Supporting Information

Dynamic Bonds Enable High Toughness and Multifunctionality in Gelatin/Tannic Acid-based Hydrogels with Tunable Mechanical Properties

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Table S1. The GPC test result of gelatin

	M_n	M_w	M_p	M_z	M_{z+1}	Polydispersity
Gelatin	6308	21588	9024	42854	82771	3.422

Table S2. G/T gel formulations

Control	code	Concentration of gelatin solution (wt %)	Concentration of TA solution (wt %)	Adding amount of Gelatin solution (ml)	Volume of TA solution (ml)	Initial TA/Gelatin（wt/wt）
Initial TA/Gelatin（wt/wt）	$G10/T1_{2.67}$	10	1	6	160	2.67
	$G10/T1_{2}$	10	1	8	160	2
	$G10/T1_{1.6}$	10	1	10	160	1.6
	$G10/T1_{1.33}$	10	1	12	160	1.33
	$G10/T1_{1.14}$	10	1	14	160	1.14
	$G10/T1_{1.07}$	10	1	15	160	1.07
Concentration of TA solution	$G10/T0.8_{1.6}$	10	0.8	10	200	1.6
	$G10/T1_{1.6}$	10	1	10	160	1.6
	$G10/T2_{1.6}$	10	2	10	80	1.6
	$G10/T3_{1.6}$	10	3	10	53	1.6
Concentration of gelatin solution	$G6.25/T1_{1.6}$	6.25	1	16	160	1.6
	$G7.7/T1_{1.6}$	7.7	1	13	160	1.6
	$G10/T1_{1.6}$	10	1	10	160	1.6
	$G14.2/T1_{1.6}$	14.2	1	7	160	1.6
	$G20/T1_{1.6}$	20	1	5	160	1.6

Measurements

Water content measurements

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

$$C_{wt} = 1 - \frac{m_D}{m_S} \tag{S1}$$

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

Rheological test

Rheological tests were performed using an AR2000ex rheometer (TA Instruments). The disc-shaped samples with thicknesses of ≈1.2 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 10–70 °C, with fixed gap distance during the test. Since the top and bottom of the tested gel was fixed to the parallel-plates by glue, the volume of the gel during measurement was considered to be fixed. Thus, following the time-temperature superposition principle, the modulus-scale shift factor b_T was set as 1 and the master curve was constructed accordingly.

The apparent activation energy E_a is obtained from an Arrhenius equation:

$$a_T = Ae^{Ea/RT}, \tag{S2}$$

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^{-1}, with the starting temperature of 10 °C and a heating rate of 2 °C min^{-1}.

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}.

Ultraviolet visible spectrophotometer (UV-Vis)

The UV-Vis was measured by Cary 5000 UV-Vis spectrophotometer (Agilent co. Ltd.). The testing scale was from 200-800 nm.

Scanning electron microscope (SEM)

The G10/$TA1_{1.6}$ gel was firstly frozen and brittle fractured in liquid nitrogen. Then the frozen G10/$TA1_{1.6}$ gel free was freeze dried using the freeze dryer (LGJ-12A, Beijing Sihuan Tech. co. Ltd.). The fracture edge was observed using the scanning electron microscope Vega 3 SBH (Tescan).

Small-angle X-ray scattering (SAXS)

The SAXS measurements were performed using the 'Xeuss 2.0' Small-angle X-ray scattering (Xenocs Co.). The wavelength of X-ray was 1.34 Å and the camera length was 1.2 m. The data acquisition time was 5 min. The two-dimension (2D) scattering images were analysed with Fit2D software from European Synchrotron Radiation Facility.

Cytotoxicity and biocompatibility

The cytotoxicity test of the G10/$T1_{1.6}$ hydrogel was determined by a direct contact method between the gel and Chinese Hamster Lung (CHL) Cell Beads via MTT assay. The sterilized G10/$T1_{1.6}$ gel was immersed in DMEM complete medium at a standard of 1 cm^2/mL, and incubated in a 37°C, 5% CO_2 incubator for 24 hours to obtain the gel extracted solution.

Supplemented with 10% fetal bovine serum (Gibco), the dulbecco's modified eagle medium (DMEM) (Gibco), 1.0 × 105 U/L penicillin (Hyclone), and 100 mg/L streptomycin (Hyclone) were used as the complete growth medium. CHL cells were seeded in 96-well plate at a density of 2500 cells/well. After CHL cells adhered to the plate for 5 h, the growth medium was replaced by 300 uL DMEM and 100 uL gel-extracted solution to further incubate the cell for the MTT assay, the gel disks were placed in the well to incubate the cell to evaluate the adhesion of the living cells on the gel. For the MTT assay, after being co-incubated for 1, 3 and 5 days, 20 μL of MTT (5 mg mL^{-1} in PBS) was added for 5 h to allow for the formation of formazan crystal. After removal of the supernatant, 150 μL DMSO was added to each well to dissolve the formazan crystal, and the absorbance was measured at 490 nm using a microplate reader. The results were expressed as percentages relative to the data obtained with the blank control. Six samples were tested for each group.

After being co-incubated with the gel disk for 1, 3 and 5 days, the gel disks were taken out and observed under a laser scanning confocal microscope (Primo vert, Carl Zeiss) to observe the living cells on the gel disks.

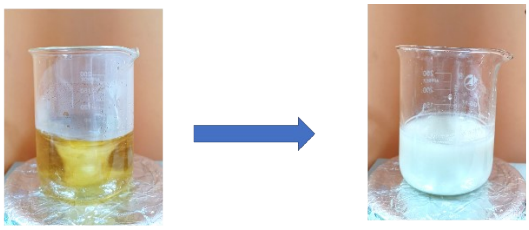

Figure S1. The appearance of the TA solution (1 wt %, 160 ml) after adding 10 ml of 10 wt % gelatin solution under stirring.

The determination of the real weight ratio of TA to Gelatin in the G/T gels

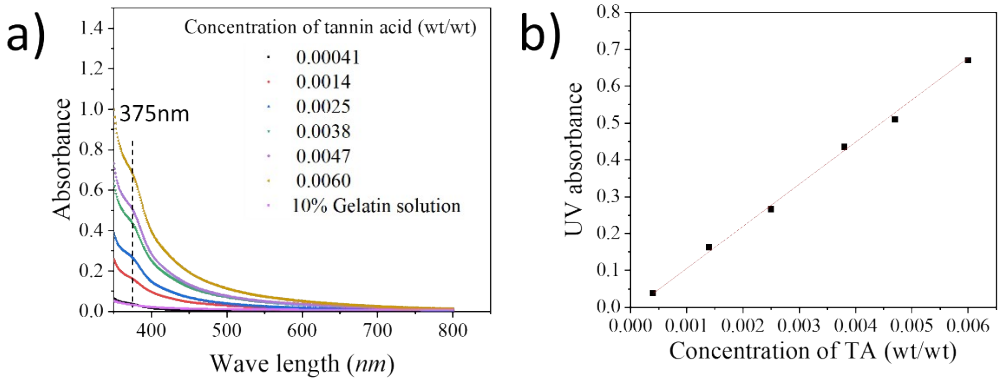

Figure S2. a) The UV-visible spectra of aqueous calibration solutions loaded with a specified concentration of tannic acid; b) the calibration curve of UV absorbance to tannic concentration.

As gelatin solution was added to a large amount of tannic acid solution and the gelatin long chains interacted with tannic acid to form a coacervate immediately when gelatin was dropped into the tannic acid solution, the clear filtrate was considered to contain minimal gelatin. The concentration of the residual tannic acid in the upper clear solution was estimated from the UV absorbance according to the calibration curve (figure S2b). The weight ratio of the tannic acid to gelatin in G/T gel (TA/Gelatin in gel) was calculated according to:

$$(TA/Gelatin\ in\ gel) = (m_{T0}\text{-}m_{Ts})/m_g \quad \text{(S3)}$$

Where m_{T0}, m_{Ts} and m_g are the weight of tannic acid in the tannic acid solution, the weight of tannic acid in the filtrated solution after the addition of gelatin solution and the weight of gelatin in the added gelatin solution, respectively.

Figure S3. Image of the bulk of loosely crosslinked G/T conservate, when gelatin solution was added to the 3 wt % tannic acid solution.

Table S2. The water content and mechanical properties of $Gx/T1_z$ gels

Gel code	Water content (wt %)	Young's modulus (MPa)	Fracture strain (mm/mm)	Fracture stress (MPa)	Fracture energy (kJ m^{-2})
$G10/T1_2$	37±1	83.7±4.1	0.11±0.02	2.0±0.1	-
$G10/T1_{1.6}$	37±4	59.4±4.8	10.3±0.9	2.1±0.2	24.7±2.3
$G10/T1_{1.33}$	37±5	45.0±1.5	11.4±3.0	1.9±0.6	17.0±4.0
$G10/T1_{1.14}$	33±1	20.4±1.0	16.8±5.7	2.0±0.4	16.1±1.2
$G10/T1_{1.07}$	34±1	6.5±0.7	23.8±2.2	1.4±0.2	15.6±0.8

All the data are averages for 3 experimental tests.

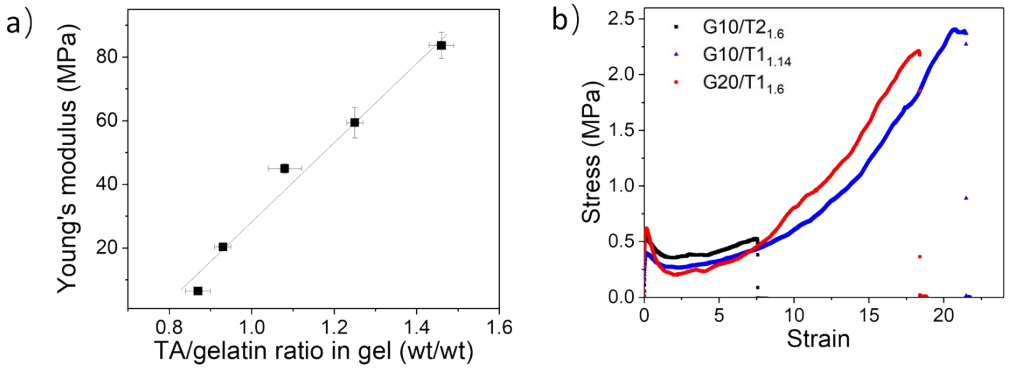

Figure S4 a) The Young's modulus vs the weight ratio of TA/gelatin in the G10/T1$_z$ gels. b) the tensile stress-strain curve of the G10/T2$_{1.6,}$ G10/T1$_{1.14}$ and G20/T1$_{1.6}$ gels with the similar TA/gelatin weight ratio in gel.

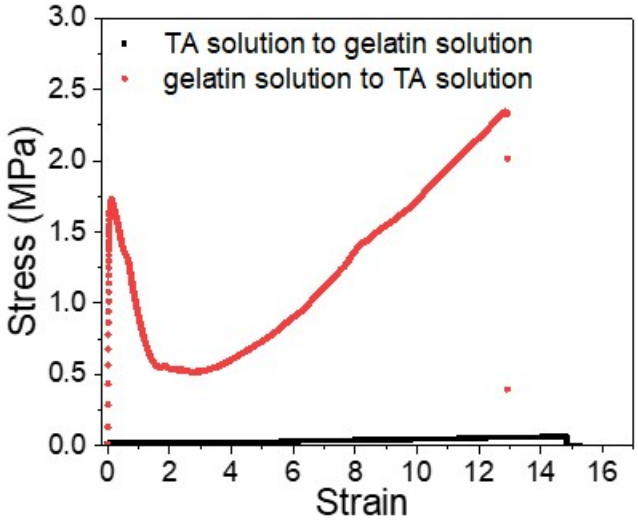

Figure S5. Tensile stress-strain curves of the G10/T1$_{1.6}$ gel (made by adding gelatin solution to TA solution) and the gel with the same recipe made by adding TA solution to gelatin solution (T/G gel).

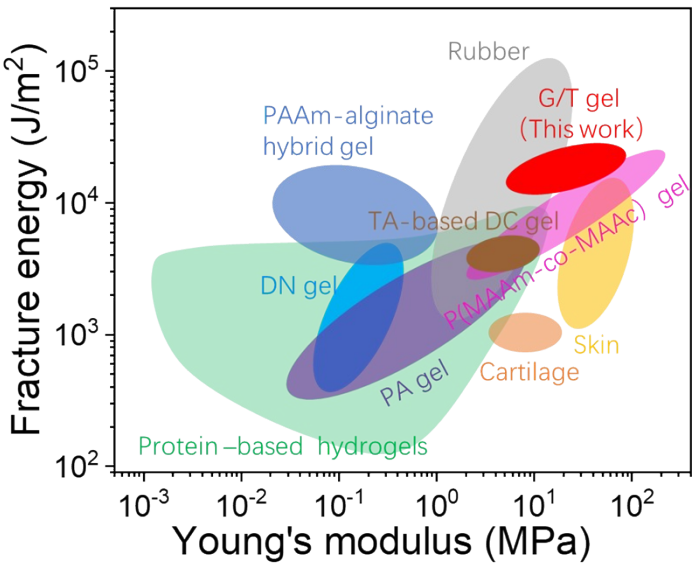

Figure S6 An Ashby plot that compares the mechanical properties of G/T gels (red region) with protein-based hydrogels, tough synthetic polymer-based hydrogels, rubber, cartilage, and skin. [1, 2, 5, 8, 26, 32-38]

SAXS analysis

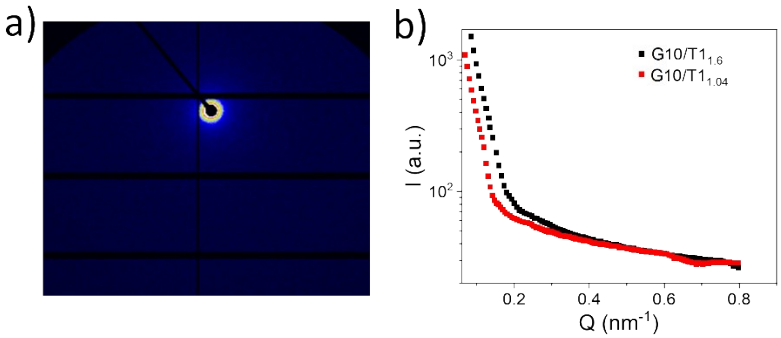

Figure S7 a) the 2D SAXS image of the G10/T1$_{1.6}$ gel; b) the 1D SAXS profile of the G10/T1$_{1.6}$ and G10/T1$_{1.04}$ gels.

The calculation of R_g

According to the equation:

$$I(Q)=I_0 \exp(-\frac{Q^2 R_g^{\,2}}{3}) \qquad \text{S1}$$

R_g is the effective size of the scattering "particle". The slope of the Guinier plot (Ln[$I(Q)$] vs Q^2) is $-Rg^2/3$.

FT-IR evidence of the formation of hydrogen bonds between gelatin and tannic acid.

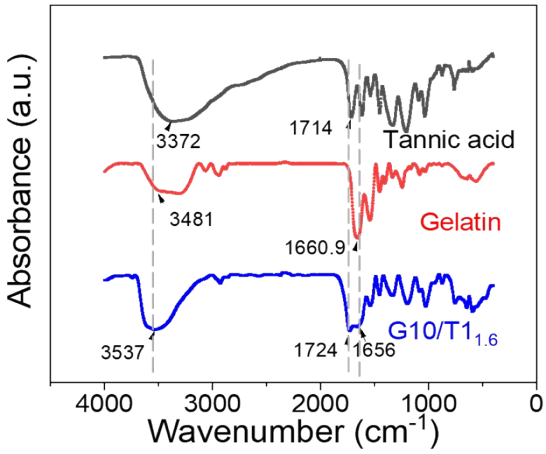

Figure S8. FT-IR spectra of freeze-dried gelatin gel, $G_{10}/T1_{1.6}$ gel, and tannic acid.

As shown in the FT-IR spectra, the carbonyl stretching resonances of the amide groups on gelatin and the ester group on tannic acid are observed at 1660 and 1714 cm^{-1}, respectively. For the $G_{10}/T1_{1.6}$ gel, two distinct absorption peaks are observed at 1724 and 1656 cm^{-1}, which are attributed to the stretching resonances of the carbonyl groups from tannic acid and gelatin, respectively. The stretching resonances corresponding to -OH on tannic acid and -NH, $-NH_2$ and gelatin are observed at 3372 and 3481 cm^{-1} respectively. After the formation of $G_{10}/T1_{1.6}$ gel, the stretching resonances corresponding to these groups are observed at 3537 cm^{-1}. Compared to the FTIR spectra of tannic acid and gelatin, the stretching resonances of all the functional groups for hydrogel bond formation shifted after the formation of the $G_{10}/T1_{1.6}$ gel, indicating the formation of hydrogen bonds between gelatin and tannic acid.

Dissolution of the $G_{10}/T1_{1.6}$ gel in urea solution

Figure S9. Images of the $G10/T1_{1.6}$ gel being soaked in the 4 M urea solution for 0 h, 0.5 h, 6 h, and 48 h respectively.

Necking of the gel after being stretched.

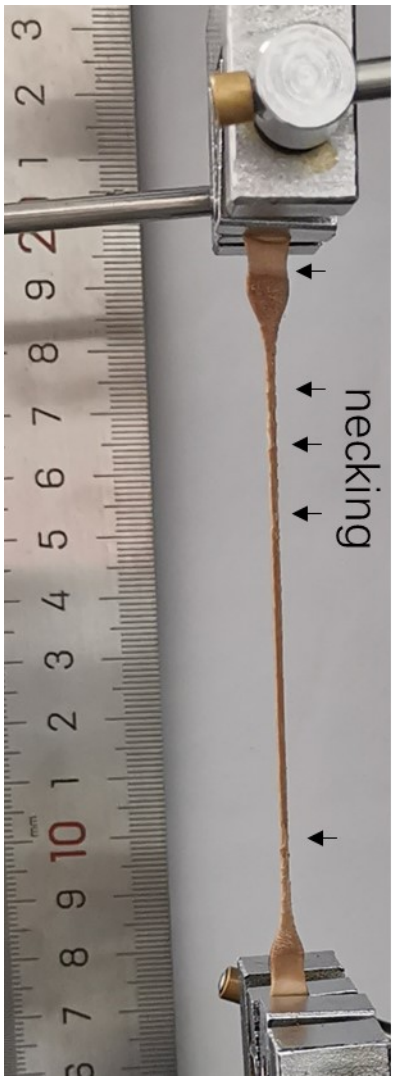

Figure S10. Image of the stretched G10/T1$_{1.6}$ with obvious necking events denoted.

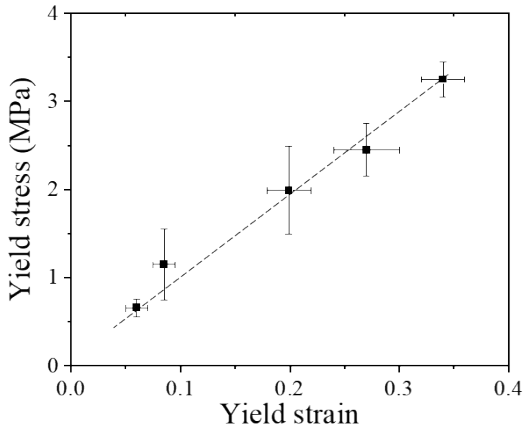

Figure S11. The yield stress versus yield strain of the G10/T1$_{1.6}$ gel tested at different strain rates of 0.028, 0.07, 0.14, 0.28 and 0.7 s^{-1}, respectively.

The calculation of τ* from the yield stress and strain with different strain rates, according to the following relationship:

$$\sigma_y = \sigma_{y,0} ln(\dot{\varepsilon}/\dot{\varepsilon}^*)^{24} \quad \text{(S4)}$$

$$\sigma_y = \sigma_{y,0} (ln(\dot{\varepsilon}) - ln(\dot{\varepsilon}^*)) \quad \text{(S5)}$$

where $\dot{\varepsilon}^*$, σ_y, $\sigma_{y,0}$, and $\dot{\varepsilon}$ are the critical strain rate, the yield stress, the characteristic yield stress, and the strain rate, respectively.

By the logarithmic fitting of the yield stress versus stain rate plot through the formula $y=alnx-b$, $\dot{\varepsilon}^*$ was calculated from the a and b value.

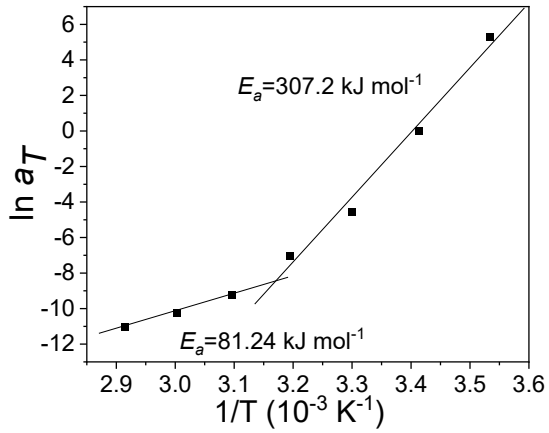

Figure S12. The Arrhenius plots for the shift factors of the G10/T1$_{1.6}$ gel. The apparent activate energy value E_a in the figure was calculated from the slope of the gel.

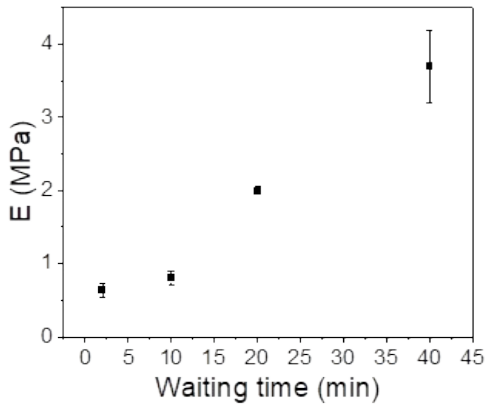

Figure S13. The waiting time dependence of the Young's modulus.

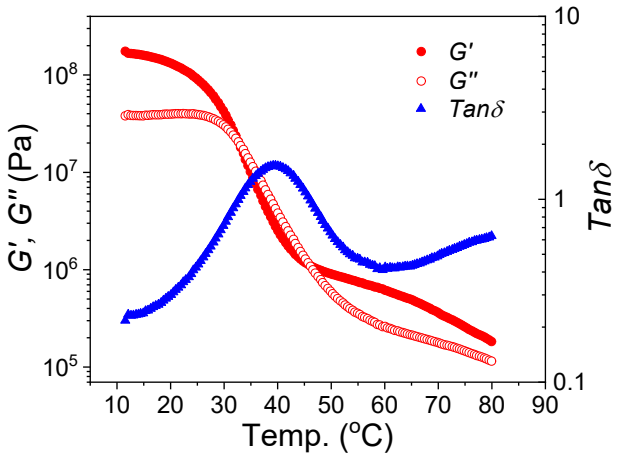

Figure S14. Temperature dependence of *G', G''* and *tanδ* at a frequency of 6.28 rad/s of the G10/T1$_{1.6}$ gel.

Video S1. The G10/T1$_{1.6}$ gel with dimension of 1.5 cm × 1.5 cm × 1 mm was stretched to a transparent thin film.

Video S2. The recovery of the stretched G10/T1$_{1.6}$ gel in 40 ^{o}C water.

Video S3. Self-healing of the G10/T1$_{1.6}$ gel.

Video S4. The self-glued G10/T1$_{1.6}$ gel with heart shape built from the gel fragments.

Video S5. The recovery of the twisted G10/T1$_{1.6}$ gel (shaped at 25 ^{o}C) at 40 ^{o}C.

Video S6. The erasable shape memory of the G10/T1$_{1.6}$ gel. From the initial state, a new state can be memorized by heating to 70 ^{o}C, followed by cooling to room temperature. A large number of dynamic bonds relax and maintain their new shape after cooling. Modifying the shape at 25 ^{o}C can result in a new temporary shape, and increase the temperature to 40 ^{o}C returns the gel to the memorized state. Further heating back to 70 ^{o}C erases the memorized shape, returning the sample to the initial shape.