Electronic Supplementary Information for

Wrinkled Double Network Hydrogel via Simple Stretch-Recovery

Chen Wang [a,c], Shaohua Yang [b], Qirui Guo [a,c], Liju Xu [a,c], Ye Xu [b], Dong Qiu [a,c] *

a. Beijing National Laboratory for Molecular Sciences (BNLMS), CAS Research/Education Centre for Excellence in Molecular Sciences, Institute of Chemistry, Chinese Academy of Sciences, Beijing 100190, China. E-mail: dqiu@iccas.ac.cn

b. School of Mechanical Engineering and Automation and Centre of Soft Matter Physics and its Applications, Beihang University, Beijing 100191, China

c. University of Chinese Academy of Sciences, Beijing 100190, China

* Correspondence author: dqiu@iccas.ac.cn

This PDF file includes:

Other supplementary materials for this manuscript include the following:

Captions for Movies S1 to S3

Movie S1. Video shows that the formation of wrinkles by in-situ observation. Movie is shown at real-time speed (15 frames per second).

Movie S2. Video shows that the two-dimensional wrinkles can be achieved by stretching W-gel in two different directions. Movie is shown at real-time speed (15 frames per second).

Movie S3. Reusability of W-gel. Movie is shown at real-time speed (15 frames per second).

Materials and Methods

Materials

Monomers including acrylamide (AM), acrylic acid (AA) and sodium p-styrene sulfonate (SS), N, N'-methylenebis (acrylamide) (MBAA), ammonium persulfate (APS), sodium alginate (Alg) and polyacrylic acid (PAA-10w, Mw = 100000) were purchased from Sigma. Ethylene glycol (EG), PVA 1799, ferric chloride ($FeCl_3$) and glycerol were purchased from J&K.

Methods

Preparation of W-gel: W-gel in this study was prepared by mixing v-PVA-gel and e-PAM-gel. In a typical recipe, PVA 1799 (10 g) and AM (10 g) were first dissolved in a mixed solvent of water (50 ml) and ethylene glycol (50 ml), and stirred at 90 ℃ for 2h till a clear solution was obtained. The solution was cooled to room temperature under nitrogen protection. Then the cross-linker MBAA (100 mg) and initiator APS (60 mg) were added into the above solution, which was transferred into a closed mold and kept at -30 ℃ for 12 hours to obtain the physical network (v-PVA-gel). Finally, the preformed hydrogel was transferred to the UV curing chamber (Yuntong Instrument, XM210, China) and irradiated for different time to form the chemical network (e-PAM-gel). The W-gel can be obtained after the formation of the above two networks.

In order to demonstrate the universality of this mechanism, the above monomers can be replaced by acrylic acid, p-phenylene sulfonic acid. W-gel (AA) and W-gel (SS) are abbreviated accordingly.

Preparation of C-PVA-gel@e-PAM-gel (C@e): C@e was prepared by mixing C-PVA-gel and e-PAM-gel. PVA 1799 (10 g) and AM (10 g) were first dissolved in water (100 ml), and stirred at 90 ℃ for 2h till a clear solution was obtained. The solution was cooled to room temperature under nitrogen protection. Then the cross-linker MBAA (100 mg) and initiator APS (60 mg) were added into the above solution, which was transferred into a closed mold. The solution were repeatedly frozen at -30 ℃ for 8 hours and thawed at 25 ℃ for 3 hours. This process was repeated 5 cycles to form the C-PVA-gel. Finally, the preformed hydrogel was transferred to UV curing chamber and irradiated 1.5 h to form the e-PAM-gel.

Preparation of elastic-elastic interpenetrated networks (e@e): e@e was prepared by mixing e-Alg-gel and e-PAM-gel. Sodium alginate (2 g) and AM (10 g) were first dissolved in a mixed solvent of water (50 ml) and ethylene glycol (50 ml), and stirred at 60 ℃ for 2h till a clear solution was obtained. The solution was cooled to room temperature under nitrogen protection. Then the cross-linker MBAA (100 mg) and initiator APS (60 mg) were added into the above solution. And the solution was transferred to the UV curing chamber and irradiated 1.5 h to form the chemical network. Finally, the preformed hydrogel was immersed in a 500 ml of 1M $FeCl_3$ at room temperature for 24 hours.

Preparation of viscous-viscous interpenetrated networks (v@v): v@v was prepared by mixing polyacrylic acid and v-PVA-gel. Briefly, PVA 1799 (10 g) and PAA-10w (10 g) were dissolved in a mixed solvent of water (50 ml) and ethylene glycol (50 ml), and stirred at 90 ℃ for 2h till a clear solution was obtained. Then the solution was transferred into a closed mold and kept at -30 ℃ for 12 hours to obtain the v@v.

Preparation of viscous-elastic non-interpenetrated networks (v-e): v-e was prepared by mixing v-PVA-gel and micron-scale freeze-dried e-PAM-gel powers. AM (10 g), cross-linker MBAA (100 mg) and initiator APS (60 mg) were first dissolved in water (100 ml), and stirred at 0 ℃ for 2h till a clear solution was obtained under the protection of nitrogen. Then the solution was transferred to the UV curing chamber and irradiated 1.5 h to form the hydrogel in a closed mold. e-PAM-gel powers can be achieved by freeze-drying and mechanical grinding of the above hydrogel. Then e-PAM-gel powers (5 g) and PVA 1799 (10 g) were dissolved in a mixed solvent

of water (50 ml) and ethylene glycol (50 ml), and stirred at 90 ℃ for 12h to obtain a mixed solution. v-e can be achieved by transferring the solution into a closed mold and kept at -30 ℃ for 12 hours.

The glycol in the above methods can be replaced by glycerol in equal volume. Detailed recipes are summarized in Table S1.

Wrinkles measurement

The formation of wrinkles by in-situ observation

We use the small mechanical stretching device and combine it with confocal microscope (Nikon Eclipse Ti-E). Image stacks are acquired using this system. We use a 4X object lens (Plan Fluor) with a numerical aperture (NA) of 0.13, depending on the experimental requirements for the spatial resolution, imaged with a Prime BSI CMOS camera. The samples move by the home-built stretching device with a minimum step size of 0.05 μm and a micrograph are taken continuously. The samples with a thickness of 1 mm were cut into stripes (15 mm in length, 5 mm in width). The sample are tested by a miniature loading stage (MTI Instrument, Inc., USA) (450 N capacity) with a loading rate of 0.05 mm/s.

Observation of wrinkles under different strains

Samples with a thickness of 2 mm were cut into stripes (100 mm in length, 10 mm in width). The different strains are achieved by using the Instron 5567 instrument with a 100 N load cell at a rate of 100 mm/min. The gauge length between the clamps was 30 mm. The deformed samples are transferred to the sample stage of confocal microscope to observe the wrinkles. The quantitative data of wrinkles are collected by software (Image J).

Cytotoxicity test

The cytotoxicity of the W-gel was evaluated using Cell Counting Kit-8 (CCK-8, Dojindo Laboratories, Kyushu Island, Japan) according to the manufacturer's instructions. In brief, W-gel was separately incubated in alpha Minimum Essential Medium (αMEM, Gibco, Gaithersburg, MD) at concentrations of 200 mg/mL for 24 h at 37 ℃. The supernatants were collected and filtered

through a 0.22 μm filter (Millipore, Billerica, MA). The extractions were designated as 100% extractions. Then, the 100% extractions were diluted to two times and four times denoted as 50% extractions and 25% extractions, respectively. W-gel extractions and αMEM supplemented with 10 v/v% foetal bovine serum (FBS, ScienCell, San Diego CA, USA) and 100 U/mL penicillin–streptomycin (Gibco, Gaithersburg, MD) were used for cell culture in the following experiments. MC3T3-E1 were seeded into a 96-well plate (1×10^4 cells/well) and incubated for 24 hours at 37 °C in 5% CO_2. The culture medium was replaced with W-gel extractions or αMEM complete medium. At 1, 3 and 5 days of culture, the cells were treated with 10% CCK-8 reagent medium solution and incubated at 37 °C in 5% CO_2 for 1 hours. The optical density (OD) value was measured at 450 nm with a reference wavelength of 630 nm. Three individual experiments were performed, and each sample was conducted in triplicate.

Attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) measurement

ATR-FTIR spectroscopy were collected in the wavenumber ranging from 400 cm^{-1} to 4000 cm^{-1} on a Bruker Einox 55 instrument assisted by ATR attachments. Measurements were conducted at ambient temperature. Required parts of the W-gel (without further treatment) were placed on the crystal chip of ATR attachments and collected the information from 400 cm^{-1} to 4000 cm^{-1}. For the analysis of ATR-FTIR, we used software (Origin) to integrate the two peaks (wavenumber ranging from 1550 cm^{-1} to 1750 cm^{-1} and wavenumber ranging from 3000 cm^{-1} to 3500 cm^{-1}) to calculate the areas representing relative content of -CO-NH_2 and -OH respectively. Then, we calculated the change in the ratio of the area of -OH to -CO-NH_2 to estimate the content change of v-PVA-gel and e-PAM-gel on the surface of W-gel.

<u>In-situ attenuated total reflection Fourier transform infrared spectroscopy (ATR-FTIR) spectroscopy of W-gel</u>

We use the small mechanical stretching device and combine it with Bruker Einox 55 instrument assisted by ATR attachments. The test and analysis of ATR-FTIR are consistent with the above.

X-ray scattering

X-ray scattering measurements were carried out on Rigaku (D/MAX 2500) instrument with an X ray of $\lambda = 0.154$ nm, operated at 40 kV and 200 mA. Data was collected for 2θ values between 5° to 55° with a speed at 1 °/min.

Mechanical characterization

Tensile test

The tensile test was carried out using the Instron 5567 instrument with a 100 N load cell at a rate of 100 mm/min. Samples with a thickness of 2 mm were cut into stripes (100 mm in length, 30 mm in width). The gauge length between the clamps was 30 mm. The tensile stress was obtained through dividing the force by the cross-sectional area. The strain was obtained through dividing stretched length by the original length.

Table S1. Detailed recipe of samples.

sample	PVA 1799 (g)	AM (g)	MBAA (mg)	ethylene glycol (ml)	water (ml)	APS (mg)	Alg (g)	AA (ml)	PAA-10w (g)	SS (g)	$FeCl_3$ (1 M) (ml)	e-PAM-gel powers (g)	irradiation time (h)	radiation intensity (W/cm^2)
W-gel	10	10	100	50	50	60							1.5	0.6
Low-UV	10	10	100	50	50	60							1.5	0.18
Low-C	10	10	10	50	50	60							1.5	0.6
e@e		10	100	50	50	60	2				500		1.5	0.6
v@v	10			50	50				10					
v-e	10			50	50							5		
W-gel (AA)	5		100	50	50	60		10					1.5	0.6
W-gel (SS)	5		100	50	50	60				10			1.5	0.6
C-PVA-gel	10				100									
W-gel (6:1)	5	30	300	50	50	100							0.75	0.24
W-gel (15:2)	4	30	300	50	50	100							0.75	0.24
W-gel (10:1)	3	30	300	50	50	100							0.75	0.24
e-PAM-gel		10	100		100	60							1.5	0.6

Supplementary Figure S1 to S13

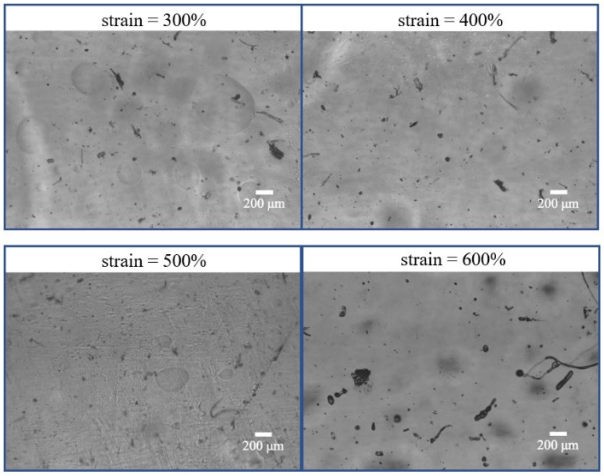

Figure S1. The optical photos of C@e surface at different strains.

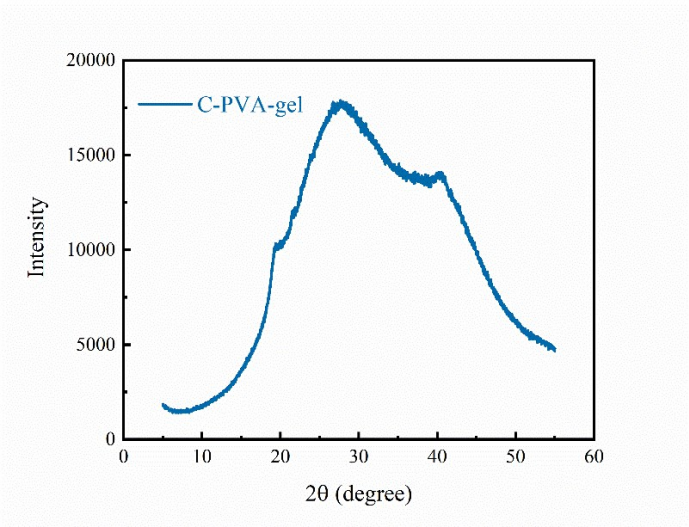

Figure S2. XRD diffraction pattern of C-PVA-gel.

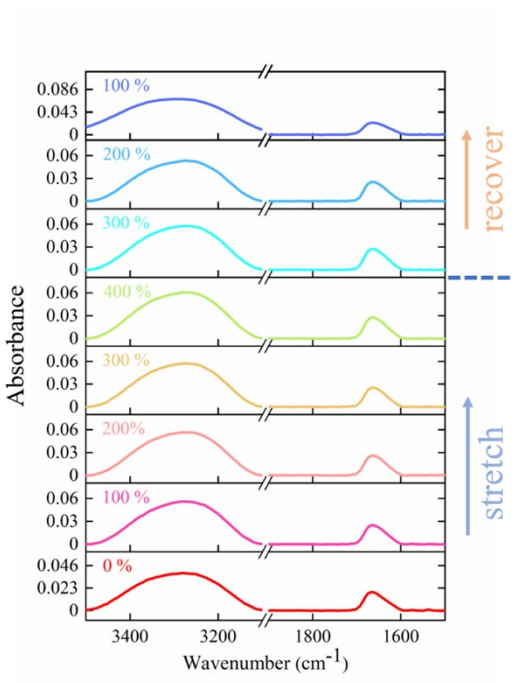

Figure S3. In-situ ATR-FTIR test of the surface of W-gel during the process of stretch-recovery.

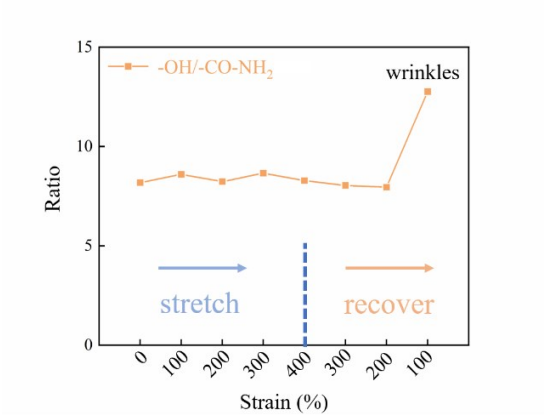

Figure S4. Ratio of -OH to -CO-NH_2 on W-gel surface during the process of stretch-recovery.

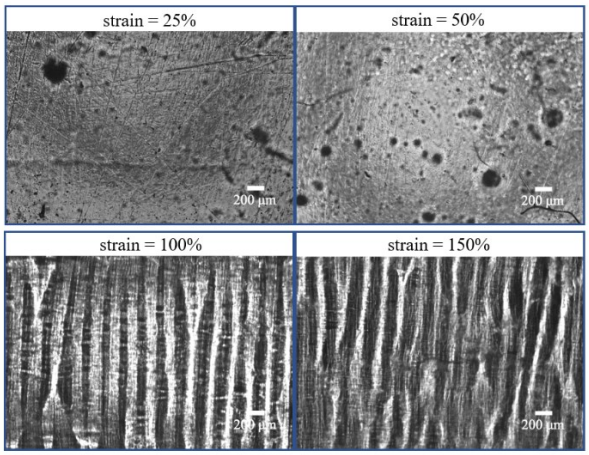

Figure S5. The optical photos of W-gel (AA) surface at different strains.

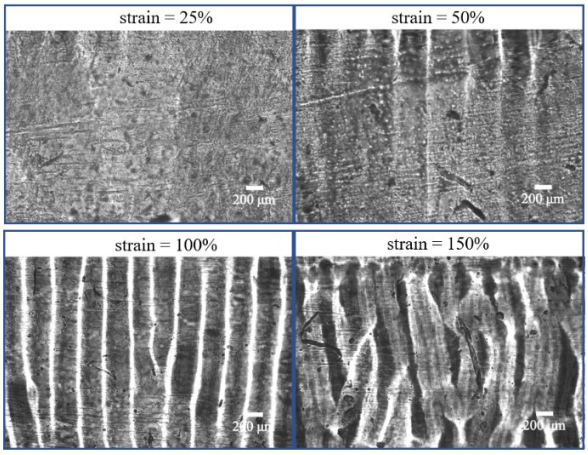

Figure S6. The optical photos of W-gel (SS) surface at different strains.

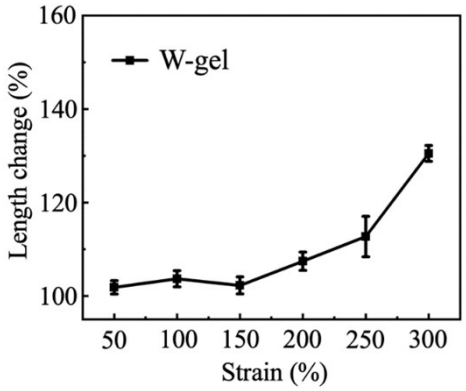

Figure S7. The length change of W-gel in the direction of stretching at different strains.

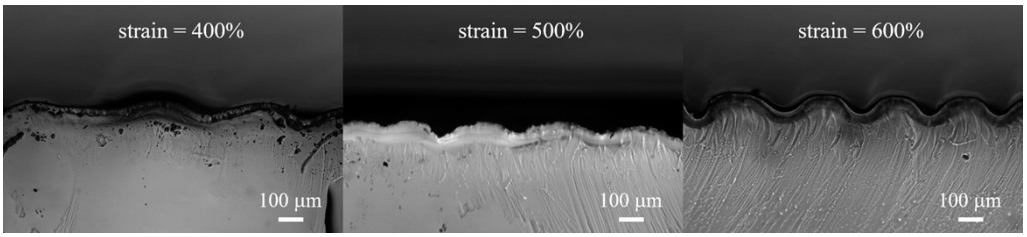

Figure S8. The optical photos of thick W-gel (named t-W-gel) surface with the thickness of 3.5 mm at different strains.

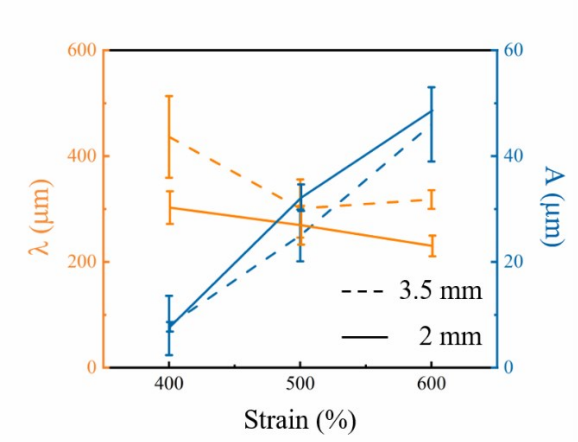

Figure S9. The change of amplitude and wavelength of wrinkles for W-gels with different thickness (3.5 mm and 2 mm) at different strains.

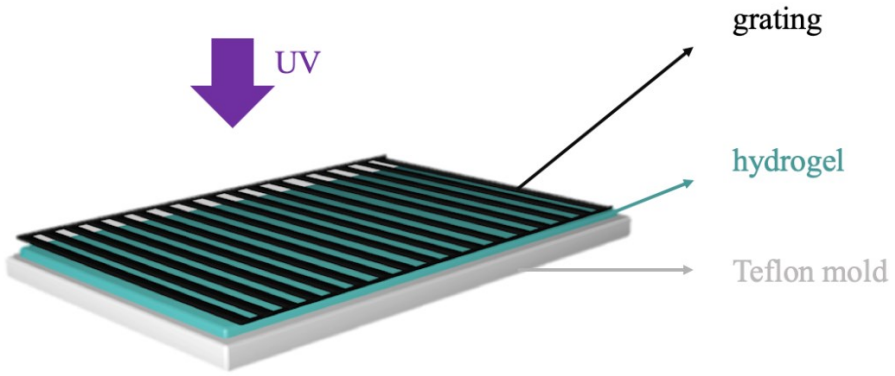

Figure S10. Schematic diagram of device to produce unevenly distributed internal stress inside the hydrogel.

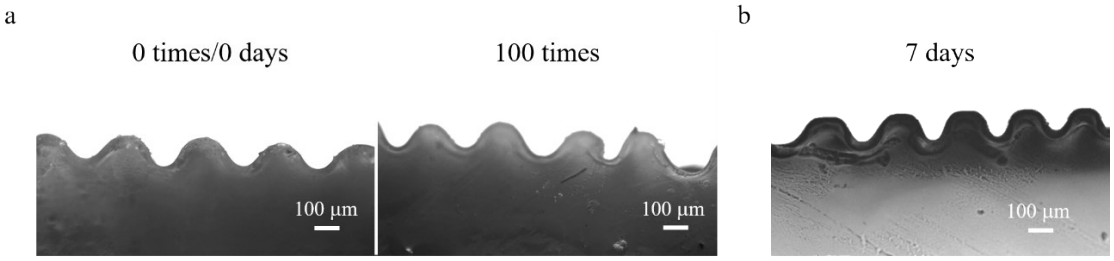

Figure S11. (a) The optical photos of W-gel for stretching 0 times and 100 times. (b) The optical photo of W-gel after stored in 25 ℃ for 7 days.

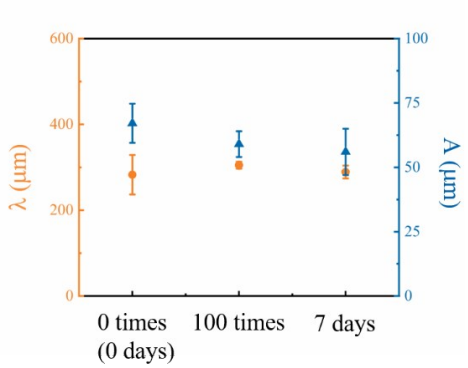

Figure S12. The change of amplitude and wavelength of wrinkles for W-gel after stretching 100 times under the strain of 200% and stored in 25 ℃ for 7 days.

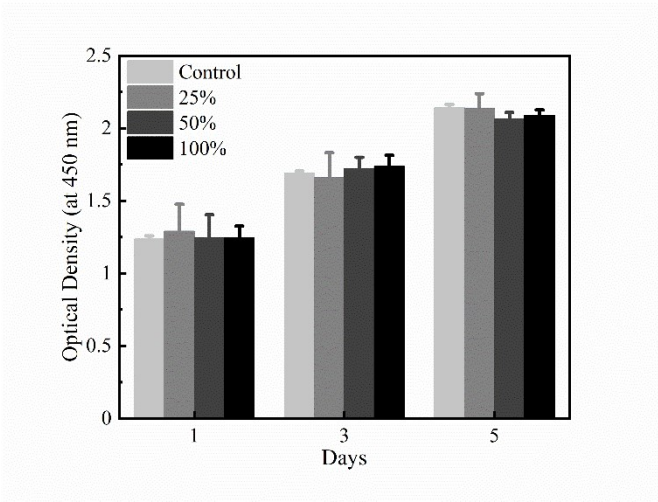

Figure S13. CCK-8 assay results showed that the W-gel has no cytotoxicity.