Supporting Information

Proton Transfer Hydrogels: Versatility and Applications

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General Methods and Materials

4-arm PEG thiol (M_w = 10000), **1**, was purchased from Laysan Bio Inc. Poly(ethylene glycol) diglycidyl ether (M_n = 500), **2**, pentaerythritol tetrakis(3-mercaptopropionate), **3**, diglycidyl 1,2-cyclohexanedicarboxylate, **4**, lithium hydroxide (LiOH), 1-methylimidazole, and triethylamine (TEA) were purchased from Sigma Aldrich. Phosphate buffered saline (PBS) (pH = 7.5, 0.1 M) and bicine buffer (pH = 9.0, 0.1 M) solutions were purchased from Biosesang. Photobase 1,2-Diisopropyl-3-[Bis(dimethylamino)methylene]guanidium2-(3-benzoylphenyl)propionate, **5**, was purchased from Wako Pure Chemical industries.

IR analyses were carried out using a Varian 640 IR spectrophotometer and an attenuated total reflection (ATR)-Fourier Transform infrared (FTIR) spectrometer with Resolution Pro as software. X-ray photoelectron spectroscopy (XPS) analysis was carried out with SIGMA PROBE (ThermoVG, U.K.) using a monochromatic Al-K(α) source (15kV, 100W, 400 micrometer). Narrow scan analyses were carried out with a pass energy of 20 eV and a step size of 0.1 eV. XPS spectra were analyzed by Avantage (ThermoVG) software.

Preparation of Hydrogels

In PBS (47.8 wt% precursor concentration): 4 arm PEG thiol **1** (0.5 g, 0.05 mmol) was added to 0.6 mL of PBS, and the mixture was stirred till it became a clear solution. Subsequently, PEG diglycidyl ether **2** (50 mg, 0.1 mmol) was added to the solution and the mixture was warmed up to 37 °C. The heating was continued until the solution became a gel. The gelation time was measured by test-tube inverting method using a stopwatch. An hour after the gelation, the gel was placed in ethanol and stirred for overnight, and then washed thoroughly with ethanol and water for several times. This washed hydrogel was then freeze-dried before any further use.

In Bicine (47.8 wt% precursor concentration): Same method and amounts were used as described for PBS with the exception that the gelation occurred at room temperature.

In Water (47.8 wt% precursor concentration): The procedure and amounts are the same as described for PBS. The difference being that PBS was replaced by pure water. And, LiOH, 1-methylimidazole, or triethylamine was added as a catalyst. For 1:1.5 (SH:Cat) molar ratio, 0.3 mmol of the catalysts was used. For lower catalyst ratios of 1(SH):0.5(Cat) and 1(SH):0.05(Cat), 0.1 and 0.01 mmol of the catalyst was used.

In 10% aq. THF (47.8 wt% precursor concentration): Same procedure as described for PBS was used. However, instead of PBS, same amount of a 10% (vol/vol) aqueous THF was used as a medium. The catalysts amounts were same as described in case of pure water as the gelation medium.

Imprint lithography (ambient conditions): Hydroxylated silicon wafer with patterns (width: ca. 700 nm, height: ca. 200 nm) was used as a hard master. Pentaerythritol tetrakis(3-mercaptopropionate) **3** (0.2 g, 0.409 mmol) and poly(ethylene glycol) diglycidyl ether **2** (0.4093 g, 0.819 mmol) were added to the 0.66 mL of 10% aq. THF, and was stirred till the solution became clear. TEA (0.25g, 2.46 mmol) was added to the mixture and the solution was stirred to get a homogenous solution. Stamps were prepared by drop casting this solution onto patterned silicon wafers and allowing for the solution to gel. The gelled films could be peeled off by hands or tweezers from the hard master and then analyzed by atomic force microscopy (AFM, Veeco Nanoscope V).

Photochemical synthesis (Bulk): To a solution of pentaerythritol tetrakis(3-mercaptopropionate) **3** (0.2 g, 0.409 mmol) and poly(ethylene glycol) diglycidyl ether **2** (0.4093 g, 0.819 mmol) in 0.66 mL of 10% aq. THF or 0.66 mL acetonitrile was added photobase generator **5** (0.0203 g, 0.0409 mmol) in dark. This mixture was covered with aluminum foil and stirred to obtain a clear solution. This mixture was transferred into a quartz cell of 20 mm diameter and 10 mm length, and cured under a UV light source with power intensity of 2 mW/cm^2. For **1**+**2** system, aqueous THF or acetonitrile were not used. In this case pure water was used as a gelation medium.

Photo-imprint lithography: Similar procedure as described for imprint lithography under ambient conditions was used. However, instead of TEA, photobase generator **5** (0.0203 g, 0.0409 mmol) was used. And dark conditions were used to prepare the solution and for drop

casting onto the hard silicon master. Furthermore, aqueous THF could be replaced with acetonitrile as the solvent.

Mechanical Tests

The measurement of mechanical properties of the hydrogels was conducted on a universal testing machine using an Instron 5567A at a rate of 0.5 mm/min^{-1} and at room temperature. The hydrogels were prepared and immersed in distilled water until they became fully swollen. Diameter and thickness of the swollen hydrogels were measured. The swollen hydrogels were covered with silicon oil to prevent any unwanted dehydration from the air and put on the compression plate.

Swelling Studies

Hydrogels were immersed into distilled water and then freeze-dried. The initial mass of dried hydrogels was measured and then they were immersed in distilled water until they reached the equilibrium state. Swollen hydrogels were weighed, and swelling was calculated according to the equation, Water Uptake (%) = [(mass of swollen hydrogel – mass of dried hydrogel)/mass of dried hydrogel] × 100.

In Vitro Degradation

Degradation of hydrogels was investigated in 0.1 M PBS solution (pH = 7.5) at 37 °C. Every 10 days, the hydrogels were taken out of the buffer, washed with deionized water, freeze-dried and measured in terms of their weights to calculate the mass loss. This was calculated according to the equation, degradation (wt%) = (Mass of degraded hydrogel / Mass of initial dried hydrogel) × 100.

Rheological Characterization

The sol-gel transition of hydrogels was determined by a rotational rheometer (AR2000, TA Instruments, USA). The polymer aqueous solution was placed between parallel plates of 40 mm diameter and a gap of 0.5 mm at room temperature or at 37 °C. Elastic (G') and viscous (G'') moduli were investigated in the small amplitude oscillatory shear (SAOS) mode with a frequency of 1 Hz and 1 % strain amplitude under linear viscoelasticity conditions.

Dynamic Mechanical Analysis (DMA) Tests

DMA experiments were done at a temperature range of -150 to 80 ºC on DMA Q800 (TA) in a tension mode (Strain: 0.1%; Frequency: 1Hz, Sample: height 30mm, width 5mm, thickness 0.3 ~ 0.5 mm). Through these measurements, the glass transition temperature was measured to be -16, 50, and 55 ºC for samples made up of **2**+**3**, **1**+**2**, and **1**+**4**, respectively.

Determination of the Crosslinking Density

Cross-linking density is calculated in accordance with the theory of rubber elasticity (L. E. Nielsen, J. Macromol. Sci. 1969, C3, 69) using the equation $v = E_r'/(3RT)v$ (S Parker, R Reit, H Abitz, G Ellson. Macromol. Rapid Commun. 2016, 37, 1027-1032) in moles per liter. Here, E_r' is the rubbery elastic storage modulus (MPa), R is the ideal gas constant ($L\ MPa\ K^{-1}mol^{-1}$), and T is the absolute temperature at which the rubbery elastic modulus was measured. For measurement of rubbery elastic modulus, a torsion test was carried out as shown in Figure S6. Here, the temperature of 130 ºC was chosen which is beyond the glass transition temperature of the hydrogels. The strain was 0.1%, angular frequency was 0.1 ~ 10 (rad/s), sample height was 30 mm, width was 5 mm and thickness was 0.3 ~ 0.5 mm.

Thermogravimetric (TGA) Analysis

The decomposition profile of the hydrogel was analyzed with a TA Instruments (Utah) Q50. 8mg of a hydrogel sample was placed in a platinum sample pan and heated from 25 to 700 ºC under a nitrogen atmosphere at a heating rate of 10 ºC/min, and the weight loss was recorded as a function of temperature.

Gel Functionalization

Hydrogel Alkylation (Method 1): The dried hydrogel was suspended in distilled water, and iodoacetamide (1 molar) was added. The mixture was stirred at room temperature for 24 hr. Afterwards, alkylated hydrogel was dialyzed against deionized water for 24 hr, followed by freeze-drying.

Hydrogel Alkylation (Method 2): The dried hydrogel was suspended in DMF, and bromoethane (1 molar), or bromopentane (1 molar) was added. The solution was covered with foil, stirred at room temperature for 24 hr, and dialyzed against deionized water for 24 hr, followed by freeze-drying.

Antibacterial Properties

A live/dead Baclight bacterial viability kit (L-7012, Molecular Probes, USA) was used to assess the bacterial cell viability on the hydrogel surface with *E. coli* as the model bacteria. In this assay, the red-fluorescent nucleic acid staining agent propidium iodide, which only penetrates damaged cell membrane, was used to label dead bacterial cells on the hydrogel surface (excitation: 485 nm, emission: 630 nm). The SYTO 9 green-fluorescent nucleic acid staining agent, which can penetrate cells both with intact and damaged membranes, was used to label all the bacterial cells (excitation: 485 nm, emission: 530 nm). The freeze-dried hydrogel sample, which was cut into pieces of 0.3 cm × 0.3 cm at the thickness of about 1 mm, was immersed in 200 μL of *E. coli* suspension (10^6 cells/mL) and then incubated at 37 °C for 24 h. After incubation, the supernatant was removed and all samples were rinsed with sterile PBS buffer for three times. The hydrogel samples were subsequently stained with 200 μL of a dyes-containing solution, which was prepared by adding 3 μL of SYTO (3.34 mM) and 3 μL of propidium iodide (20 mM) to 2 mL of PBS buffer, at room temperature in the dark for 15 min. The stained bacterial cells on hydrogel surfaces were examined under a laser scanning confocal microscope (CLSM, LSM 700, Carl-Zeiss, Germany). Images of three random locations on each sample were obtained by using an oil immersed 40× object lens under the same conditions.

Cancer Cell Studies

A human ovarian cancer cell line, SKOV3, was provided by Korea Basic Science Institute and cultivated in RPMI1640 with 10% FBS and 1% antibiotic (penicillin-streptomycin) in a humidified 5% CO_2 incubator at 37 ^oC. The tested cells (8×10^5 cells/dish) were seeded onto the gel substrate (**1**+**2** system/PBS medium) in 60-mm round dishes and incubated for 8 d, during which a fresh medium was recharged after washing with PBS (pH 7.4) every other day. Prior to the imaging experiment, the cells were washed twice with PBS (pH 7.4) to remove the remnant growth medium, placed onto 35-mm cover glass bottom dishes, and stained with a Live/Dead assay kit (calcein AM, 0.2 μM; EthD-1, 0.4 μM). The cells were then imaged using a LEICA DMI3000B equipped with a Nuance FX multispectral imaging system (CRI, USA).

Table S1. Gelation in Water

	Sample	Precursor Concentration (wt%)	SH: Catalyst	Reaction Temperature (ºC)	Bulk Gelation Time (measured by tube-inverting method)	Gel point by Rotational Rheometer
1	**1**+**2**-Bicine	47.8	-	25	30 minutes	33.5 minutes
2	**1**+**2** PBS		-	37	40 minutes	43.8 minutes
3	**1**+**2** H_2O-IM		1 : 1.5	25	50 minutes	46.6 minutes
4	**1**+**2** H_2O-TEA				≈ 3 minutes	≈ 2 minutes
5	**1**+**2** H_2O-LiOH				≈ 1 minute	≈ 2 minutes
6	**1**+**2** H_2O-IM		1 : 0.5		110 minutes	-
7	**1**+**2** H_2O-TEA				6 minutes	-
8	**1**+**2** H_2O-LiOH				≈ 2 minutes	-
9	**1**+**2** PBS	31.4	-	37	70 minutes	-
10	**1**+**2** Bicine		-	25	44 minutes	-
11	**1**+**2** H_2O-IM		1 : 1.5		65 minutes	-
12	**1**+**2** H_2O-TEA				4.6 minutes	-
13	**1**+**2** H_2O-LiOH				≈ 3 minutes	-
14	**1**+**2** H_2O-IM		1 : 0.5		100 minutes	-
15	**1**+**2** H_2O-TEA				5 minutes	-
16	**1**+**2** H_2O-LiOH				≈ 3 minutes	-
17	**1**+**2** H_2O-TEA	47.8	1 : 0.05		10 minutes	7.8 minutes
18	**1**+**2** H_2O-LiOH				10 minutes	6.8 minutes

Bicine = bicine buffer, PBS = phosphate buffer solution, IM = 1-methylimidazole, TEA = triethylamine, LiOH = lithium hydroxide. SH:Catalyst is a molar ratio between a thiol group and the catalyst.

Table S2. Modularity of Precursor and Application of Organic Co-Solvent

	Sample	Precursor Concentration (wt %)	SH:Catalyst	Reaction Temperature (ºC)	Bulk Gelation Time (measured by tube-inverting method)
1	**3**+**2** 10% aq. THF-TEA	31.4	1 : 1.5	25	70 minutes
2	**1**+**4** 10% aq. THF-TEA				30 minutes

Table S3. Photochemical Gelation

	Sample	Precursor Concentration (wt%)	SH:Catalyst	Reaction Temperature (ºC)	Bulk Gelation Time (measured by tube-inverting method)
1	**1**+**2** H_2O	47.8	1 : 0.05	25	15 minutes
2			1 : 0.025		30 minutes
3	**3**+**2** 10% aq. THF		1 : 0.05		4 minutes
4			1 : 0.025		5 minutes
5	**3**+**2** Acetonitrile		1 : 0.05		5 minutes
6			1 : 0.025		13 minutes

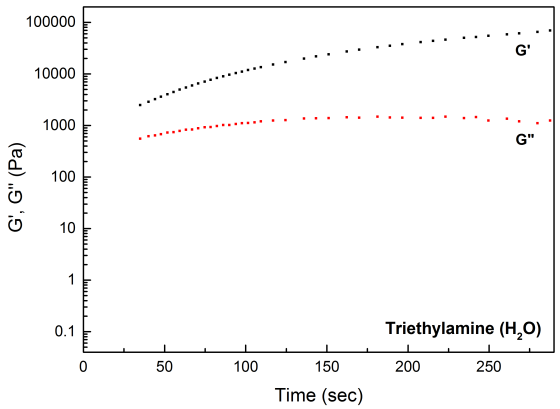


Figure S1. Gelation of precursors **1** and **2** in water using triethylamine as a base and studied with the help of a rotational rheometer (entry 4, Table S1).

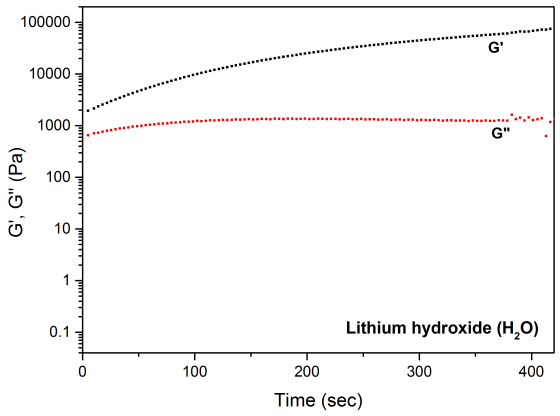


Figure S2. Gelation of precursors **1** and **2** in water using lithium hydroxide as a base and studied with the help of a rotational rheometer (entry 5, Table S1).

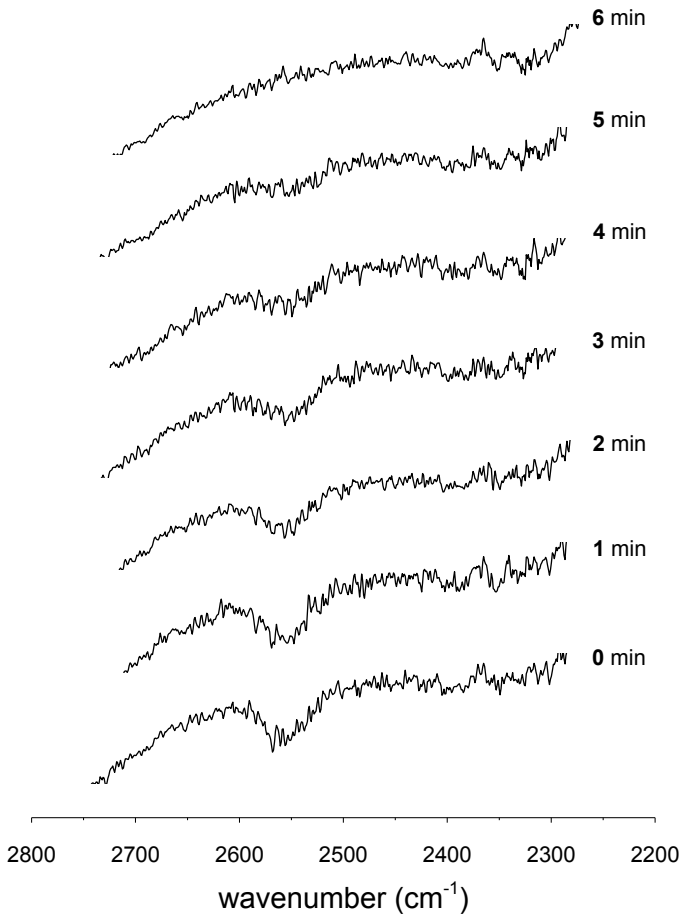


Figure S3. IR spectra showing the thiol frequency of precursor **3** as a function of irradiation time during gelation with **2**.

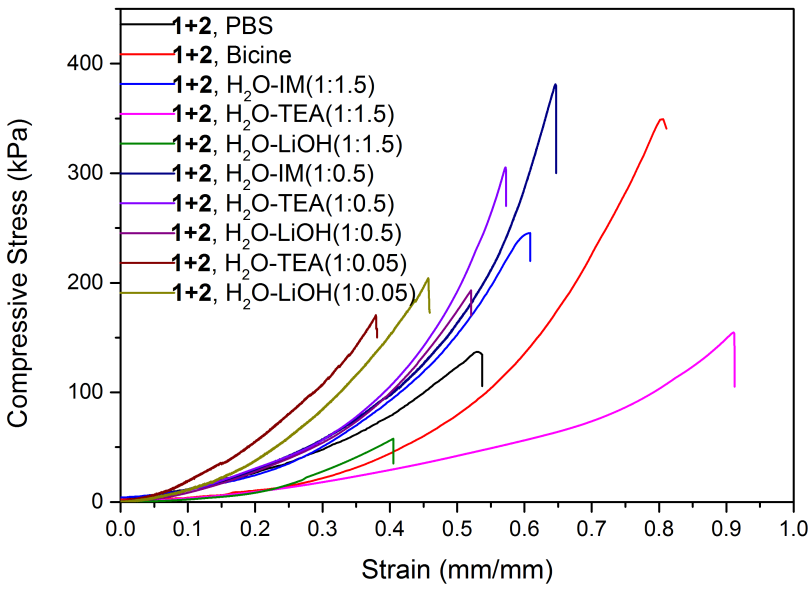


Figure S4. Mechanical properties of materials shown in entries 9-18 in Table S1.

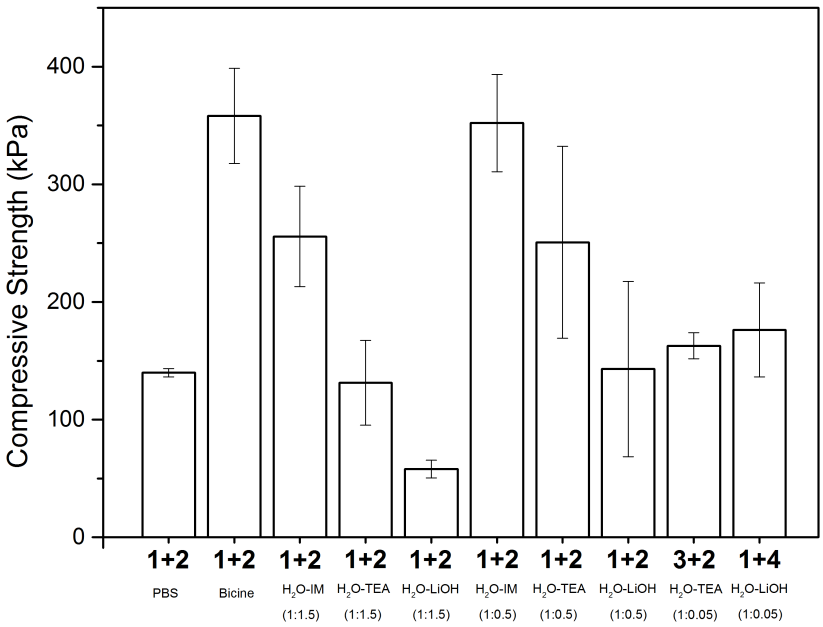
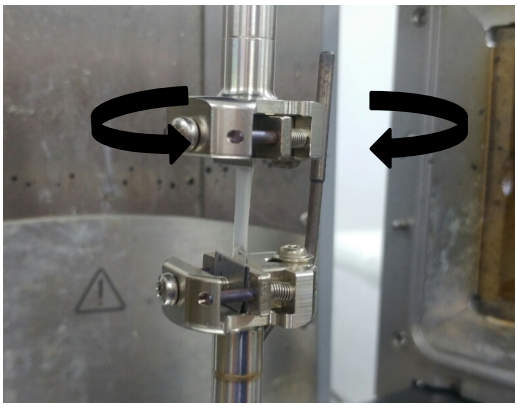


Figure S5. Mechanical properties of materials shown in entries 9-18 in Table S1.



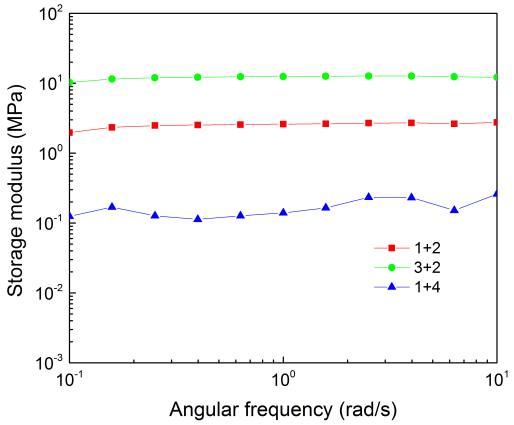


Figure S6. Digital picture of the solid rectangular sample torsion test machine and storage modulus of the three different gel systems. For further information see experimental details section heading 'Determination of the Crosslinking Density'.

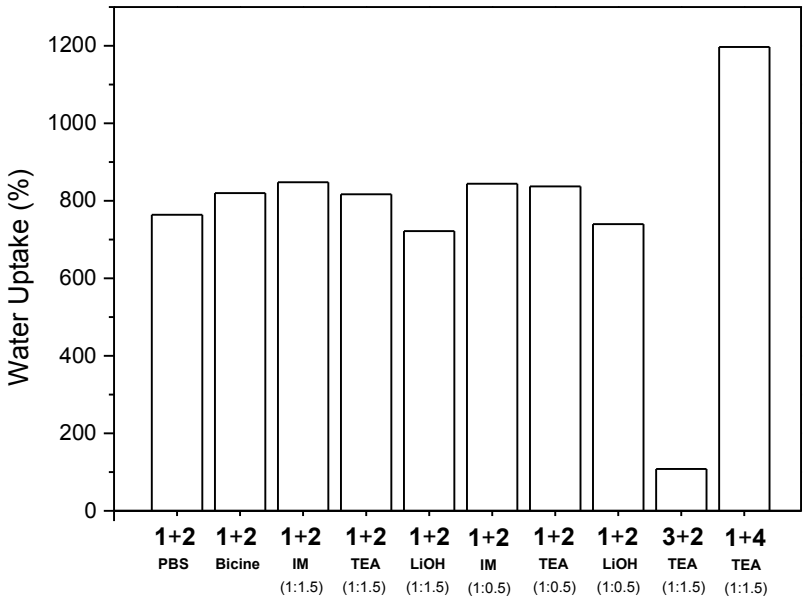


Figure S7. Water–uptake properties of materials shown in entries 1-8 in Table S1 and entries 1-2 in Table S2.

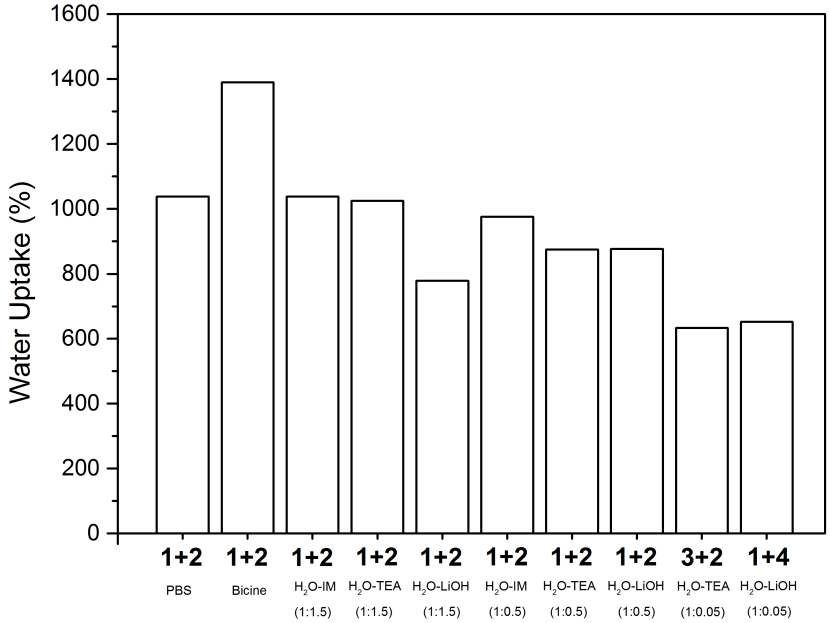


Figure S8. Water–uptake properties of materials shown in entries 9-18 in Table S1.

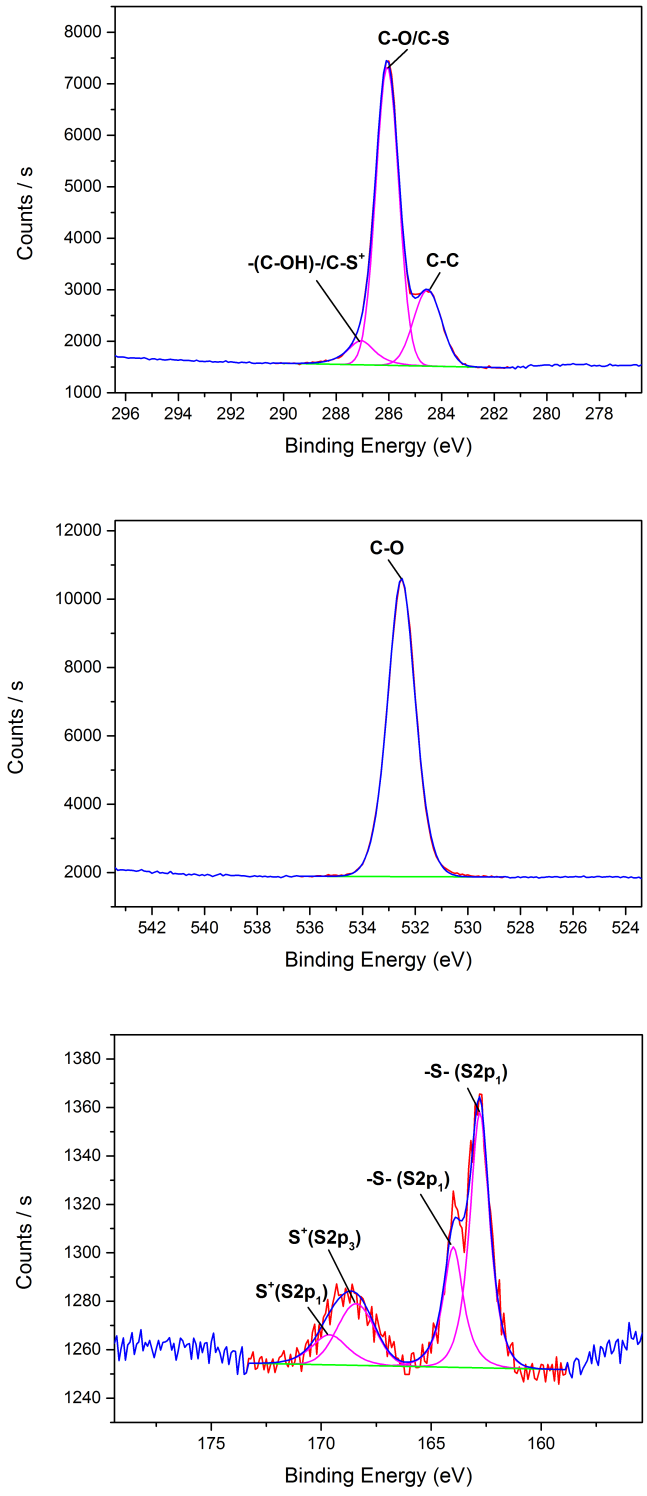


Figure S9. XPS spectrum showing binding energies of the gel after modification with bromoethane.

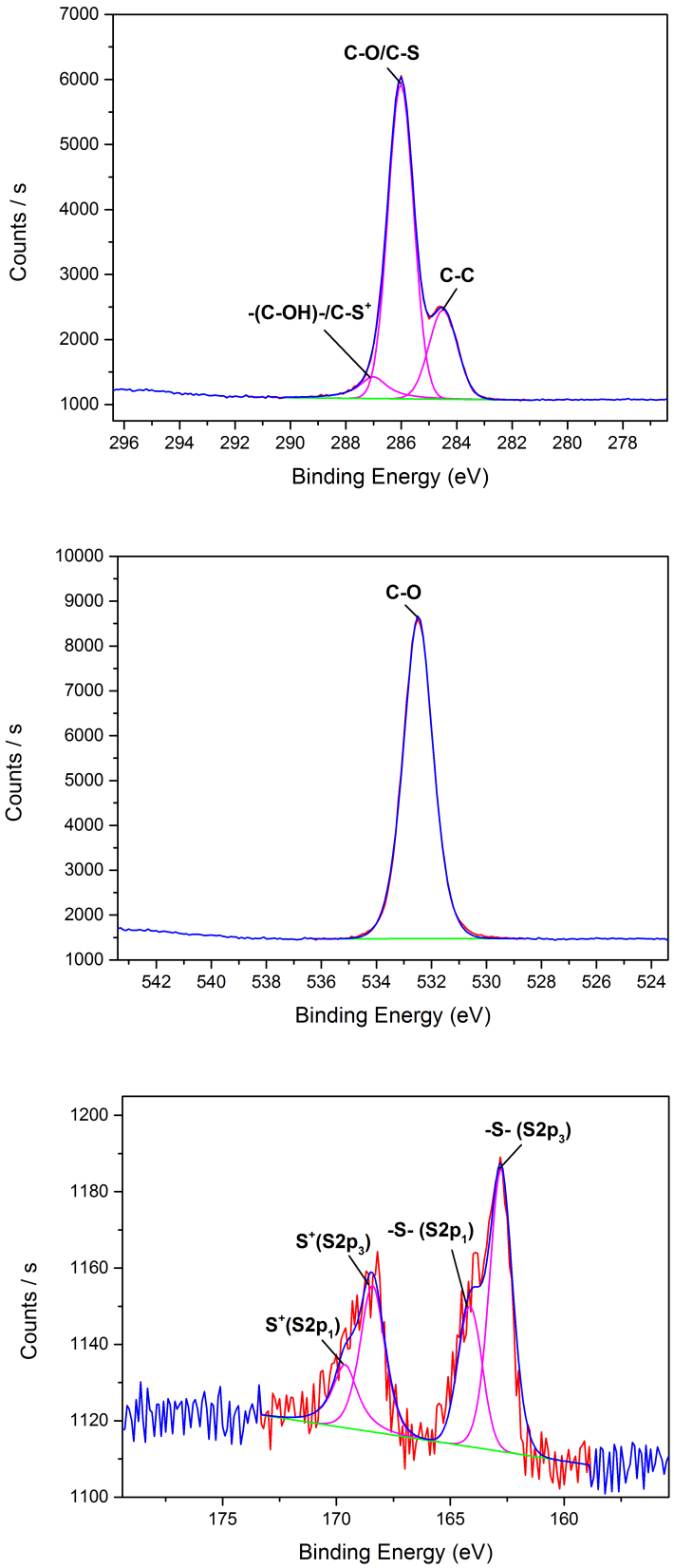


Figure S10. XPS spectrum showing binding energies of the gel after modification with bromopentane.

Table S4. XPS details for Figures 10, S6 and S7.

	Chemical state	Position	FWHM	Percent Area (%)
Unmodified Hydrogel	C-C	284.53	1.28	7.56
	C-O/C-S	286	1.16	56.81
	C bonded to OH/C-S^+	287.2	1.32	4.96
	C-O	531.93	1.24	31.01
	S2p3	162.92	1.13	0.65
	S2p1	164.12	1.08	0
Modified with Iodoacetamide	C-C	284.51	1.17	23.52
	C-O/C-S	286.14	1.1	37.9
	C bonded to OH/C-S^+	287.34	1.19	49.5
	$CONH_2$	530.73	1.44	2.01
	C-O	532.49	1.36	30.76
	S2p3	162.93; 168.4	0.9; 1.61	0.57; 0.29
	S2p1	164.11; 169.6	0.94; 1.61	0; 0
Modified with Bromoethane	C-C	284.53	1.25	14.93
	C-O/C-S	286.06	1.02	47.88
	C bonded to OH/C-S^+	287.06	1.22	5.19
	C-O	532.52	1.22	30.73
	S2p3	162.79; 168.42	1.06; 2	0.91; 0.37
	S2p1	163.99; 169.62	1.07; 1.92	0; 0
Modified with Bromopentane	C-C	284.49	1.2	15.37
	C-O/C-S	286.02	1.08	48.56
	C bonded to OH/C-S^+	287.02	1.23	4.89
	C-O	532.48	1.25	29.99
	S2p3	162.77; 168.42	1.2; 1.28	0.77; 0.43
	S2p1	164.14; 169.62	1.25; 1.32	0; 0