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PAPER

Anisotropic Swelling in Hydrogels Formed by Cooperatively Aligned Megamolecules

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Sacran is a supergiant cyanobacterial polysaccharide with an extremely high absolute molecular weight that exceeds 10^7 g/mol (molecular length: over 30 μm). Sacran forms milli-scaled orientation domains in aqueous liquid crystalline (LC) state, even in trace concentrations i.e. 0.3 wt%. Aqueous sacran films that are cast from a LC state and annealed between 70-140 °C form self-standing sheets composed of oriented hydrogels. When sacran films swell, they experience changes in size that are 70 fold higher in relation to thickness than those that occur in relation to width. Either an increase in film thickness or a decrease in sacran chain length reduces swelling anisotropy, demonstrating that stress that occurs during drying can be effectively used to propagate the cooperative alignment of LC chains on a micrometer sized scale comparable with the thickness of self-standing films.

Introduction

Anisotropic gels function as soft biomaterials and have been widely studied for possible applications in biomedical,[1-3] optical,[4-6] and electronic materials science.[7-9] Anisotropy was created using various methods such as self-assembly,[10,11] directional diffusion,[12] directional freezing,[13] elongation,[14-16] and solvent-cast.[17,18] The solvent-cast method is one of the easiest methods for preparing films, and the in-plane molecular orientation of the resulting film can be driven upon solvent removal, which causes stress to the system.[17,18] When oriented films are fixed by cross-linking and films are rehydrated in solvents, oriented gels are formed. This kind of *in-situ* hydrogelation method is useful because the dried precursor film can be easily stored for a long period of time without decay and can be reconstituted in water just prior to use. To a point, thinner films more efficiently drive the polymer chain orientation,[19] but very thin films with nano-scaled thickness generally are not self-supporting and in reality cannot be used as precursors to hydrogels. Although self-supporting films are better precursors to hydrogels, a precise molecular orientation is difficult to achieve by solvent-casting. Therefore, self-supporting films with a high degree of in-plane orientation are not easily formed by simple solvent-cast methods.

We recently developed a micron-sized megamolecule, sacran (main structure; Fig. S1†), with more than 5.0 x 10^4 sugar moieties by extracting them from *Aphanothece sacrum,* a cyanobacterium.[20-26] Elemental analyses, chromatographic, and spectroscopic studies of sacran revealed the following sugar residues; Glc, Gal, Man, Fuc, Rha, Xyl, Rib, methylated hexose, uronic acids, and trace muramic acid.[20] The carboxylate composition of sacran was 11 mol %, and substitution of sacran chain sulfate groups was favored when the sulfate composition was 22 mol % to sugar residues. In aqueous solutions, sacran adopted a nematic liquid crystalline (LC) phase even when it was present at a very low concentration i.e. 0.3 wt % (Fig. S2†; nematic Schlieren texture in Fig. S1 and Fig. S6 left†).[26] The low critical LC concentration means that sacran chains are highly rigid.[21]

Herein we prepared self-supporting sheets of sacran hydrogels by a water-casting method from LC solutions, where precursor films cross-linked upon heating to a dry state displayed in-plane orientation by the cooperative alignment of sacran rigid rods. Moreover we found that molecularly-oriented hydrogels show anisotropy in water-swelling and mechanical properties, and oriented structure of water assembly in the oriented sacran networks.

Experimental

Materials

Hydrogel preparation

The 0.5% aqueous LC solution of sacran, which was purchased from Green Science Materials Inc (Kumamoto, Japan), was prepared by heating at 80 °C with agitation for 8 hrs to create a homogenous viscous solution. The solution was poured into a polypropylene case (5 cm x 5 cm x 5 cm) and was dried in an oven at 60 °C overnight to form translucent films with a thickness ranging 40-70 μm. As a result, the water content of the film was reduced down to 15 wt%, determined by thermogravimetry. The films were cut into 5 mm x 5 mm squares with a surgical knife. The squared films were thermally-

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treated at 70, 80, 90, 100, 120, and 140 °C for 2 hrs, and then immersed in water at room temperature. The hydrogels were formed within 1 min and kept for one day to be equilibrated. After the water on the surface of hydrogels was wiped away by water-absorbable paper, the hydrogels were weighed. The swelling degree, *q*, as a weight ratio of hydrogels to dry films was calculated. The averaged data of five samples were used as data. For anisotropic swelling evaluation, thickness and width of the films and hydrogels were measured by an electronic micrometer. The averaged data of linear size change along the x- and y-directions were used as width change. Again, the averaged data of five samples were used as data.

Methods

Ultrasonication

Sacran solutions (0.5 wt%, 250 ml) were kept on ice and sonicated for 0.5-30 mins, with an ultrasonic tip 13 mm diameter made of a titanium alloy (Ti-6Al-4V) controlled by a SONICS Vibra cell VCX750 ultrasonicator (Sonics & Materials Inc. USA, Frequency: 20 kHz) whose power was adjusted to 40 % of maximum (750 W). Ultrasonicated solutions rested for 10 sec every minute to prevent overheating. Following ultrasonic irradiation, sacran solutions were filtered by syringe filter with a 5 μm mesh to remove micro-scale dust particles possibly emitted from the irradiator tip.

Multi-angle static laser light scattering

In order to determine the absolute *Mw* of ultrasonicated sacran chains, multi-angle static laser light scattering (MALLS; Dawn Heleos II, Wyatt Technology) measurements were made as follows. MALLS system (Laser wavelength: 665.2 nm) was equipped with a SEC system with the SEC columns (Shodex OHpak with a dimension of 8.0 mmID x 300 mmL, SB-807G (Guard), SB-807 HQ, and SB-804 HQ) under a column temperature of 40 °C. The concentration of injected sacran solution in $NaNO_3$ aq (0.1 M) was 0.01 % (injected volume: 100 μm), and then the samples were released at a rate of 1 ml/min. The samples in a fused silica cell were detected by refractive indices detector (RI; Optilab T-rEX, Wyatt Technology, laser wavelength; 658 nm) and LLS detector (Detecter angles: 13.0°, 20.7°, 29.6°, 37.5°, 44.8°, 53.1°, 61.1°, 70.1°, 80.1°, 90.0°, 99.9°, 109.9°, 120.1°) around 25 min flowing. The d*n*/d*c* value was measured using the sacran solution with concentrations of 0.001 %, 0.0025 %, 0.005 %, 0.0075 %, 0.01 % under a large amount of $(NH_3)_2SO_4$ aq (0.01 M) using a laser (wavelength: 658 nm) at 25 °C by the RI detector (Wyatt Technology Optilab T-rEX), to be 0.108±0.004 mL/g. We used the following parameter for calculation. Water refractive index was 1.331, water viscosity at 25 °C was 0.8945, and Rayleigh ratio was 7.3239 x 10^{-7}.

Although the original sacran concentration of injected aqueous solutions was 0.01 %, the concentration might be decreased remarkably during flowing the SEC columns. When the sacran chains were detected by RI and LLS after over 13 min flowing, the concentration was low enough presumably to regard it as a solution of an elementary sacran chain (but we cannot obtain evidence of a single chain). The molecular weight curves obtained by MALLS chromatogram are shown in Fig. S3† when the Zimm model was used. Coefficient of determination, R^2, ranged 0.979-0.998, as shown in Fig. S4†. The averaged <s> determined as z-average, R_z, ranged between 57- 641 nm (Table 2).

Differential scanning calorimetry (DSC)

Thawing of sacran aqueous solutions (1 wt%) was measured by differential scanning calorimetry (DSC6000, Seiko Instruments Inc.) at a scan ratio of 2 °C min^{-1} from - 150 °C to 20 °C under nitrogen gas. Samples (3.9 mg) were placed in aluminum pans and sealed. Samples were weighed before and after DSC measurements and no weight change was confirmed to determine that no water had vaporized.

Thermogravimetry (TGA)

Water content of the dried film was measured by thermogravimetry (STA7000, Hitachi High Tech Science Coop.). A specimen of film (4 mg) was heated from 25 to 175 °C at a rate of 2 °C/min under a nitrogen atmosphere. Weight loss around 150 °C was regarded as water content due to the plateau appearance of the curve.

Crossed-polarizing microscopy

Microscopic observations were made by a microscope (BX51, Olympus) equipped with CCD camera (DP80, Olympus). A specimen of sacran hydrogels were cut to size for microscopic observation (ca. 5mm x 0.5mm x 1mm) and put on the glass plates at 25 °C. A first order retardation plate (530 nm) was put onto the light path.

Scanning electron microscopy (SEM)

SEM observation of freeze-dried hydrogels was made by SEM system (Hitachi, S-4500) under an acceleration voltage of 0.5 kV without any sample modification.

Mechanical properties

The sacran hydrogels were mechanically tested in compression and tensile mode. A probe for compression or tensile test was set up on an Instron 3365 machine using a 5 kN load cell with a crosshead speed of 1.00 mm/min. Elastic moduli, *E*, of the hydrogels were calculated using the initial inclinations of the stress-strain curves.

Wide-angle X-ray diffraction (WAXD) imaging

WAXD patterns were measured using a graphite monochromatized CuKα radiation beam focused via a 0.5 mm pinhole collimator with a flat 20 x 20 cm^2 imaging plate (IP) detector of 1900 x 1900 pixels (Rigaku, R-AXIS IIc). A small piece of the sample with edge sizes less than 1 mm was mounted with the sample-IP distance of 10 cm. The exposure time was set to 3 minutes for each shot in a geometrical condition by directing the X-ray beam in front of samples. The orientation degrees, *f,* were calculated by Hermann's orientation function using the azimuthal angle, φ ,

$$f = \frac{3\langle \cos^2 \varphi \rangle - 1}{2} \qquad (1)$$

where;

$$\langle \cos^2 \varphi \rangle = \frac{\int_0^{2\pi} I(\varphi) \cos^2 \varphi \sin \varphi \, d\varphi}{\int_0^{2\pi} I(\varphi) \sin \varphi \, d\varphi}$$

Results and discussion

1. Gel formation

We previously demonstrated that sacran chains[21] are extremely rigid as described in the Introduction, but we were concerned that the high rigidity would make it difficult to hydrate sacran chains and they would simply disperse in solution. A 0.5 wt % rehydrated sacran solution was extremely viscous with a 1.1 x 10^5 cps; however, if sacran chains had not dissolve during rehydration but remained suspended in solution, the viscosity, η, would have been 6.8 x 10^2 cps as calculated by Guth and Gold equations for determining viscosity in suspensions containing anisotropic micron-size particles; [27]

$$\eta = \eta_0\left[1 + 0.67\alpha\phi + 1.62(\alpha\phi)^2\right] \quad (2)$$

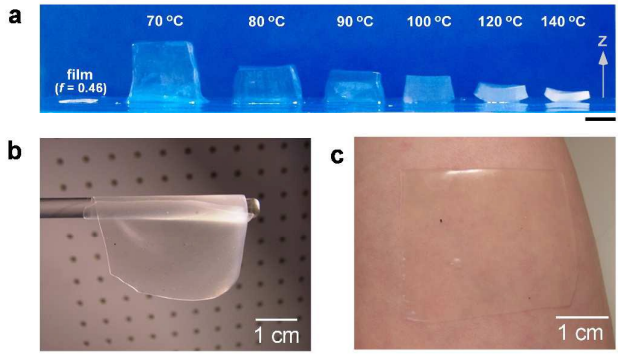

Fig.1 Photos of sacran film and hydrogels. a) Sacran film (left) and hydrogels swelling in water after the film was annealed at various temperatures (right). Scale bar: 5 mm b) Hydrogel sheet is tough enough to deform and translucent enough to scatter the white light irradiated from right. c) Hydrogel sheet put on the arm.

Table 1. Swelling and mechanical behaviors of sacran hydrogels[a]

Temp. [°C]	q [b] [g/g]	q_z/q_{xy} [b,c]	E [kPa] zx plane	E [kPa] xy plane	Anisotropy of E	σ [kPa]	ρ [mol/m^3] zx plane	ρ [mol/m^3] xy plane	Anisotropy of ρ
70	370 (±8)	69 (±4)	0.872	0.202	4.32	-	2.4	0.55	4
80	173 (±2)	51 (±2)	2.94	1.21	2.43	-	6.2	2.6	2
90	95 (±2)	38 (±2)	14.4	7.57	1.9	-	25	13	2
100	64 (±1)	33 (±1)	26.2, 22.8[d]	4.48	5.85, 5.08[d]	40[d]	40, 34[d]	6.8	6, 5[d]
120	24 (±0.8)	17 (±1)	148[d]	70	2.11[d]	153[d]	160[d]	76	2[d]
140	18 (±0.5)	13 (±0.2)	2900[d]	490	5.92[d]	503[d]	2900[d]	480	6[d]

[a] Sacran hydrogels were prepared by in-situ gelation of the water-cast films treated at temperatures shown in the 1st column. [b] The values in parentheses are standard errors estimated from five data. [c] q_z/q_{xy} refers to the anisotropy of swelling meaning the ratio of linear swelling degree of thickness direction, q_z, to that of width, q_{xy}. [d]The data were obtained as a result of elongation tests. Other mechanical data were obtained as a result of compression tests, as shown in Figure S6.

where η and η_0 are the viscosities of the suspension and dispersion medium (water, η_0 = 0.89 cps), respectively and ϕ is the volume fraction of the disperse phase (sacran) with a concentration of 0.5 wt %, which is approximately $\phi = 5 \times 10^{-3}$ based on assumptions for the suspension density, d = 1.0 g/ml. The aspect ratio, α, is the ratio of chain length to width of the dispersing sacran chains, which is approximately α = 4300 because the sacran chain length was estimated to be 8.6 x 10^4 nm {the number of sugar residues (size 0.5 nm) = 1.7 x 10^5} and the width of sacran associates was previously estimated to be 20 nm by electron microscopy.[21] By substituting these values, η_0= 0.89 cps, ϕ = 5x10^{-3}, and α = 4300 into the above equation, the viscosity was determined to be 6.8 x 10^2 cps. This calculated result demonstrates that sacran chains dissolved in water and interacted with water molecules, which was confirmed by the increase in viscosity of the solution. In fact, precipitates were not observed in the sacran solution, even after they were left standing for one year. The resulting good hydration of sacran chains enabled us to make homogeneous water-cast films.

Sacran solutions were cast on polypropylene plates at 60 °C in an oven to form dried films with thicknesses ranging 40-70 μm. Films were cut with a surgical knife into 5.0 x 5.0 mm^2 squares. Square films were heated at 70, 80, 90, 100, 120, and 140 °C and thermally-treated for 2 hrs. Weighed films were immersed in water and rehydrated to a macroscopic gel within 1 min. Hydrogels immersed in water for a day were in an almost equilibrated swollen state, and they never dissolved in water even after more than one

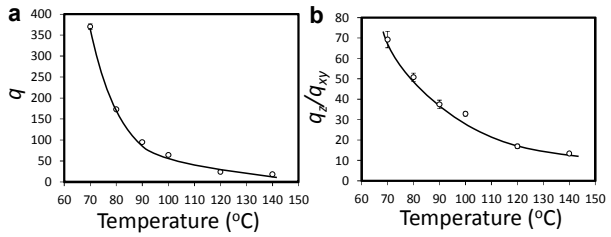

Fig. 2 Plots of hydrogel properties against annealing temperature of precursor films. a) Swelling degree, q. b) swelling Anisotropy, q_z/q_{xy}

week of immersion (hydrogel photos: Fig. 1a). On the other hand, films dried at 60 °C did not form hydrogels, but dissolved in water, suggesting that films annealed at 70 °C and higher via *in-situ* hydrogelation. Once hydrogels were weighed, we calculated the degree of swelling, q, as a weight ratio:

$$q = w_{gel}/w_{film}, \quad (3)$$

where w_{gel} and w_{film} are the weights of the hydrogels and dry films, respectively. The results are summarized in Table 1. The q values ranged from 13 to 273 g/g, meaning that the water content of the hydrogels ranged from 92.3-99.6 wt %. The annealing temperature is very effective in controlling gel volumes as shown in Fig. 1a, which illustrates that smaller gels are formed under higher annealing temperatures. It should be emphasized that standard errors of q calculated using standard deviations of 5 gel specimens were within 3.5 % which is much lower than errors for q values of any other hydrogel reported thus far.[28] This allows for the precise control of q by changing the film-annealing temperature, suggesting that the number of crosslinking junctions efficiently increased by annealing. A plot of q against the annealing temperature is shown in Fig. 2a. The plot shows that q is more sensitive to temperatures below 100 °C. We can speculate that the cross-linking mechanism is related to the vaporization of water remaining in sacran films. Water bound to sacran was estimated by DSC. A 1 wt % aqueous solution was heated at a rate of 2 °C min^{-1} from -150 °C to 20 °C. The sacran solution showed a peak when ice was melting around -1 °C with an enthalpy of 312.0 Jg^{-1} while pure water showed an enthalpy of 333.4 Jg^{-1}, meaning that 6.4 wt% of all the water was not frozen. Namely, 0.248 mg of water was bound to 0.039 mg of sacran, meaning a 6.4 fold weight ratio of water bound to sacran. Therefore sacran binds water in a way that is similar to that of other polysaccharides.[29] Once water is removed by heating, sacran chains can form multiple hydrogen bonds with one another, which leads to the physical cross-linking of the chains. If a few ester bonds of uronic carboxylic acid with hydroxyls are formed, these covalent bonds should work as chemical cross-linking points. We confirmed the stability of hydrogels derived from films pre-treated at 140 °C; hydrogels were kept at 80 °C for 48 hrs but the degree of swelling showed a small change (from 13 to 20 g/g) as shown in Fig. S5†, suggesting the possible contribution of covalent bonding to the cross-linking. We then tried to prepare hydrogels of other anionic polysaccharides such as xanthan gum (*MW*: 4.7 x10^6 g/mol), sodium alginate (*MW*: 1.5 x 10^5 g/mol), and hyaluronic acid (*MW*: 1.2 x10^6 g/mol), but only xanthan gum formed hydrogels (q ranging from 82 to 1220) at high annealing temperatures (120 and 140 °C). Out of

these polysaccharides, only sacran and xanthan gum demonstrated a LC phase, which might have an effect on hydrogel formation.

2. Anisotropic swelling

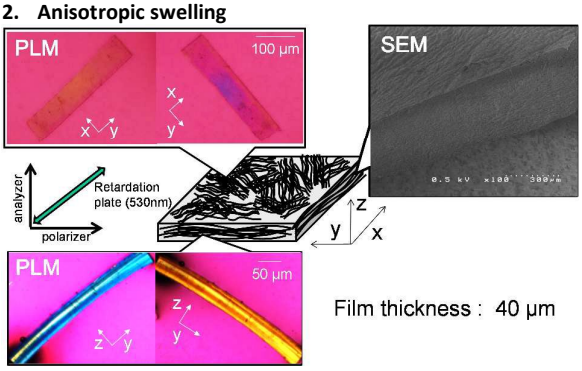

Fig. 3 Sacran film with in-plane orientation. Inset four pictures at left (upper: xy-surface, lower: zy-surface) are crossed polarizing microscopic (PLM) images under first-order retardation plate (530 nm), while inset picture at right is SEM image of freeze-dried film edge (xy-surface).

Table 2. Effects of ultrasonication on sacran molecular weight and gelation

Irradiation time[a] (min)	0 (0 kJ)	0.5 (650 kJ)	1 (1.5 MJ)	3 (4.3 MJ)	5 (6.8 MJ)	8 (10 MJ)	10 (13 MJ)	30 (42 MJ)
M_w[b] (x10^6 g/mol)	28.4 (34 %)	5.17 (9.8 %)	4.81 (7.8 %)	4.04 (2.9 %)	2.48 (2.0 %)	2.46 (1.4 %)	2.36 (1.2 %)	1.40 (1.0 %)
R_z[b] (nm)	641 (1.2%)	228 (2.6%)	166 (3.0%)	111 (2.1%)	110 (1.6%)	95 (0.7%)	64 (1.2%)	57 (1.2%)
q[c] (q_z/q_{xy})	64 (33)	62 (15)	69 (5.5)	NG	NG	NG	NG	NG

[a] Ultrasonic irradiation for sacran solution with a concentration of 0.5 wt% was performed by a titanium alloy irradiation tip. Values in parentheses are irradiation energy which was simultaneously detected by irradiation machine. [b] Weight-average molecular weight, M_w, and z-average, R_z, were determined by a SEC-MALLS system. Values in parentheses were errors. [c] Swelling degrees, q, of hydrogels prepared from the films annealed at 100 °C for 2 hrs were estimated as a weight ratio of water swellon gels to the dried films. Values in parentheses were anisotropy of swelling. NG means "not gelated" in this condition.

Fig. 1a reveals that the film face did not change much in size when compared with the above mentioned q value changes; this demonstrates anisotropic swelling in water. We quantified the anisotropy of swelling, q_z/q_{xy}, by estimating the ratio of the linear swelling degree in the z-direction, q_z, (thickness change) to the linear swelling degree in the xy-direction, q_{xy} (width change defined in Fig. 3). The degree of linear swelling was calculated as L_g/L_f, where L_g is the hydrogel length (thickness or width) and L_f is the precursor film length. The anisotropy value of q_z/q_{xy} was around 70 for the gel derived from the film annealed at 70 °C, meaning that the film swelled 70 fold more in the z-direction than in the xy-direction. The anisotropy gradually decreased by increasing the annealing temperature of the film (Fig. 2b), similar to q behaviour, but remained at more than 10 regardless of the temperature. The behaviour similarity of anisotropy to q suggests that water swelling was responsible for almost all of the thickness increase. Actually, the linear degree of swelling in the xy-direction ranged from 1.01 to 1.79 while that in the z-direction varied widely from 13.6 to 120 depending on the annealing temperature of the film (Table S1†).

As shown in the picture in Fig. 1b, hydrogels were not transparent and scattered white light. From this observation, we concluded that liquid crystalline domain boundary was maintained in hydrogels where the sacran content ranged between 0.3 and 7.1 wt%, which was within the LC concentration range of sacran solutions. Fig. 3 shows a microscopic photo of sacran hydrogels derived from films pre-treated by annealing at 140 °C, taken under a cross-nicol polarimetry using a first-order retardation plate (λ = 530 nm) inserted in the direction of the upper right to the lower left in the light path as shown in Fig. 3. The polarized microscopic observation of the hydrogel's zy-surface reveals a clear orientation.

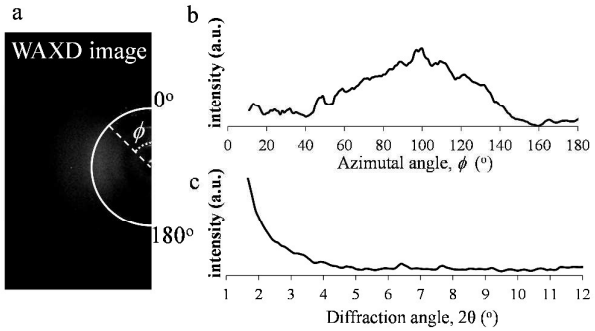

Fig. 4 X-ray diffraction of the sacran dry film. a) Wide-angle X-ray diffraction image, b) Intensity diagram scanned azimutally around 2θ = 20 ° to draw diagram, c) Intensity diagram in a smaller 2θ angle range from 1 to 12°.

The hydrogel birefringence is positive, as evidenced by both subtractive birefringence (blue colour) in the hydrogel lying from the upper right to the lower left and additive birefringence (orange colour) in the hydrogel lying from the upper left to the lower right. Observations of zx-surfaces also showed positive birefringence (data not shown). The positive birefringence strongly suggests that sacran chain backbones lie along x- and y-axes. On the other hand, the xy-surface observation does not show such an orientation. Thus, microscopic images revealed that hydrogels oriented in plane, as schematically shown in the middle illustration of Fig. 3. WAXD studies of hydrogels did not detect evidence of molecular orientation presumably due to a high water content (more than 93 wt % to total weight of the hydrogels). However WAXD images of films revealed a molecular orientation showing an arc of a broad halo as a result of X-ray irradiation to zx-edge surfaces of the films (Fig. 4a) and no smaller diffraction appeared suggesting no smectic layer was formed (Fig. 4c). Since WAXD studies of xy-surfaces of the films showed no orientation, the films oriented in-plane. From the azimuthal scan (Fig. 4b), we calculated an orientation factor, f=0.46, which is in the range of a general orientation factor (f = 0.4-0.7) of nematic LC.[30]

The mechanism of formation for in-plane orientation structure of the hydrogel is illustrated in Fig. 5a. In a sacran solution with a concentration of 0.5 wt %, sacran chains, whose maximum length was estimated to be more than 30 μm based on the molecular weight, were in the LC state and formed self-oriented domains based on the excluded volume theory.[31] Before drying, several domains in sacran solution with a concentration of 0.5 wt% were observed on the quartz-solution interfaces at the wall and the bottom (Fig. S6 left†). The domain sizes of such big molecules ranged over millimeter scale as demonstrated previously[25]. The solution was placed in an oven at 60 ˚C under atmospheric pressure to evaporate water gradually. Just before the drying was completed, the transmitted light intensity increased in the whole liquid phase, and the orientation domains apparently integrated in an in-plane orientation state where molecules were parallel to the gas-liquid interfaces and solid-liquid interface on the bottom (Fig. S6 right†). The in-plane orientation domain was very thick around 4 mm, although the alignment is disordered around the side walls. Finally, almost all chains became oriented along the substrate because of the tendency of LC chains to form in-plane oriented films with a thickness of around 40 μm due to the cooperative orientation effect. SEM images of freeze-dried

hydrogel edges are shown in a photo inset on the right side of Fig. 3. The zx-surface shows a texture almost pointing in the x-direction and its roughness seems lower than that of the xy-surface, suggesting in-plane orientation.

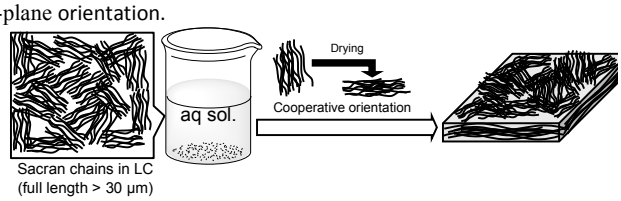

Fig. 5 Orienting behaviour of sacran chains by drying process to form a film with a microscale thickness.

3. Size effects

In order to support the prior discussion on in-plane orientation, sacran chains were disrupted by ultrasonication and an attempt was made to form hydrogels from films by a similar *in-situ* hydrogelation method. Sacran solutions (0.5 wt%) were ultrasonically irradiated for different periods of time, and SEC-MALLS measurements of the resulting sacran were performed in order to determine if ultrasonication had an effect on the molecular weight of the sacran chains. As shown in Table 2, an increase in irradiation time or energy efficiently reduced M_w values of the sacran chains to 1.4 x 10^6 g/mol. R_z values corresponding to radii of gyration also decreased monotonously by ultrasonication, suggesting that the sacran chains were disrupted. After 0.5 and 1.0 minutes of ultrasonication (corresponding to 650 kJ and 1500 kJ, respectively), sacran molecular weights became 5.5 and 5.8 fold shorter than their original length (M_w: 5.17 and 4.81 x 10^6 g/mol) and their lengths at the fully-extended state were around 14 and 13 μm, respectively. The sonicated sacran still formed hydrogels from their films (annealed at 100 °C for 2 h) but, as expected, they showed less anisotropy (q_z/q_{xy}, of 15 and 5.5, respectively) than non-sonicated gels (q_z/q_{xy} = 33). The q_z/q_{xy} reduction by sonication was derived from the increase in the degree of linear swelling in the x- or y-direction, from 1.18 for the non-sonicated sample to 1.43 for sacran sonicated for 0.5 min and to 3.45 for sacran sonicated for 1.0 min. The q values were unaffected by ultrasonication, meaning that the efficiency of formation of cross-linking points during film annealing did not depend on molecular weight in this range. On the other hand, hydrogels did not form in instances where the ultrasonication time was 3 min or longer. A clear reason was not found but a shoulder around 15 min appeared on the RI chromatogram of a sample irradiated for 3 min (Fig.S3†), which suggests that the pattern of chain disruption might differ under these conditions. Contact points between sacran chains can be disrupted after 3 min of ultrasonication. As for xanthan gum which has a similar molecular weight as sacran, when it was sonicated for 1 min, a hydrogel did not form with an annealing temperature of 100 °C for the precursor film but a very soft hydrogel with a q of 1220 (standard deviation, SD: ±350) and q_z/q_{xy} of 82 (SD: ±20) was formed at an annealing temperature of 120 °C. The high anisotropy is derived from the high q. However this gel was actually not self-supporting, making further research difficult. A tougher hydrogel of xanthan gum with a q of 52 (SD: ±5) and q_z/q_{xy} of 54 (SD: ±5) was formed at an annealing temperature of 140 °C, to give rise to anisotropic hydrogels.

The molecular size effects on anisotropization were also investigated by using thicker sacran films with thicknesses of 50, 60, and 70 μm. These films also formed hydrogels but with q_z/q_{xy} values ranging from 23 to 29, which were lower than those of hydrogels from films of 40 μm thickness although q was unaffected by changes in thickness (Table S2†). These additional experiments led us to hypothesize that supergiant sacran chains with lengths of more than several tens of micrometers can form in-plane orientation films that can be prepared as a result of the cooperative effect; rigid sacran chains synchronously align along the substrate surface, showing remarkable anisotropy after successive swelling in aqueous solvents.

4. Mechanical property and structures

If networks of sacran chains are oriented in hydrogels, some properties should be anisotropic. We conducted mechanical compression tests on the hydrogels in the x- or y-direction. We were unable to measure the mechanical stress at breakage because the hydrogels became too thin at the breaking point to properly evaluate their strength (Fig.S7). On the other hand, the mechanical modulus, E, was easily evaluated from the initial slope of the stress-strain curves. The results are summarized in Table 1. E values of the compression tests on the xy-surfaces increased from 0.2 to 490 kPa with an increase in the annealing temperature of the precursor films, suggesting that the hydrogel hardness can be controlled by varying the annealing temperature.

Next, we conducted mechanical tests of the zx-surfaces. Although compressive mechanical moduli were obtained for hydrogels from films pre-treated at 70, 80, 90, and 100 °C, it was impossible to conduct compression tests on hydrogels from films pre-treated at 120 and 140 °C because they were too thin. We then tested hydrogels from films pre-treated at 100, 120 and 140 °C in a tensile mode but were unable to test samples at 70 and 80 °C since their edges were too soft to pinch. Mechanical strength at breakage was measured in the tensile mode and the results are summarized in Table 1. One can easily confirm that the mechanical moduli of hydrogels from films annealed at 100 °C in both the compression and tensile modes were comparable. Next, the mechanical moduli were directly compared for all hydrogels and it was found that the mechanical moduli for zx-surfaces were higher than those for xy-surfaces. The ratio of the zx-surface to xy-surface mechanical moduli ranged from 2.1 to 5.9. This result suggests that the anisotropic network structure, which was confirmed by birefringence of the hydrogels, was effective in the mechanical moduli in spite of the presence of many amorphous water molecules. To evaluate the structure of the polymer network, the cross-linking density, ρ, was estimated by the following equation: [32]

$$\rho = E / RT\, q^{-1/3} \qquad (3)$$

where R and T are the gas constant and absolute temperature, respectively. The results are summarized in Table 1. The ρ values calculated using E in either the xy or zy planes decreased with an increase in the annealing temperature, indicating the degree of cross-linking increased as the temperature increased. Since E was used instead of the storage moduli, G', the absolute values of ρ are not exact. However the comparison of ρ values for two different planes was important to evaluate the structure of oriented hydrogels. The results show the network density along the z-direction (thickness) is lower than that along the x- or y-direction (width).

Finally, we discuss the actual use of anisotropic hydrogels, which showed mechanical strength of around 500 kPa for hydrogels from films annealed at 140 °C. The strength is sufficient so that the hydrogel can behave as a sheet, as shown in Fig. 1c. We anticipate that sacran hydrogels can be effectively applied as easily-handled films. Previously it was found that sacran solutions demonstrated

anti-inflammatory effects in an allergic mouse model, comparable to steroidal drugs such as hydrocortisone, and demonstrated anti-inflammatory efficacy for dry skin in human volunteers.[33] Hydrogel sheets could thus be useful as topical agents applied directly to the damaged skin of patients, but chemical gels including un-reacted cross-linkers are possibly not suited to this type of application. However physical sacran gels devoid of additives (or other irritants) such as chemical cross-linkers or metal ions have not yet been developed.

Conclusions

In-plane oriented films with a mean thickness of 40 μm were obtained when LC aqueous solutions of *Aphanothece sacrum* polysaccharides, sacran, which formed an LC orientation domains with a millimetre scale size at an extremely low concentration of 0.3 wt %, were air-dried at a temperature of 60 ^{o}C. The degree of orientation of films was evaluated as f=0.46 by WAXD imaging, which was comparable with the general value for nematic solutions. Films annealed at temperatures ranging from 70 to 140 ^{o}C, thus demonstrating *in-situ* hydrogelation to form anisotropic hydrogels; films swelled 70 fold at maximum more in thickness than in width. Additional treatment by sonication of sacran decreased the molecular weight to around 1.4 x 10^5 g/mol, inducing less anisotropy in swelling. Similarly, the swelling anisotropies of hydrogels from thicker films in the 50-70 micrometer range which was presumably longer than the length of sacran chains were decreased. We theorized that such in-plane orientation of films with microscale thickness was effected by synchronous alignment, of microscale LC rigid chains onto substrates propagated by drying stress. Anisotropically swollen hydrogels were composed of oriented network structures confirmed by crossed polarizing microscopy and an estimation of the molecular weight between cross-linking points was done by using the degree of swelling and mechanical moduli.

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Table of Contents

A cyanobacterial polysaccharide, sacran, which is a supergiant rod with an extremely high molecular length over 30 μm, form in-plane oriented film by casting. The film showed auto-gelation to create self-standing and uniaxially-swelling hydrogels with a micrometer-scale thickness.