

Supporting Information

Sea Cucumber Mimicking Bacterial Cellulose Composite Hydrogel with Ionic Strength-sensitive Mechanical Adaptivity

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Method

Materials

Bacterial cellulose (BC) hydrogels were obtained from Fujicco Co., Ltd., Japan (Japan). *N,N*-dimethyl formamide (DMF) and triethylamine (TEA) were purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Acryloyl chloride (AC), sodium *p*-styrene sulfonate (SS), sodium hydroxide and sodium chloride were all purchased from Wako Chemicals (Osaka, Japan). Ammonium persulfate (APS) was purchased from Sigma-Aldrich (St. Louis, USA). All chemicals used were chemically pure grade, and deionized (DI) water was used when water was involved.

Purification and quantification of BC

BC gels were firstly thoroughly washed by DI water, then purified in 0.2 M NaOH aqueous solution at 80 °C for 4 h, finally washed with DI water again until the pH value was constantly at 7.0. In this work, all the BC gels were counted as a composition of 1 wt% cellulose and 99 wt% water. The concentrations of solutions were the final values after adding BC gels when BC was involved.

Modification of BC with AC

Prior to modification, the BC hydrogels were subjected to a solvent exchange, which replaced interior water in BC by DMF completely. After that, distilled TEA was added into the mixture of BC and DMF, excess AC (a mole ratio of 10:1 to the hydroxy groups in cellulose) was added dropwise in the mixture while stirring in an ice bath. The reaction was conducted at room temperature. After 24 h, deionized water was added to terminate the reaction, the resultant AC modified BC (ACBC) gels were purified by washing with deionized water completely.

Grafting of PSS from ACBC

The PSS was grafted from ACBC via free-radical polymerization using APS as initiator. The required amount of SS and 1.0 mol% APS (corresponding to SS monomer) were added to the mixture of ACBC and deionized water. The mixture was well homogenized by stirring for 24 h at room temperature and deaerated by using a vacuum pump. Then the free-radical polymerization was initiated by transferring the mixture into a 70 °C oil bath. The polymerization was continued for 24 h. After that, the obtained BC composite gels (BC-g-PSS) were completely washed with deionized water before characterizations. The BC samples were denoted as BC-*x*, where *x* represents the concentration of SS in grafting polymerization, *i.e.* BC-0 is pristine BC gel and BC-1.0 means the grafting polymerization of BC-g-PSS gel was conducted under a SS concentration of 1.0 mol·L⁻¹.

Determination of the composition of BC-0 and BC-g-PSS gels

The composition of BC gels was determined as following. For BC-g-PSS gels, the initial weight of the used pristine BC gels was first recorded before modification. After grafting with PSS, the weight of corresponding composite gels was measured again. Then, the BC-g-PSS gels were dried in an oven at 80 °C up to a constant mass, and the dried weights were measured. For BC-0 gels, the wet and dry weight were also measured.

The mass percent of water (R_w), cellulose nanofibers (R_c) and PSS (R_p) in BC gels was calculated according to Eqs. (1), (2) and (3), respectively.

$$R_w = \frac{W_0 - W_d}{W_0} \times 100\% \quad (1)$$

$$R_c = \frac{W_c}{W_0} \times 100\% \quad (2)$$

$$R_p = \frac{W_d - W_c}{W_0} \times 100\% \quad (3)$$

In these equations, W_d is the dry weight and W_0 is the wet weight of gels. W_c is the weight of cellulose nanofibers in gels. For BC-0 gels, no PSS is involved, W_c is exactly the W_d . However, it is unable to determine W_c in BC-g-PSS gels by measuring the weight directly. Therefore, the W_c in BC-g-PSS gels was estimated on the basis of initial wet weight (pristine BC gels before modifications) and the R_c of BC-0 gels.

Characterization of BC gels

Attenuated total reflection Fourier transform infrared (ATR-FTIR) spectroscopy (Thermo Scientific Nicolet

is 5, USA) was used to confirm the successful preparation of BC-g-PSS composite hydrogel. The samples were frozen by liquid nitrogen and then dried in a vacuum freeze-drier for ATR-FTIR.

The vertical sections of the BC and BC-g-PSS gels were observed using scanning electron microscopy (SEM) (Hitachi SU3500, Japan). The gel samples were freeze-dried and sputtered with gold by using an MSP-1S magnetron sputter (Vacuum Device Inc, Japan). The samples were observed under high vacuum at an acceleration voltage of 1.50 kV.

Compression test

For compression test, the samples were cut into cylinders (10 mm in diameter and 10 mm in height). As regards practical applications, the compressing testing of BC gels in this study was performed by applying the respective compressive stress along the growth direction (weaker direction). The required strain to achieve a deformation speed of $3.0 \text{ mm}\cdot\text{min}^{-1}$ was measured in a 500 N load cell with a material tester (Shimadzu EZ Graph, Japan) at room temperature. At least 5 specimens were tested for each sample. Besides, the compression strain at 0.01 MPa stress ($\varepsilon_{0.01}$) was regarded as the end of soft state and the start of linear elastic deformation region in respective compression stress-strain curve. The compression modulus was determined as the slope of the linear elastic deformation region from $\varepsilon_{0.01}$ to $\varepsilon_{0.01} + 10\%$ as shown in Fig. S4.

Ionic responsive behavior of BC-g-PSS gels

The original volumes of BC-g-PSS gels in DI water were firstly measured via volume displacement method. Subsequently, BC-g-PSS gels were separately immersed in a series of sodium chloride solutions with different concentrations at room temperature. After 24 h, took out the BC gels, gently removed the excess of solution and measured their equilibrium volumes. Afterwards, the compression properties of samples were measured according to the aforementioned method. The volumetric shrinkage ratio (VSR) of BC gels in ionic responding was calculated as followed:

$$VSR = \frac{V_0 - V_e}{V_0} \times 100\% \quad (4)$$

Where the V_e is the equilibrium volume of BC gels after being immersed in NaCl solutions and V_0 is the original volume of BC-g-PSS gels in DI water.

Drying-swelling ability

First, the initial wet weights of pristine BC (BC-0) and BC-1.0 gels were measured before drying. The

samples were taken out from deionized water and dried in an oven at 80 °C up to a constant mass. Then, the samples were immersed in water again for 4 h at room temperature and weighed. The same procedure was repeated several times. The mass ratio of gels during the drying and reswelling process was determined by dividing the current mass to the original mass.

Results

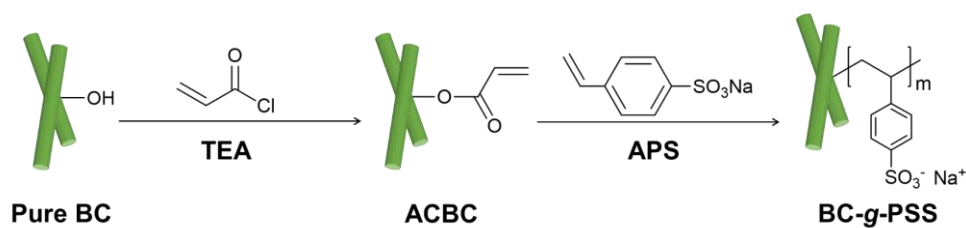


Fig. S1 Schematic presentation of the preparation of BC-g-PSS: acylation with AC (first step), graft polymerization of SS (second step).

Table S1 Compositions of BC-0 and BC-g-PSS gels

| Sample | Water content (wt%) | Cellulose content (wt%) | PSS content (wt%) |
|--------|---------------------|-------------------------|-------------------|
| BC-0 | 99.4 | 0.6 | 0 |
| BC-0.5 | 99.3 | 0.6 | 0.1 |
| BC-1.0 | 98.7 | 0.6 | 0.7 |
| BC-2.0 | 98.0 | 0.5 | 1.5 |

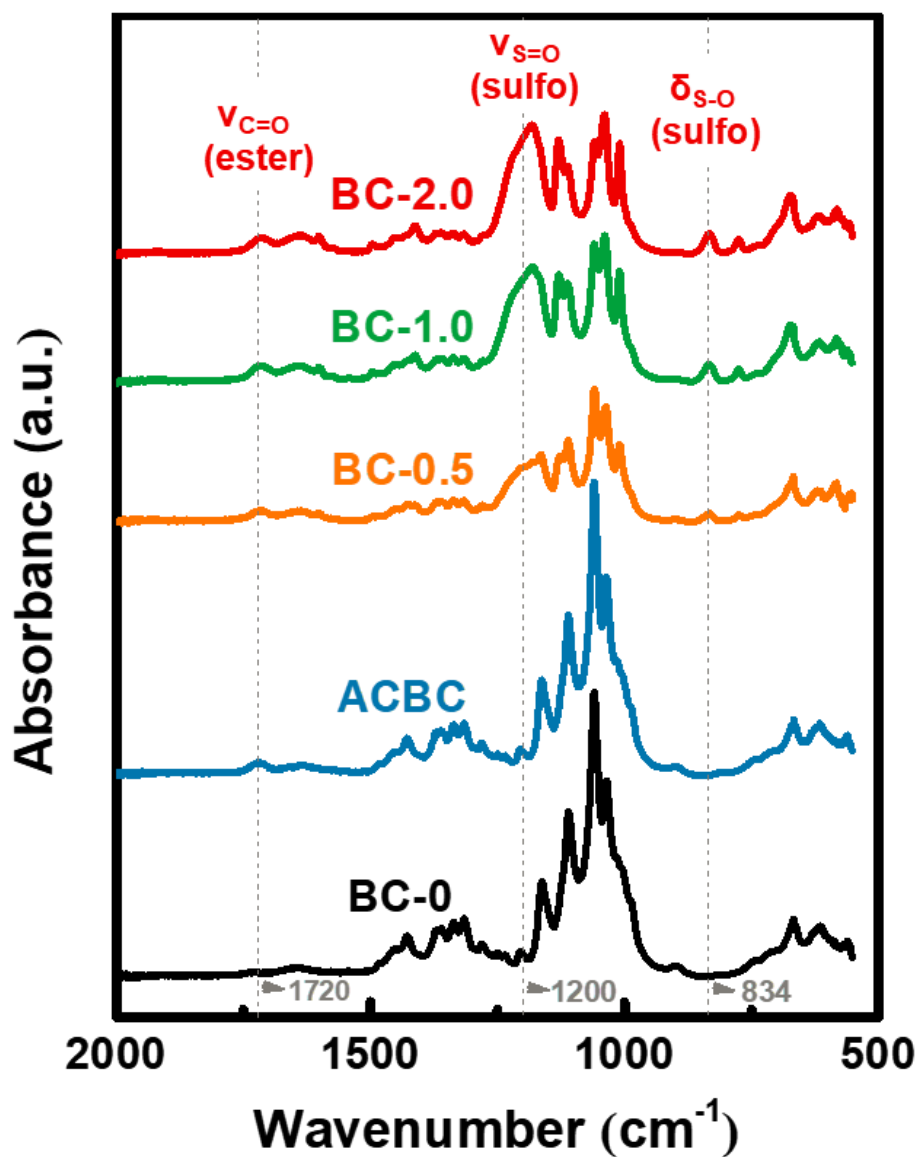


Fig. S2 ATR-FTIR spectra of BC-0, ACBC and BC-g-PSS gels.

Comparing the spectrum of AC-modified BC (ACBC) with BC-0, a new peak at 1720 cm^{-1} was observed and assigned to C=O stretching in acrylate groups, confirmed the successful reaction of BC with AC. Concerning BC-g-PSS samples, the spectrum contained two new absorption peaks at 1200 and 834 cm^{-1} , as characteristic of S=O stretching vibration and S-O bending vibration in sulfonic acid groups of the grafted PSS. Moreover, the characteristic peaks of PSS enhanced with the increase of monomer concentration in preparation.

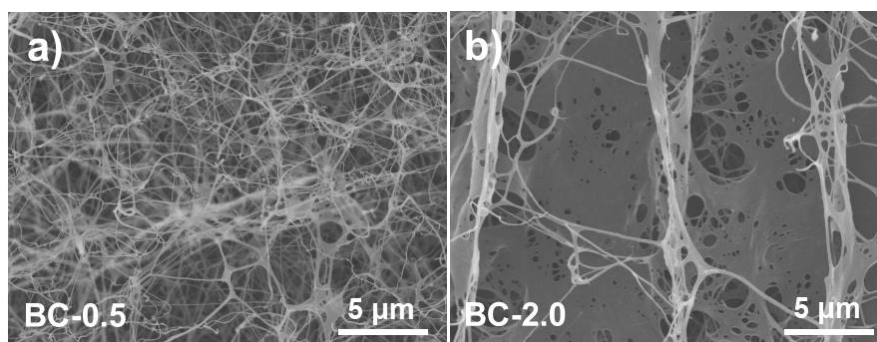


Fig. S3 SEM images of freeze-dried (a) BC-0.5 and (b) BC-2.0 at 3000 \times magnification.

The coverage of PSS on BC nanofibers in the micrograph of BC-0.5 could be observed in Fig. S3a, while most areas still appear very similar to the pristine BC. A more compact composite structure of BC with higher PSS content was obtained in BC-2.0 (Fig. S3b), the grafted PSS almost completely bound the adjacent cellulose nanofibers and filled the voids in BC-2.0, making the pores much less and more inhomogeneous.

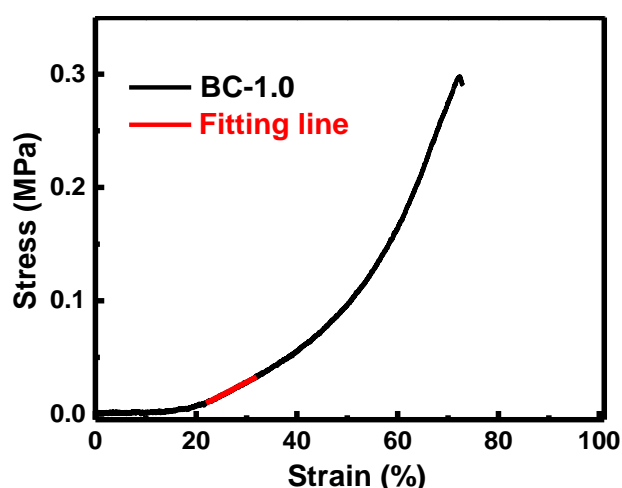


Fig. S4 Schematic diagram of compression modulus estimation.

The compression stress-strain curves of BC gels always showed a low modulus plateau (the modulus stayed constantly under 1 Pa) in the low strain region. Therefore, the compression strain at 0.01 MPa stress ($\epsilon_{0.01}$) was recorded and regarded as the end of soft state and the start of linear elastic deformation region in respective compression stress-strain curve. The compression modulus was determined as the slope of the linear elastic deformation region from $\epsilon_{0.01}$ to $\epsilon_{0.01} + 10\%$. In Fig. S4, BC-1.0 was taken as an example to show the compression modulus estimation method. For some soft gels, no valid compression modulus could be obtained.

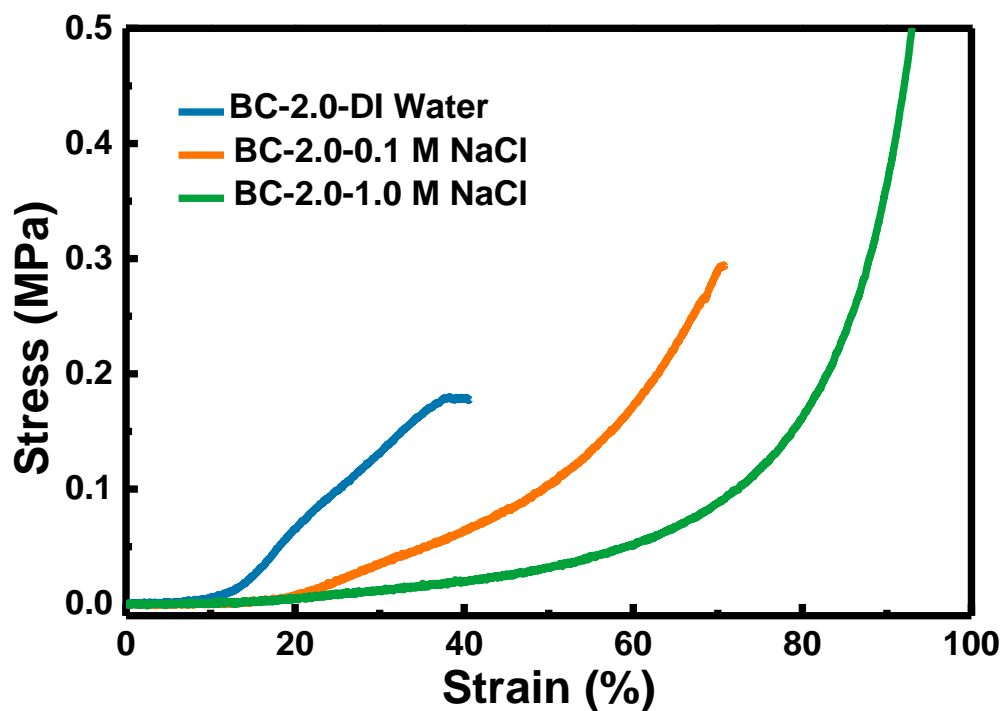


Fig. S5 Ionic strength-responsiveness of BC-2.0 gels in compression property.

The ionic strength-responsiveness of BC-2.0 in compression property is shown in Fig. S5 and Table S2. As same as BC-1.0, BC-2.0 softened by increasing ionic strength without obvious volumetric change. But BC-2.0 still kept a relative high compression modulus of 0.7 MPa in 1 M NaCl solution, even the osmotic pressure in BC-2.0 decreased to a low level. This was because the abundant PSS in BC-2.0 gels bound the nanofibers together and filled the inherent voids and gaps in BC network, eventually formed a mechanically strong network in BC-2.0 gels. Thus, the stiffness change of BC-2.0 gels in responding to ionic strength was not as significant as BC-1.0 showed.

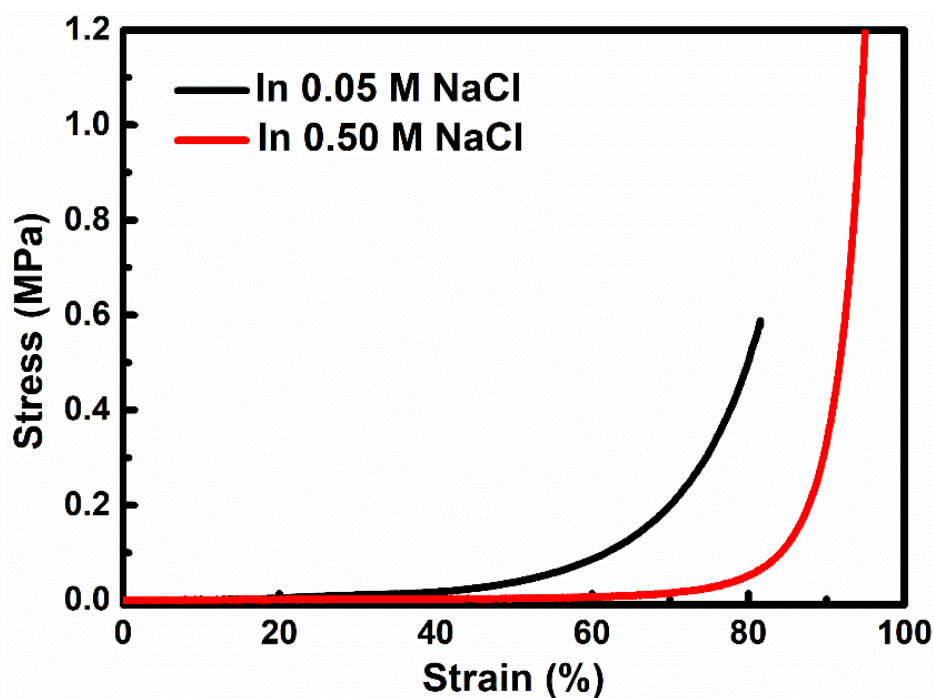


Fig. S6 Compression stress-strain curves of BC-1.0 gels in 0.05 and 0.50 M NaCl solutions.

Table S2 Properties of BC gels in DI water and in different ionic strength solutions.

| Samples | Compression modulus (kPa) | Fractured strain (%) | Compression strength (MPa) | Strain at 0.01 MPa stress (%) | Volumetric shrinkage ratio (%) |
|--------------------|---------------------------|----------------------|----------------------------|-------------------------------|--------------------------------|
| BC-2.0-0.10 M NaCl | 2.9 ± 0.3 | 70.4 ± 3.1 | 0.3 | 21.5 ± 1.8 | 3.3 ± 0.4 |
| BC-2.0-1.00 M NaCl | 0.7 ± 0.1 | - | - | 28.7 ± 2.1 | 15.3 ± 1.2 |
| BC-1.0-0.05 M NaCl | 1.6 ± 0.1 | 75.6 ± 6.4 | 0.6 ± 0.1 | 34.8 ± 4.8 | 1.3 ± 0.2 |
| BC-1.0-0.50 M NaCl | - | - | - | 65.4 ± 4.6 | 12.9 ± 0.9 |

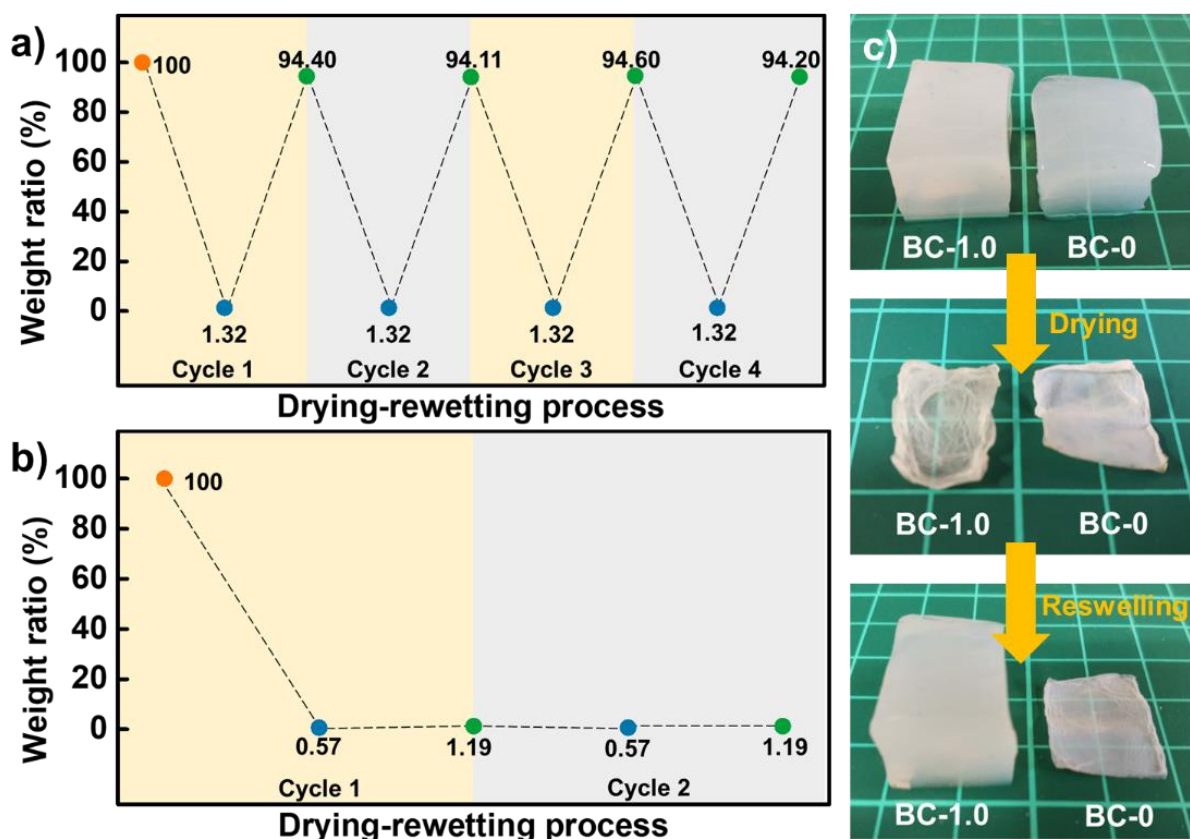


Fig. S7 Current weight ratios of BC-1.0 (a) and BC-0 (b) in cyclic drying-reswelling. (c) the original, dried and reswollen states of BC-1.0 and BC-0.

As shown in Fig. S7a, after a completely drying-reswelling cycle, BC-1.0 could reswell to over 94 % of its original weight. Moreover, this reswelling behavior was not limited to a single drying-swelling cycle, but was stable in several cycles. By contrast, no significant reswelling was observed after immersing the dried BC-0 in water (Fig. S7b). The tremendous difference between the drying-reswelling behaviors of BC-1.0 and BC-0 was shown in Fig. S7c. After drying, BC-1.0 and BC-0 gel both uniaxially shrank into a thin film, due to the anisotropic structure of the internal cellulose network. Then, the two films were immersed in abundant water for 4 hours. After that, the BC-1.0 reswollen to the original gel state, while the BC-0 still retained the film state. Besides, the BC-1.0 gel could reswell from a completely dried state to 89.84% of its original weight in a 1 M NaCl solution.