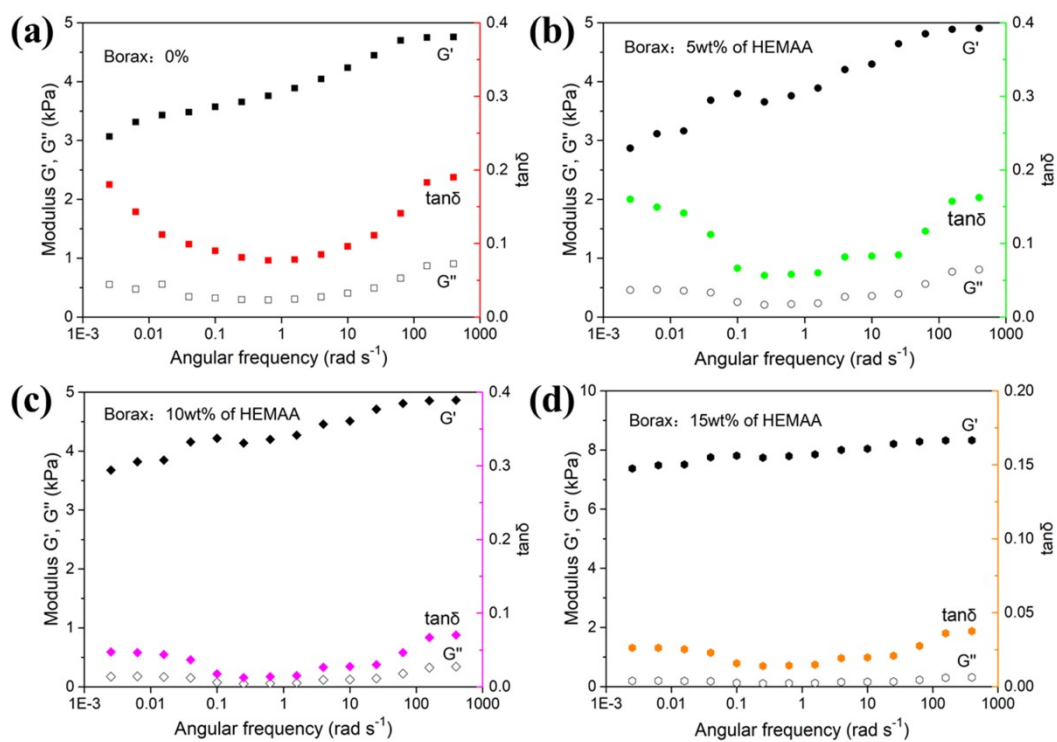


Figure S4. Rheological behaviors of hydrogels with 25 wt% HEMA, 15 w/v% casein and (a) 0 borax; (b) 5 wt% borax; (c) 10 wt% borax; (d) 15 wt% borax.

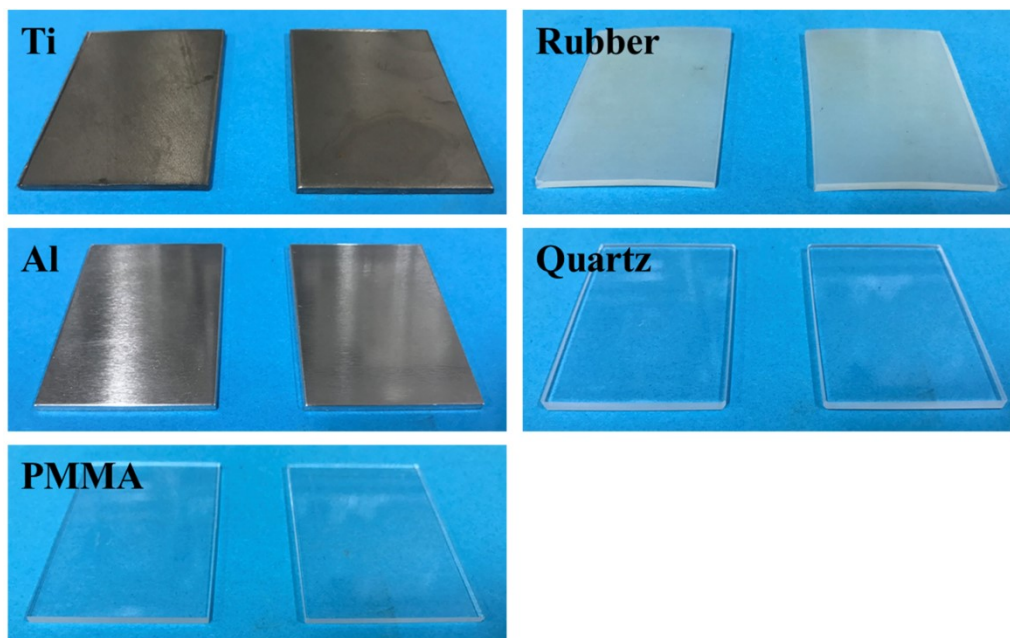


Figure S5. Schematic images of various adherends including Ti, rubber, Al, quartz, and PMMA panel.

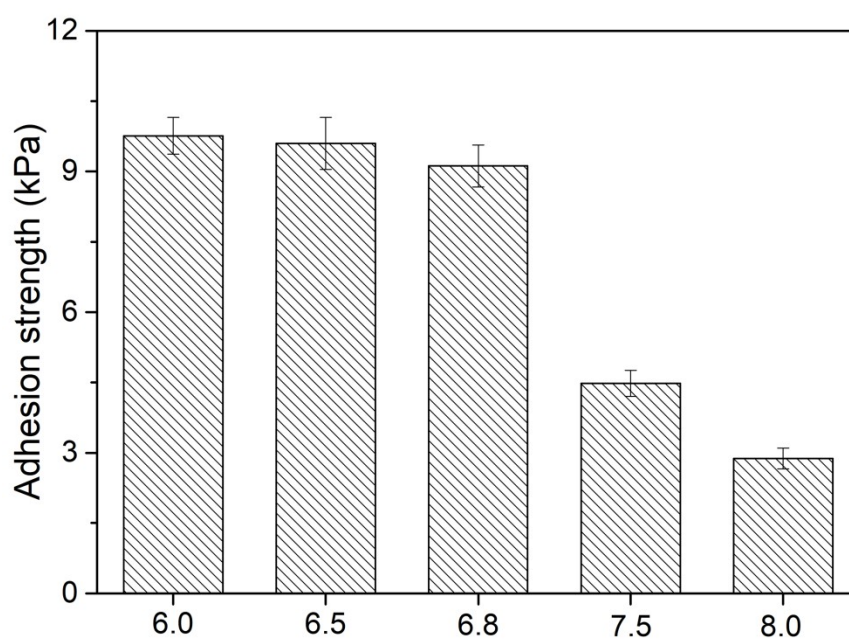


Figure S6. Effects of pH on the adhesion strength of designed hydrogels with 25 wt% HEMA, 10 wt% borax, 10 w/v% casein.

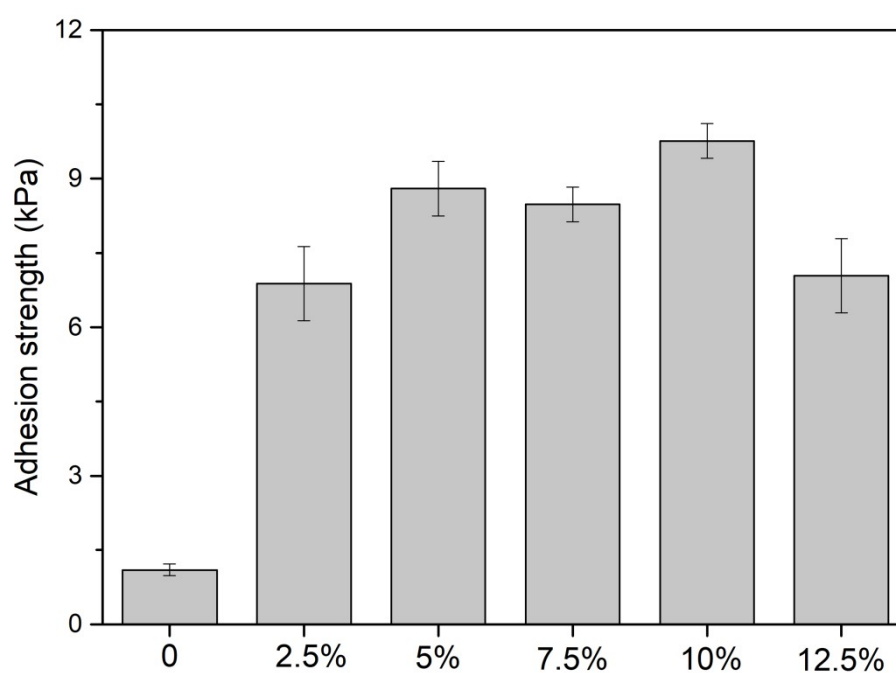


Figure S7. Effects of casein contents on the adhesion strength of as-designed hydrogels with 25 wt% HEMA and 10 wt% borax.

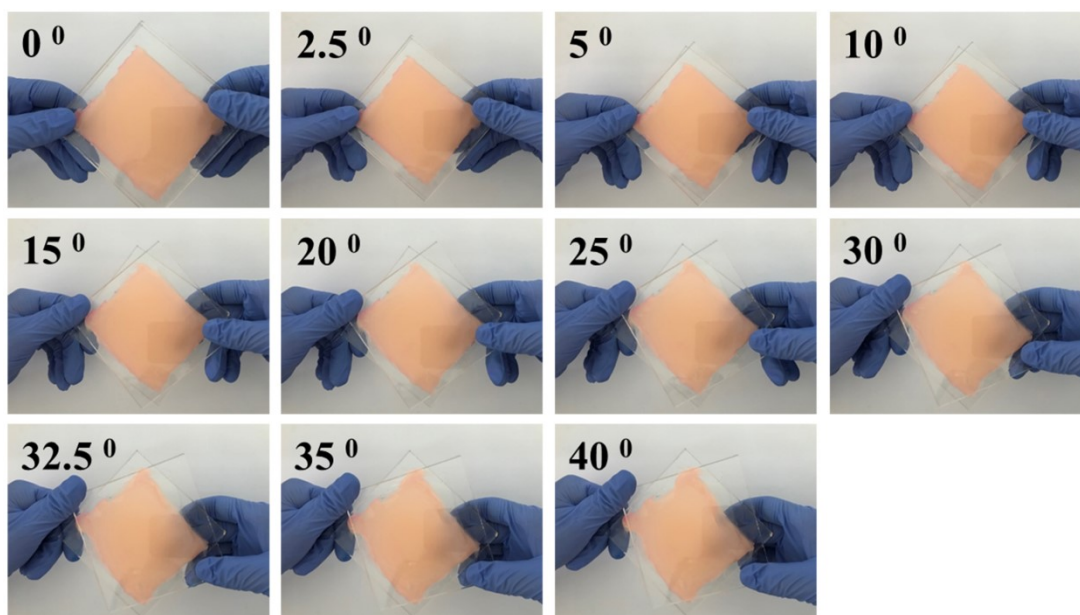


Figure S8. Schematic images of hydrogel (sandwiched with two glass plates) assemblies twisted forcibly for different angles. The designed hydrogel was fabricated with 25 wt% HEMA, 10 wt% borax, 10 w/v% casein.

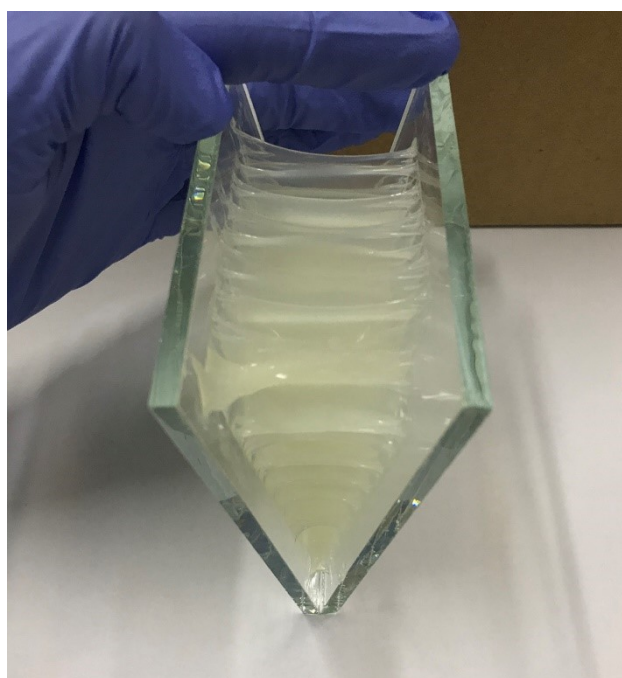


Figure S9. Schematic images of the two glass panels glued by the designed hydrogel and fiercely pulled apart for 30 mm. The designed hydrogel was fabricated with 25 wt% HEMA, 10 wt% borax, 10 w/v% casein.

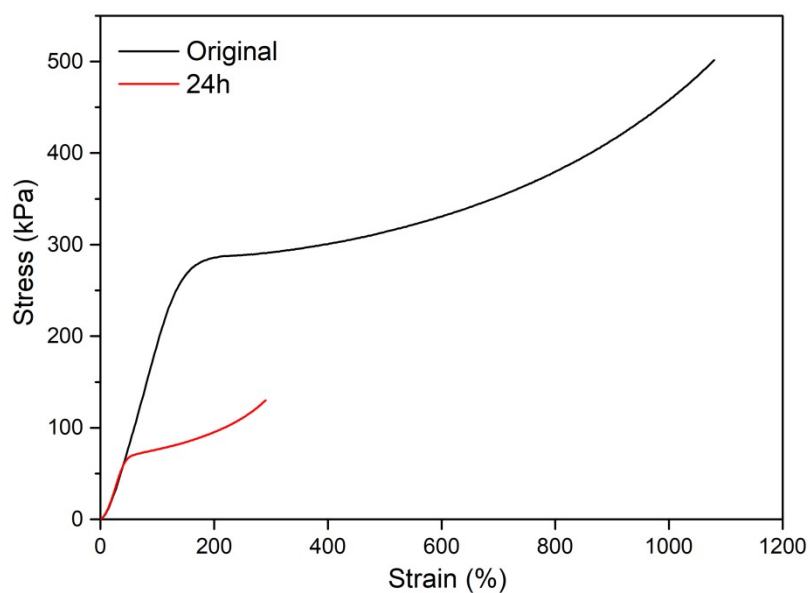


Figure S10. Stress-strain curves of original and self-healed hydrogel fabricated with 25 wt% HEMA, 10 w/v% casein but without borax.

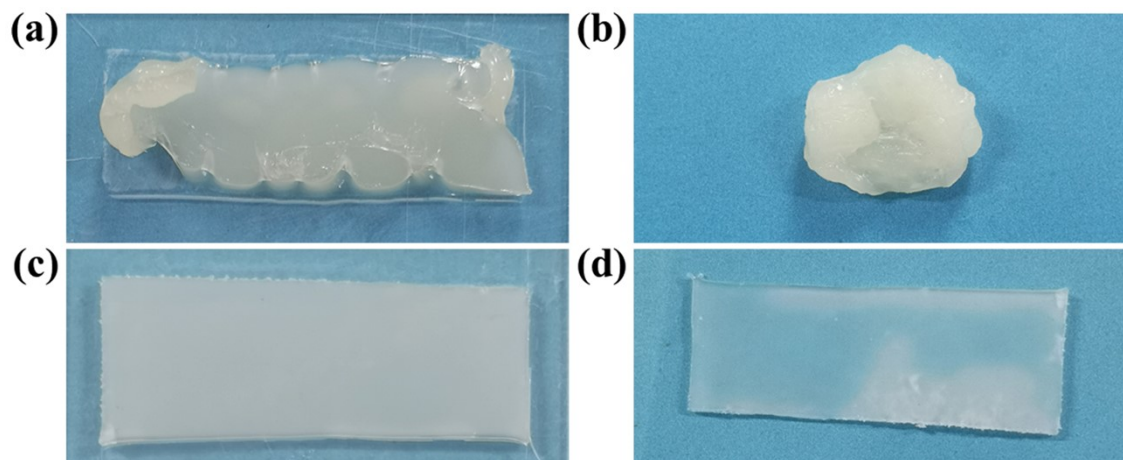


Figure S11. Schematic pictures of hydrogels. The pictures of 25 wt% PHEMA hydrogel without borax (a) in glass mold consisting of one slide with a silicone spacer; (b) after the removal from glass mold. Pictures of 25 wt% PHEMA hydrogel with borax (c) in glass mold consisting of one slide with a silicone spacer; (d) after the removal from glass mold. The pictures indicate that PHEMA hydrogel without borax can not self-stand. PHEMA hydrogel with borax can self-stand.

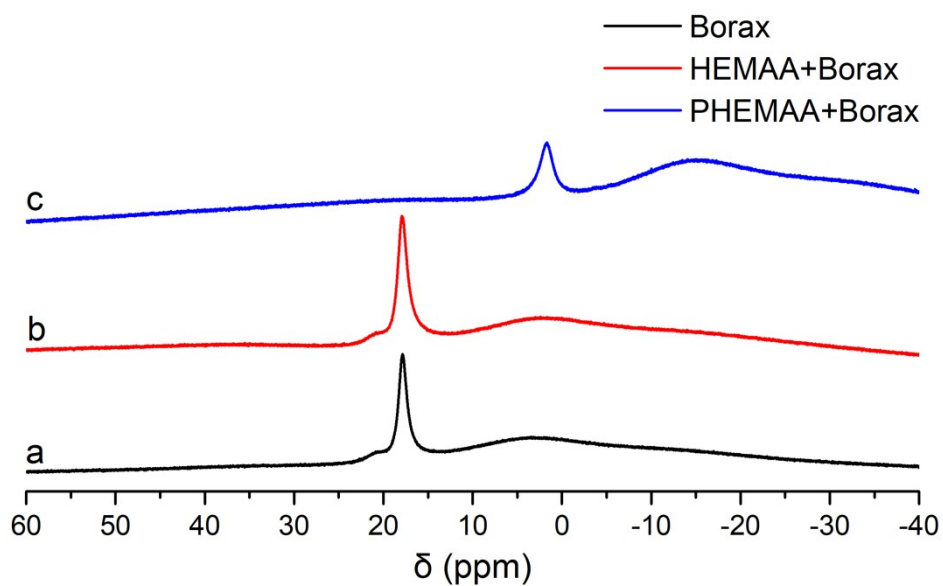


Figure S12. ^{11}B NMR spectra of the borax, HEMAA/borax/DEAP mixture without and with a UV radiation, respectively, in D_2O , 50 mg/mL polymer concentration.

Table S1. Compressive properties of hydrogels (25wt% HEMAA) with different casein and borax contents.

Casein/w/v%	0	2.5	5	10	15	20
	Compressive strain/stress					
0	91.55%	94.06%	95.07%	96.52%	99.39%	91.73%
	2.4MPa	4.61MPa	7.62MPa	11.56MPa	14.9MPa	12.07MPa
5	78.54%	83.26%	88.32%	96.43%	99.5%	92.79%
	2.96MPa	4.76MPa	10.45MPa	18.76MPa	25.7MPa	23.08
10	75.57%	84.83%	89.10%	95.39%	99.5%	93.95%
	5.31MPa	11.14MPa	17.55MPa	29.22MPa	25.7MPa	34.74MPa
15	67.38%	70.02%	72.24%	65.06%	61.43%	53.51%
	7MPa	7.66MPa	8.06MPa	7.26MPa	8.4MPa	6.06MPa

Table S2. Tensile properties of hydrogels (25wt% HEMAA) with different casein and borax contents.

Casein/w/v% Borax/wt%	0	2.5	5	10	15	20
	Tensile strain/stress					
0	1014%	1327%	1520%	1540%	1445%	1158%
	15kPa	21kPa	74kPa	125kPa	222kPa	185kPa
5	1124%	1239.2%	1446.8%	1546.9%	1500.8%	1159%
	109kPa	271.2kPa	314kPa	440.6kPa	511kPa	398kPa
10	860.8%	1135.6%	1215%	1386%	1458.6%	1135.1%
	160kPa	279kPa	345kPa	518kPa	615kPa	386kPa
15	327%	405%	485%	311%	158%	103%
	229kPa	288kPa	371kPa	230kPa	104kPa	80kPa

Table S3. Tensile toughness of hydrogels (25wt% HEMAA) with different casein and borax contents.

Casein/w/v% Borax/wt%	0	2.5	5	10	15	20
	Tensile toughness/MJ·m ⁻³					
0	0.1	0.21	0.63	1.4	1.79	1.2
5	0.68	2	2.5	3.74	4.22	2.76
10	0.87	2.12	2.69	4.69	5.98	2.57
15	0.52	0.79	1.22	0.49	0.1	0.05

References:

1. W. Xue, M. B. Huglin and T. G. J. Jones, *Eur. Polym. J.* 2005, **41**, 239.
2. K. Lei, K. Q. Wang, Y. L. Sun, Z. Zheng and X. L. Wang, *Adv. Funct. Mater.*, 2021, **31**, 2008010.