Supporting Information For

Developing Super Tough Gelatin-based Hydrogels by Incorporating Linear Poly(methacrylic Acid) to Facilitate Sacrificial Hydrogen Bonding

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Materials

Food grade gelatin from porcine skin, type B, with the bloom value of 160 was obtained from Henan Boyang Gelatin Co., Ltd. (China). Methylacrylic acid (AR, >99%) and α-Ketoglutaric acid (AR, >98%) were all obtained from Shanghai Aladdin Bio-Chem Technology Co., LTD, and was used as received.

Synthesis of the G-M gels

Firstly, the gelatin was dissolved in pure water at 45^{o}C to prepare the gelatin solution with the concentration of 10 wt %. Then, methylacrylic acid (MAA) and 0.02 mol % (relative to the monomer) α-Ketoglutaric acid as photo-initiator was added to the gelatin solution at 45 ^{o}C to prepare gelatin/MAA solutions with MAA concentrations of 1.6 wt %, 4 wt %, 8 wt %, 12 wt % and 16 wt %. The gelatin/MAA solutions were poured into a rectangular mold with dimensions of 130 mm × 130 mm × 1.5 mm and cooled to 8 ^{o}C to introduce gelation. The gelatin/MAA solutions with MAA concentration of 8 wt % was also cooled to 2 ^{o}C and 12 ^{o}C, respectively to introduce gelation. After that, the gels were irradiated with 395 nm UV LED lamp for 6 h at their gelation temperature (2 ^{o}C, 8 ^{o}C or 12 ^{o}C) to synthesize PMAA within the gelatin gel. The as-prepared gels were completely dried at room temperature for at least 12 h and then reswollen in pure water at room temperature for at least 12 h to reach the final equilibrium gelatin-PMAA

gels. The gelatin-MAA gel synthesized with C_{MA}A of x wt % at y ^{o}C was coded as G-Mx-y^{o}C for simple.

As control, the 8 wt % MAA and 0.02 mol % (relative to the monomer) α-Ketoglutaric acid solution was also irradiated under 395 nm UV LED lamp at 35 ^{o}C for 6 h to synthesize PMAA within the gelatin solution.

Measurements

Uniaxial tensile tests

Uniaxial tensile tests were performed on dumbbell-shaped samples with the standard JIS-K6215-7 size (12 mm (gauge length) × 2 mm (width) × ≈1 mm (thickness)) using an UTM-2102 Electronic universal testing machine (Shenzhen Co., Ltd. China). The initial distance L_0 between the two clamps of the tester was 12 mm and the tensile deformation was performed at the strain rate of 0.14 s^{-1} unless specifically indicated. Cyclic tensile tests were also performed on the same samples at the strain rate of 0.14 s^{-1} with maximum strain of 1 mm/mm. The tensile tests were all performed at 22^{o}C unless specifically indicated.

Water content measurement

Water content of gels was measured using a moisture balance MOC-120H (Shimadzu Co.). The dry sample was obtained by heating the sample at 120 °C until sample weight becomes constant. Water content C_{wt} is defined as

$$C_{wt} = 1 - \frac{m_D}{m_S}$$

where m_D and m_S are weights of the dry sample and the swollen sample, respectively.

Tearing test

Tearing tests were all performed at 22 °C to characterize the fracture energy of the gels. The gel samples were cut into rectangular shape (35 mm × 15 mm) with 10 mm initial notch at the midpoint of the short edge. The two arms of a test piece were clamped, and then the upper arm was pulled upward at constant velocity 100 mm/min while the tearing force F was recorded. The fracture energy T was calculated at a constant tearing force F using the relation $T = 2F/w$, where w is the thickness of the sample.

Rheological test

Rheological tests were performed using an AR2000ex rheometer (TA Instruments). The disc-shaped samples with thicknesses of ≈1 mm and diameters of 13 mm were adhered to the plates with glue and surrounded by water.

The rheological temperature-frequency sweep test was performed with frequency sweeping from 0.628 to 100 rad/s and a shear strain of 0.1% in the parallel-plates geometry, in a temperature range of 2–42 °C, with fixed gap distance during test. As the top and bottom of tested gel was fixed on the parallel-plates by glue, the volume of gel during measurement was considered as being fixed. Thus, following the time-Temperature Superposition principle for the construction of the master curve, the modulus-scale shift factor b_T was set as 1. The apparent activation energy E_a is obtained from an Arrhenius equation:

$$a_T = Ae^{Ea/RT},$$

where a_T is the shift factor, R is the ideal gas constant, and A is a constant. [S1]

The rheological temperature sweep test was performed at frequency of 6.28 rad/s, with the starting temperature of 2 °C and heating rate of 2 °C/min.

Fourier transform infrared spectroscopy (FT-IR)

FT-IR was measured by VERTEX 70 FTIR spectrometer (Bruker co. Ltd.). The testing scale was from 400-4000 cm^{-1}, with the resolution of 4 cm^{-1}.

The FT-IR evidence of the formation of hydrogen bonds between gelatin and PMAA

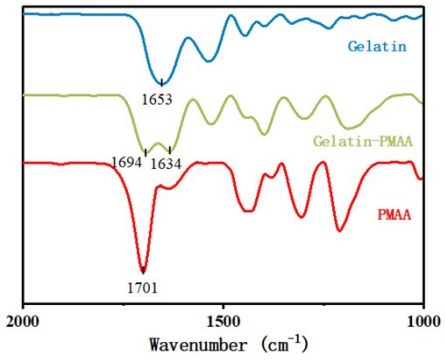


Figure S1 FT-IR spectra of freeze-dried gelatin gel, PMAA solution, and gelatin-PMAA coacervates.

The gelatin-PMAA coacervates were obtained by directly mixing the gelatin and PMAA solution. The gelatin gel, PMAA solution and the gelatin-PMAA coacervates were freeze-dried for the FT-IR measurement. As shown in the FT-IR spectra, the carbonyl stretching resonances of the amide groups on gelatin and the carboxylic acids group on PMAA are observed at 1653 and 1701 cm^{-1}, respectively. For the Gelatin-PMAA, two distinct absorption peaks are observed at 1694 and 1634 cm^{-1}, which are attributed to the stretching resonances of carbonyl group from PMAA and gelatin, respectively. Compared to the FTIR spectra of PMAA and gelatin, the absorption peak corresponding to carbonyl stretching resonance of Gelatin-PMAA shows a red shift, indicating the formation of the hydrogen bond between the amide groups of gelatin and the carboxylic acids of PMAA.

Gel-sol transition temperatures of gelatin/MAA solutions

The gel-sol transition, which describes the crossover between the viscous solution behavior and elastic gel behavior, can be defined as the intersection of the storage G' and loss G'' moduli at specific frequency. The gel-sol transition temperatures ($T_{s/g}$) of gelatin/MAA solutions were determined by the rheological test, under the temperature sweep model at frequency of 6.28 rad/s, with the starting temperature of 2°C and heating rate of 2 °C/min, with a shear strain of 0.5% in the parallel-plates geometry. The dimensions of all the samples were 20 mm in diameter, 1mm in thickness.

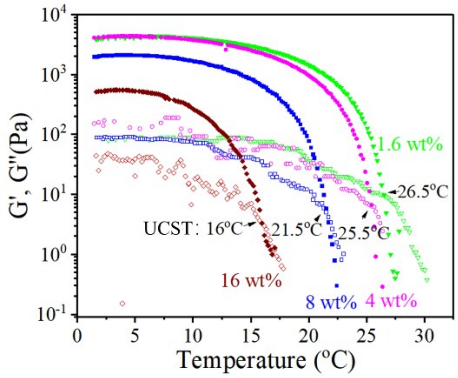
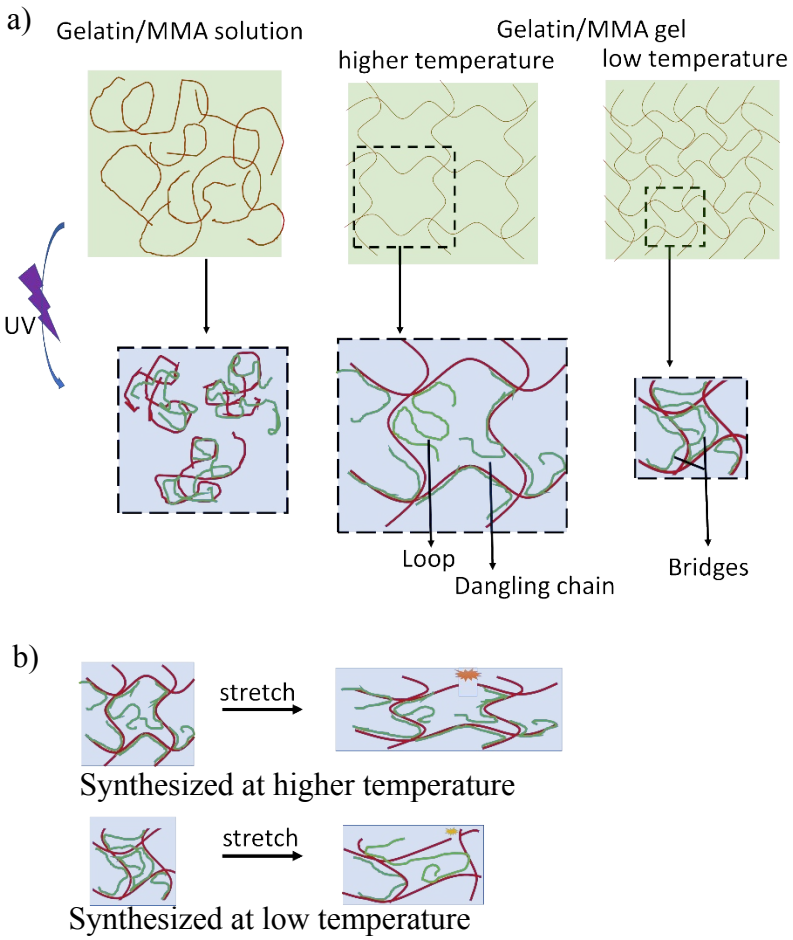


Figure S2 Temperature dependence of storage (filled) and loss (unfilled) modulus for gelatin/MAA solutions with the concentration of MAA of 1.6 wt %, 4 wt %, 8 wt %, 16 wt %, respectively.

From figure S1, it can be seen that the UCST of gelation/MAA solutions were 26.6 °C, 25.5 °C, 21.5 °C and 16 °C for 1.6 wt %, 4 wt %, 8 wt %, 16 wt % MAA, respectively.

The polymerization of MAA in gelatin/MAA solutions at 37^{o}C

Figure S3 The photo of the half-separated PP films (the film between solution and glass mode to prevent the adhesion of the polymerized product on the glass mode) and the product after the polymerization of MAA in Gel/MAA solution with 8 wt % MAA at 30 ^{o}C.



Scheme S1 a) Structures of Gelatin-PMAA gel after polymerization at different states and temperatures; b) Fracture of the network of G-M gels after Stretching.

Table S1 The water content, Young's modulus, fracture stress. fracture strain and tearing energy of G-M gels

Gel code	C_{MAA} （wt%）	$n_{COOH}/n_{CO\text{-}NH}$	T_{syn} （°C）	C_{wt} (%)	E (MPa)	σ_b (MPa)	ε_b （mm/mm）	T (kJ/m^2)
G-M1.6-8°C	1.6	0.19	8	87±3	0.33±0.19	0.15±0.03	1.31±0.28	0.071±0.008
G-M4-8°C	4	0.48	8	71±3	1.19±0.34	0.76±0.10	1.84±0.39	0.90±0.11
G-M8-8°C	8	0.99	8	55±2	5.34±0.52	1.39±0.28	2.96±0.20	3.65±0.65
G-M12-8°C	12	1.53	8	69±1	0.74±0.18	0.29±0.12	4.62±1.34	0.46±0.19
G-M16-8°C	16	2.12	8	81±1	0.56±0.02	0.10±0.03	4.37±1.29	0.21±0.04
G-M8-2°C	8	0.99	2	45±2	11.3±1.7	4.93±0.44	4.81±1.36	8.52±0.66
G-M8-12°C	8	0.99	12	62±2	1.07±0.61	0.60±0.12	3.02±0.04	1.11±0.27

C_{MAA}, $n_{COOH}/n_{CO\text{-}NH}$, T_{syn}, C_{wt}, E, σ_b, ε_b, T stand for the concentration of MAA, the group ratio of -COOH on PMAA to the -CO-NH- on the gelatin in the gel, the synthesis temperature, water content of the gel, Young's modulus, fracture tensile stress, fracture tensile strain and fracture energy, respectively. All the datas are averages for 3 experimental tests.

The calculation of $n_{\text{-COOH}}/n_{\text{-CO-NH-}}$ in the G-M gels:

The gelatin was from porcine skin. According to the composition of amino acids in the collagen of porcine skin, [S1] the average molecular weight per amino acid unit of the gelatin was estimated as 89.33 g/mol. As one amino acid unit corresponded to one -CO-NH-, knowing that the molecular weight of MAA (86.09 g/mol) and each MAA monomer contains one -COOH group, the molar ratio $n_{\text{-COOH}}/n_{\text{-CO-NH-}}$ can be easily estimated from the weight concentration of gelatin and MAA in the G-M gels.

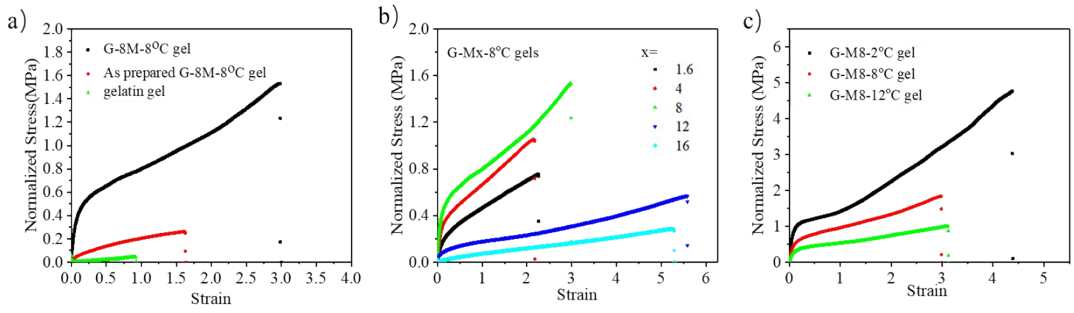


Figure S4 Tensile (normalized stress)-strain curves of a) the gelatin gel, the as-prepared G-M8-2°C gel and the G-M8-2°C gel, b) the G-Mx-8°C gels (x=1.6, 4, 8, 12, 16), c) the G-M8-2°C, G-M8-8°C, and G-M8-12°C gels.

The normalized stress was obtained by normalizing the tensile stress of the gels with their solid content.

Effect of temperature on the G-M8-2^{o}C gel

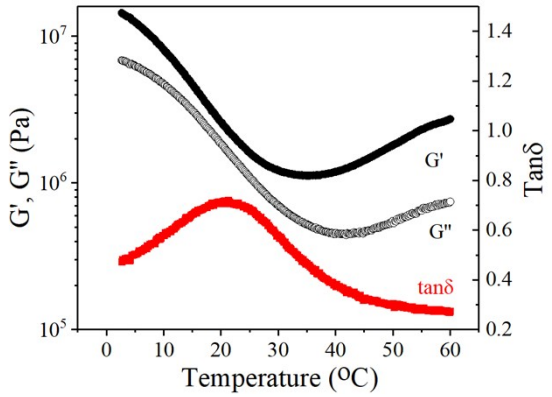


Figure S5 Temperature dependence of the storage modulus (*G'*), loss modulus (*G''*) and loss factor (*tanδ*) of B gel at 6.28 rad/s and 0.1% strain.

As shown in Figure S5, the *G'* firstly decreased with the increase of temperature and dropped to the minimum at 36 ^{o}C due to the breaking-up of hydrogen bonds through heating. Then, with the increase of testing temperature, the *G'* increased. As the MAA contains the hydrophobic methyl side group and the gelatin also contains hydrophobic side groups like -CH_3, -CH-$(CH_3)_2$, and -CH(CH_3)-CH_2-CH_3, the increase of *G'* with the increase of temperature was considered to be due to that heating promoted the formation and the enhancement of hydrophobic interactions. The maximum of *tanδ* appeared at around 21 ^{o}C which was consistent with the $T_{s/g}$ of the original Gel/MAA solution. Moreover, the minimum *G'* still reached MPa, indicating the high strength of the G-M8-2^{o}C gel.

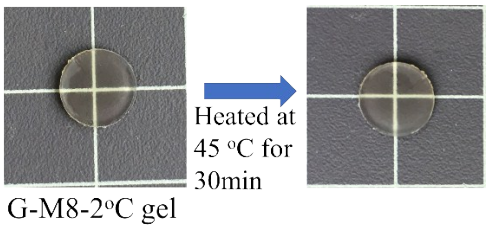


Figure S6 The photos of the G-M8-2°C gel before and after being heated at 45 °C for 30 min

Dissolution of the G-M8-2°C gel

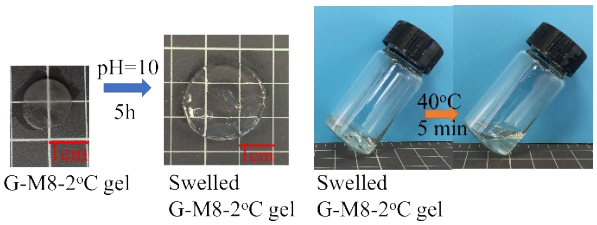


Figure S7 The images demonstrated the dissolution process of the G-M8-2°C gel

As shown in Figure S7, the G-M8-2°C gel was completely dissolved in water by firstly swelling the gel in a Na_2HPO_4/ NaH_2PO_4 buffer solution with the pH value of 10 and then heating the gel at 40 °C for 5 min.

Biocompatibility

Mouse fibroblast cells (L929 cells) were obtained from Cell Bank of the Chinese Academy of Sciences (Shanghai, China). The cells were cultured at 37 ^{o}C in a humidified atmosphere with 5% CO_2 in Dulbecco's Modified Eagle's Medium-high high glucose with 4500 mg/L glucose, L-glutamine, sodium pyruvate, and sodium bicarbonate (HyClone Laboratoryis, Utah, USA). G-M8-2^{o}C gel was cut into squares and sterilized with an autoclave. After sterilization, the scaffolds and the medium were put in the wells of non-treated 6-well PS microplates in a SW-CJ-1FD super clean bench (Airtech Asia ltd., Tianjin, China) and the scaffolds were immersed in the medium for 2 days at 37 ^{o}C. Then, the L929 cells were seeded onto the tops of the medium-immersed scaffolds in the microplates with the cell density of 10^4-10^5 cells/cm^2. 3 points of the cells on each scaffold were observed using a RX5 microscope (Sunny Optical Technology (Group) Co., LTD, China) at the time of 14 h, 2 days and 3 days after seeding. The cell number was counted and analyzed using the Image pro-Plus and SPSS Statistics 21 software. The medium was exchanged after each observation.

Reference

S1. Liu, G. L. W., *College Chemistry*. China Light Industry Press Ltd. : Beijing, China, 2013.