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High Tensile Strength Regenerated α-1,3-Glucan Fiber and Crystal Transition

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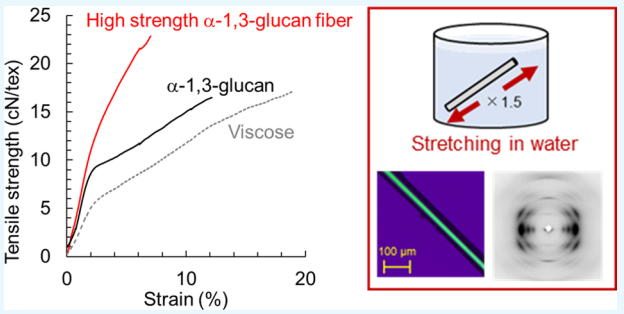
ABSTRACT: α-1,3-Glucan is a linear and crystalline polysaccharide which is synthesized by *in vitro* enzymatic polymerization from sucrose. A previous study reported that regenerated fibers of α-1,3-glucan were prepared using a wet-spinning method. However, the mechanical properties were poorer than cellulose regenerated fibers. Then, in this study, the mechanical properties of the regenerated α-1,3-glucan fiber were improved by the transformation of the crystal structure and stretching. The regenerated fiber stretched in water and dehydrated by heating showed high tensile strength (18 cN/tex) that is comparable with that of viscose rayon. Moreover, the crystal structures of the regenerated fibers were investigated using wide-angle X-ray diffraction (WAXD). To date, four crystal polymorphs of α-1,3-glucan from polymorph I to IV have been reported. This study revealed that the regenerated α-1,3-glucan fibers had two different polymorphs, polymorph II (hydrated form) and polymorph III (anhydrous form), depending on post-treatment methods of stretching and annealing procedures. Furthermore, the obtained distinctive 2D-WAXD patterns suggested that polymorph III is identical to polymorph IV.



1. INTRODUCTION

Fibers are indispensable for various applications including clothing, medical supplies, and marine products because they are strong and have a large surface area. Many fibers are produced from synthetic polymers such as nylon and polyester, which are inexpensive and strong.[1,2] However, the production of synthetic fibers from petroleum resources is problematic because it involves the depletion of fossil resources and contributes to global warming. Therefore, to overcome these environmental problems, fibers produced from sustainable biomass resources have been offered as alternatives.

Polysaccharides are representative materials of biomass resources.[3] They are produced by plants and microorganisms and have various forms depending on their constituent monosaccharides and linkage positions. For instance, potatoes contain starch (α-1,4- and α-1,6-glucan),[4] the cell walls of plants contain cellulose (β-1,4-glucan),[5,6] and species of the genus *Euglena* produce paramylon (β-1,3-glucan).[7] Recently, our group succeeded in the *in vitro* enzymatic polymerization of completely linear and pure α-1,3-glucan comprising only glucose units bound through α-1,3-glycosidic linkages.[8] Pure α-1,3-glucan can be obtained from sucrose in one-pot under mild conditions without any toxic reagents, and the molecular weights can be adjusted by controlling conditions such as the reaction time and temperature. This enzymatic polymerized α-1,3-glucan is highly crystalline.[9]

Again, there are many types of polysaccharides, and each has different physical characteristics depending on the linkage positions.[6] Therefore, the polysaccharides constitute a promising resource for biomass-based fibers. However, there is one significant problem with processing the polysaccharides into fibers: they comprise numerous hydroxyl groups and have strong inter- and intramolecular hydrogen bonds. Therefore, they have little thermoplasticity and cannot be molded by heat-melting methods such as melt-spinning, but they can be useful thermoplastics by esterification. For example, paramylon esters can be melt-spun,[10] dextran esters can be used as hot-melt adhesives,[11] and nigeran (α-1,3-*alt*-α-1,4-glucan) esters can be used as melt-press films.[12] However, the biodegradability of polysaccharide ester derivatives decreases as the degree of substitution increases.[13,14] This decrease in biodegradability is undesirable because it exacerbates environmental pollution from microplastics.

Wet-spinning is a traditional method to process the chemically unmodified polysaccharides. In this method, the polysaccharide is solubilized in an aqueous alkaline solution, either by temporary derivatization or complex formation.[15] The resulting solution is extruded, drawn, and coagulated in an

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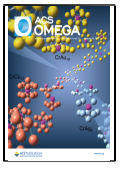

aqueous acidic solution, and then, the polysaccharide is regenerated and processed into fibers. During the regeneration process, the chemical structure of the polysaccharide returns to its original state, and thus, the regenerated fiber retains its inherent biodegradability[14] and biocompatibility.[16]

Conventionally, regenerated cellulose fibers have been commercially produced as viscose, cuprammonium rayon, and lyocell by using various types of cellulose−solvent systems. Notably, the crystal structures and the properties of these regenerated celluloses can be changed from those of natural fibers such as cotton.[2,17] However, the commercial utilization and research for regenerated fibers have been limited mainly to cellulose, despite the wide variety of polysaccharides.

Recently, our group has focused on the regeneration fibers of the other polysaccharides than cellulose and succeeded in wet-spinning of curdlan (β-1,3-glucan) fibers using an ionic liquid system.[18] The resulting regenerated fibers of curdlan showed higher water absorption (∼86%) and elongation capabilities (20−50%) than conventional regenerated cellulose fibers. Moreover, there is some research about α-1,3-glucan regenerated fibers via the viscose method.[19,20] Although they show high tensile strength 17−23 cN/tex, the viscose method required harmful reagents such as carbon disulfide. Then, our group developed the wet-spinning system of α-1,3-glucan using an 8 wt % lithium chloride (LiCl)/*N*,*N*-dimethylacetamide (DMAc) solution.[21] The mechanical properties and crystal structures of the regenerated α-1,3-glucan fibers were found to depend on the type of used coagulation baths: a fiber coagulated in a water bath has a hydrated crystal form and fragile mechanical properties, whereas a fiber coagulated in an ethanol bath has an anhydrous form and a higher tensile strength of 11 cN/tex.[19,20] However, the maximum tensile strength of the regenerated α-1,3-glucan fiber coagulated in ethanol is approximately half that of a regenerated cellulose fiber such as viscose (22 cN/tex).

Herein, we developed a post-treatment methods to enhance the mechanical properties of regenerated α-1,3-glucan fibers spun using the LiCl/DMAc solution. Generally, it is known that the increases in the orientation of the molecular chains and crystallinity contribute to improve the mechanical strength of fibers.[22] However, the regenerated α-1,3-glucan fibers in our previous work could not be subjected to further stretching processes because of the fragility and rigidity. Then, the unique ability of the crystal transition between anhydrous and hydrated forms of α-1,3-glucan was focused on. We assumed that the molecular chains of the α-1,3-glucan could be more movable during the crystal transition induced by a solvent substitution of the anhydrous-form-fiber with water, thus enabling the further drawing process. Furthermore, the resultant hydrate crystal structure of the well-stretched fiber can also be reversible by an additional drying process. Then, in this study, based on the above hypothesis, the effects of the solvent substitution, stretching in the solvents, and drying processes on the mechanical properties of the regenerated α-1,3-glucan fibers were investigated, and we successfully developed a post-treatment process, providing a regenerated α-1,3-glucan fiber with sufficiently high mechanical strength (18 cN/tex).

2. RESULTS AND DISCUSSION

2.1. Wet-Spinning of α-1,3-Glucan and Post-treatment of the Regenerated Fibers. A 10 wt % solution of the α-1,3-glucan in 8 wt % LiCl/DMAc was wet-spun using two types of coagulation baths of EtOH and water as reported previously. It has been confirmed in the previous study that the molecular weight of the fibers was the weight-average molecular weight (M_w) = 0.8−1.0 × 10^5.[21] After the wet-spinning, Regenerated Fiber (EtOH) was immersed in water, and Regenerated Fiber (Water) was immersed in ethanol to investigate the transitions in the morphologies, crystal structures, and mechanical properties of the fibers before and after solvent substitution. Pictures of the regenerated α-1,3-glucan fibers after solvent substitution are shown in Figure 1a,b. There were no significant changes in appearance during solvent substitution. Images of the regenerated α-1,3-glucan fibers after stretching in water and air-drying or oven-drying are shown in Figure 1c,d. The fibers did not change significantly in appearance when they were stretched in water, although their diameters decreased by stretching.

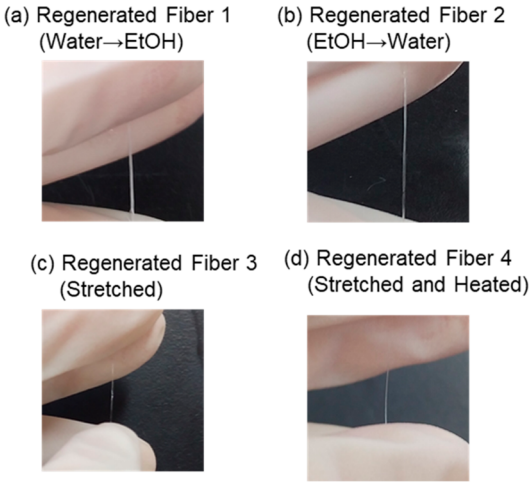

Figure 1. Pictures of regenerated α-1,3-glucan fibers: (a) Regenerated Fiber 1 (Water → EtOH), (b) Regenerated Fiber 2 (EtOH → Water), (c) Regenerated Fiber 3 (Stretched) which was dried at room temperature after stretching in water, and (d) Regenerated Fiber 4 (Stretched and Heated) which was dried at 105 °C after stretching in water.

2.2. Microscopic Observations. Optical microscope images of the four types of regenerated α-1,3-glucan fibers are shown in Figure 2. The images of the fibers after solvent substitution, i.e., Regenerated Fiber 1 (Water → EtOH) and Regenerated Fiber 2 (EtOH → Water), are shown in Figure 2a and b, respectively. There was little significant difference in transparency and appearance between Regenerated Fiber 2 (EtOH → Water) and Fiber (EtOH) which was examined in our previous study.[21] However, the diameter of Regenerated Fiber 2 (EtOH → Water) increased when it was immersed in water owing to swelling, as shown in Table 1. On the other hand, Regenerated Fiber 1 (Water → EtOH) was opaque and white (Figure 2b), whereas Regenerated Fiber (Water) was semitransparent which was examined in our previous study, but the diameter of Regenerated Fiber 1 (Water → EtOH) was not changed by immersion in EtOH.[21] Moreover, optical microscope images of the fibers after stretching in water—i.e., Regenerated Fiber 3 (Stretched) and Regenerated Fiber 4 (Stretched and Heated)—are shown in Figure 2c and d. The transparency of the fibers was not changed by stretching and heating, but the diameters decreased due to stretching from that of Regenerated Fiber (EtOH), as shown in Table 1.

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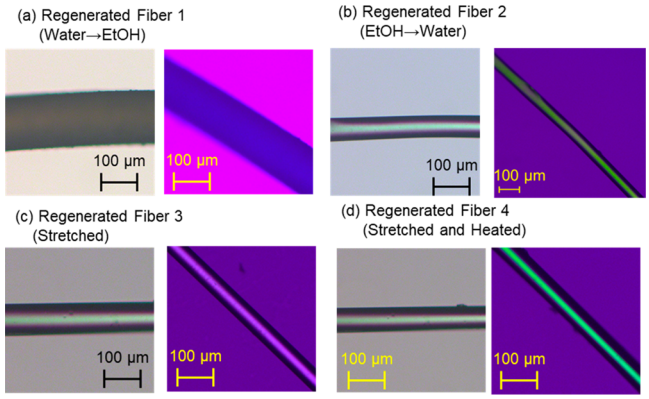

Figure 2. Optical microscope and POM images of regenerated α-1,3-glucan fibers of (a) Regenerated Fiber 1 (Water → EtOH), (b) Regenerated Fiber 2 (EtOH → Water), (c) Regenerated Fiber 3 (Stretched) which was dried at room temperature after stretching in water, and (d) Regenerated Fiber 4 (Stretched and Heated) which was dried at 105 °C after stretching in water.

Table 1. Characteristic of the Regenerated α-1,3-glucan Fibers

Fiber	Diameter (μm)	Δn (×10^{-3})	f_c (%)	X_c (%)
Regenerated Fiber (EtOH)[a]	120 ± 16	13 ± 5	76	20
Regenerated Fiber (Water)[a]	124 ± 28	5 ± 1	48	44
Regenerated Fiber 1 (EtOH → Water)	156 ± 8	9 ± 0.5	74	25
Regenerated Fiber 2 (Water → EtOH)	129 ± 12	–	–	49
Regenerated Fiber 3 (Stretched)	78 ± 7	20 ± 1.7	87	26
Regenerated Fiber 4 (Stretched and Heated)	58 ± 8	29 ± 4.1	80	33

[a]Reported by a previous paper.[21]

Precisely, the diameter of Regenerated Fiber 4 (Stretched and Heated) was lower than that of Regenerated Fiber 3 (Stretched), suggesting that the fixed fiber after stretching in water was further stretched during the oven-drying process.

To investigate the effects of the coagulation baths and solvent substitutions on fiber orientation, the four types of regenerated α-1,3-glucan fibers were examined using a polarized optical microscope (POM). The POM images of the regenerated α-1,3-glucan fibers are also shown in Figure 2. When the fibers were rotated 45 deg in a crossed Nicol arrangement, there was brightness due to molecular orientation along the fiber axis in all of the fibers except for Regenerated Fiber 1 (Water → EtOH), which comprised opaque fibers (Figure 2a). As shown in Table 1, the birefringence (Δn), which is an analog factor for total orientation, of Regenerated Fiber 2 (EtOH → Water) was lower than that of Regenerated Fiber (EtOH) owing to the swelling by solvent substitution in water. On the other hand, the Δn of Regenerated Fiber 3 (Stretched) was higher than that of Regenerated Fiber (EtOH) owing to stretching. Moreover, the Δn of Regenerated Fiber 4 (Stretched and Heated) increased by 1.5 times than Regenerated Fiber 3 (Stretched), suggesting that heating in a fixed state stretches the fibers because of the shrinkage derived from the dehydration.

SEM investigations were carried out to reveal the more detailed morphologies of the regenerated fibers, as shown in Figure 3. First, the effect of the type of coagulation bath on the

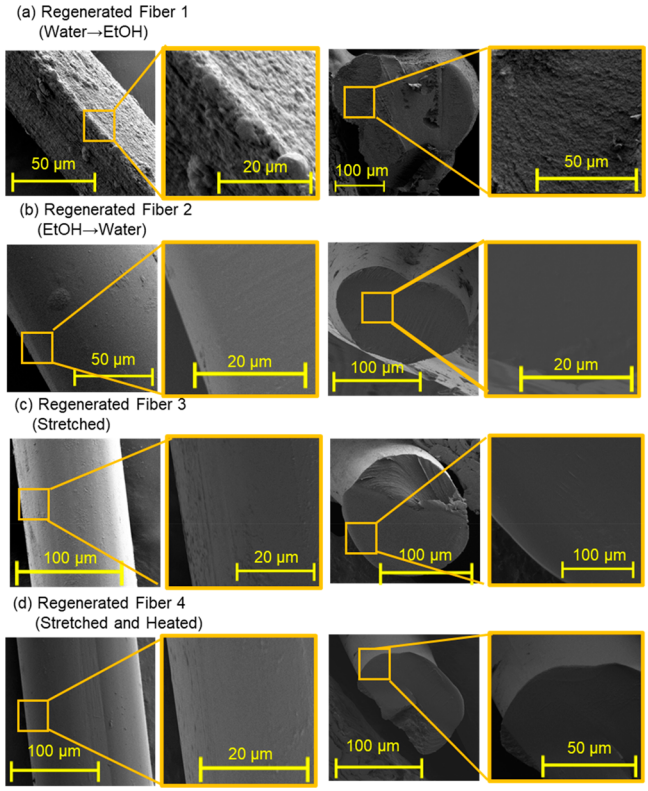

Figure 3. SEM images of the cross section and surface of regenerated α-1,3-glucan fibers of (a) Regenerated Fiber 1 (Water → EtOH), (b) Regenerated Fiber 2 (EtOH → Water), (c) Regenerated Fiber 3 (Stretched) which was dried at room temperature after stretching in water, and (d) Regenerated Fiber 4 (Stretched and Heated) which was dried at 105 °C after stretching in water.

shape of the cross section was investigated. The cross section of Regenerated Fiber 2 (EtOH → Water) was circular (Figure 3b), whereas that of Regenerated Fiber 1 (Water → EtOH) was irregular and nodulous (Figure 3a). In our previous study,[21] Regenerated Fiber (EtOH) had a circular cross section, while Regenerated Fiber (Water) had an irregular and nodulous cross section. This result is consistent with the result of the previous report which suggests that the shape of the cross section depends on the coagulation bath because of the diffusion rate and affinity between α-1,3-glucan and the coagulation bath. After stretching in water, the cross section of Regenerated Fiber 3 (Stretched) remained circular. Furthermore, Regenerated Fiber 4 (Stretched and Heated) also retained almost the same shape of the cross section after stretching and heating. Therefore, it was suggested that the shape of the cross section was determined during regeneration.

Second, the internal structures and porosities of the fibers were compared. As reported previously, Regenerated Fiber (EtOH) had a smooth surface and a dense structure,[21] and Regenerated Fiber 2 (EtOH → Water) had the same dense structure as Regenerated Fiber (EtOH) after solvent substitution with water, as shown in Figure 3b. Furthermore, Regenerated Fiber 3 (Stretched) and Regenerated Fiber 4 (Stretched and Heated) also had smooth surfaces and dense structures, suggesting that stretching and drying processes did not affect the surface shape and porosity of the fibers. Therefore, Regenerated Fiber 3 (Stretched) and Regenerated Fiber 4 (Stretched and Heated) were highly transparent and flexible after stretching and drying. In contrast, Regenerated

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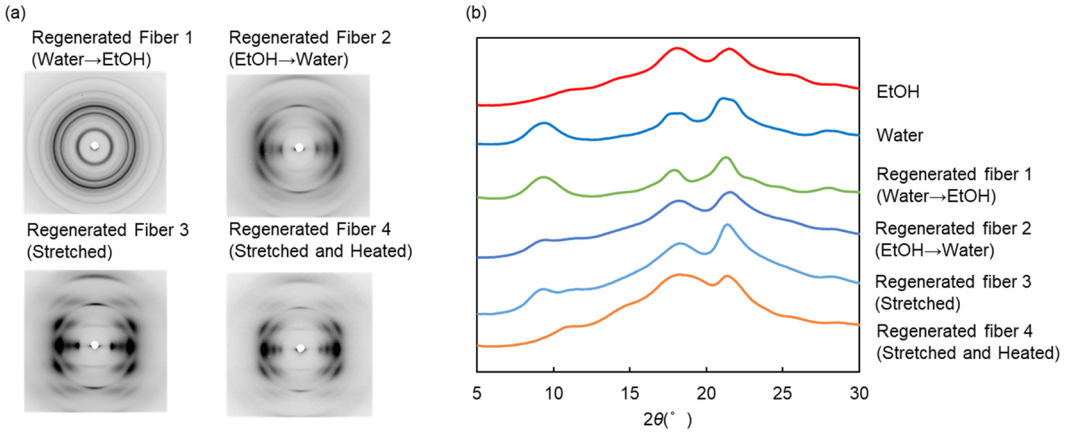

Figure 4. (a) 2D-WAXD and (b) 1D-WAXD of regenerated α-1,3-glucan fibers.

Fiber 1 (Water → EtOH) had a rough and uneven surface and, therefore, was opaque and white. These results suggest that, in addition to the shape of the cross section, the roughness and porosity of the regenerated fibers were determined during the regeneration process. In summary, the shape of the cross section, the porosity, and the roughness of the regenerated α-1,3-glucan fibers were affected by the solvent used as the coagulation bath. These morphological characteristics might be affected by the diffusion rate and the affinity between the α-1,3-glucan and the solvent surrounding the fiber during regeneration.

2.3. WAXD Analysis for Crystal Transition in Post-treatments. The 2D-WAXD patterns of the four types of regenerated α-1,3-glucan fibers are shown in Figure 4a, and 1D-WAXD profiles are shown in Figure 4b. As reported previously,[21] the 1D-WAXD profiles of the regenerated α-1,3-glucan fibers obtained by wet-spinning differed according to the type of coagulation baths (Figure 4b). Regenerated Fiber (EtOH) showed the anhydrous form, and Regenerated Fiber (Water) showed the hydrated form. The crystal structure of Regenerated Fiber (EtOH) was transformed from the anhydrous form to the hydrated form after substitution of the solvent with water, as demonstrated by the 1D-WAXD profile of Regenerated Fiber 2 (EtOH → Water). This crystal transformation from the anhydrous form to the hydrated form through hydration is consistent with that in a previous work for investigating the crystal structures of α-1,3-glucan powder from fungal cell walls.[23] However, the hydrated form of Regenerated Fiber (Water) retained the hydrated form even after solvent substitution with EtOH, as demonstrated by the 1D-WAXD profile of Regenerated Fiber 1 (Water → EtOH). This suggests that the hydrated form might be more stable than the anhydrous form, and the crystal transition from hydrated form to anhydrous form did not occur by the solvent substitution. This result is consistent with the fact that no change in diameter was observed with solvent substitution with ethanol. The stability of this hydrated crystal structure is consistent with previous reports.[24]

The degree of crystalline chain orientation (f_c) was calculated from the 2D-WAXD results. Distinctive arc-shaped reflections were observed in all the fibers (Figure 4a) except for Regenerated Fiber 1 (Water → EtOH) which was a ring pattern owing to a lack of fiber orientation. The f_c of Regenerated Fiber 2 (EtOH → Water) was 74%, and that of Regenerated Fiber 3 (Stretched) was 87%, which was higher than that of Regenerated Fiber 2 (EtOH → Water). As described in the previous section, the Δn in Regenerated Fiber 3 (Stretched) increased from that in Regenerated Fiber 2 (EtOH → Water) (Table 1). These results indicate that stretching effectively increased the degree of orientation of both the amorphous and crystalline regions in the fiber. In contrast, the f_c of Regenerated Fiber 4 (Stretched and Heated) was 80%, which was lower than that of Regenerated Fiber 3 (Stretched), although the Δn of Regenerated Fiber 4 (Stretched and Heated) was higher than that of Regenerated Fiber 3 (Stretched). Considered with the increase in the degree of crystallinity (X_c) after oven-drying (Table 1), it was considered that shrinkage of the fiber occurred during the oven-drying process, especially in the amorphous region, which induced disruption of the orientation and promoted crystallization of some of the amorphous polymer chains.

According to previous studies, four types of crystal polymorphs have been proposed for α-1,3-glucan.[9,23,24] Polymorph I is a hydrated form. This polymorph is a native crystalline form that exists in the cell walls of fungi. Polymorph II is another hydrated form that is observed after extraction of the α-1,3-glucan via dissolution in alkali solution. Polymorph II is four-chain orthorhombic with unit cell parameters a = 0.9982 nm, b = 1.9026 nm, and c (the fiber axis) = 0.8433 nm.[9] Polymorph III is an anhydrous form that is created by the dehydration of polymorph I or polymorph II. Polymorph III is two-chain orthorhombic with unit cell parameters a = 0.9217 nm, b = 1.7158 nm, and c = 0.8417 nm.[9] The reversible transformation of polymorph III into polymorph II by hydration and dehydration has also been reported.[9,23] Polymorph IV is another anhydrous form. It was reported that this polymorph was observed in α-1,3-glucan when it is an acetylated, stretched and subsequently deacetylated film.[24,25] The unit cell of polymorph IV is 2-fold helix orthorhombic with parameters a = 1.65 nm, b = 0.955 nm, and c = 0.844 nm (the fiber axis).[24]

The d-spacings of Regenerated Fiber 3 (Stretched) and Regenerated Fiber 4 (Stretched and Heated) are listed in Table S1a and S1b. The d-spacings extracted from Figure 4 were defined as observed d-spacings (d_{obs}), and the d-spacings reported previously[9] were defined as calculated d-spacings (d_{cal}). The d_{obs} values were indexed based on a piece of previous research about polymorphs II and III.[9] As shown in Table S1a, the d_{obs} of the regenerated Regenerated Fiber 3 (Stretched) agreed well with the d_{cal} of polymorph II (hydrated form) reported previously. This suggests that Regenerated Fiber 3 (Stretched) existed in the hydrated form of polymorph

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II. In contrast, the d_{obs} of Regenerated Fiber 4 (Stretched and Heated) agreed well with the d_{cal} of polymorph III (anhydrous form, Table S1b). Although the crystal structures of α-1,3-glucan polymorphs II and III were revealed by electron microdiffraction and 1D-WAXD patterns in the previous research, their distinctive 2D-WAXD pattern have not yet been reported. Therefore, the 2D-WAXD patterns are meaningful in terms of confirming the structures of polymorphs II and III.

More precisely, the 2D-WAXD pattern of Regenerated Fiber 4 (Stretched and Heated) (Figure 4a) seemed to be similar to that of polymorph IV reported by Ogawa et al. The d_{obs} of Regenerated Fiber 4 (Stretched and Heated) agreed well with the d_{cal} of polymorph IV (Table S1c).[24] Compared to polymorph III (reported by Kobayashi et al.) and polymorph IV (reported by Ogawa et al.), both were 2-fold helix and four-chain orthorhombic. The crystal lattice parameters of polymorph III were reported as a = 0.9217 nm, b = 1.715 nm, and c = 0.8417 nm, and those of polymorph IV were a = 1.646 nm, b = 0.955 nm, and c = 0.844 nm. However, these two crystal unit cells become similar when the lengths of the a axis and b axis are reversed. This similarity has not been properly considered ever, because polymorphs III and IV were investigated using different methods—i.e., 1D-WAXD, electron microdiffraction, and 2D-WAXD. It should be noted that the distinctive 2D-WAXD pattern of the anhydrous and hydrated forms of α-1,3-glucan were first obtained in this study via wet-spinning and stretching in water. The 2D-WAXD pattern of anhydrous form indicate that polymorphs III and IV have similar 2D-WAXD patterns. Therefore, Regenerated Fiber (EtOH) and Regenerated Fiber 4 (Stretched and Heated) might have the same anhydrous unit cell. More details will be obtained by electron diffraction and stereochemical model refinement in the future.

2.4. Post-treatment's Effect on the Mechanical Properties of the Regenerated Fibers. The mechanical properties of the regenerated α-1,3-glucan fibers were evaluated by tensile tests. The obtained stress−strain curves of the regenerated fibers are shown in Figure 5, and the tensile strength, Young's modulus, and elongation at break values are listed in Table 2. Compared with the mechanical properties of Regenerated Fiber (EtOH) (tensile strength 11 ± 2.9 cN/tex, strain 12 ± 3.4%, and Young's modulus 3.5 ± 1.0 GPa),[21] the tensile strength and strain of Regenerated Fiber 2 (EtOH → Water) increased, whereas the Young's modulus decreased. As shown in Table 1, the Δn of Regenerated Fiber 2 (EtOH → Water) was lower than that of Regenerated Fiber 1. It is considered that the decrease of the orientation improved the flexibility of the regenerated fibers. In contrast, Regenerated Fiber 1 (Water → EtOH) had a lower tensile strength (0.6 cN/tex) than Regenerated Fiber (Water) (2.7 ± 0.8 cN/tex).[21] As described in the previous sections, Regenerated Fiber 1 (Water → EtOH) had a rough and porous structure, which might cause the lowering of the tensile strength and strain values.

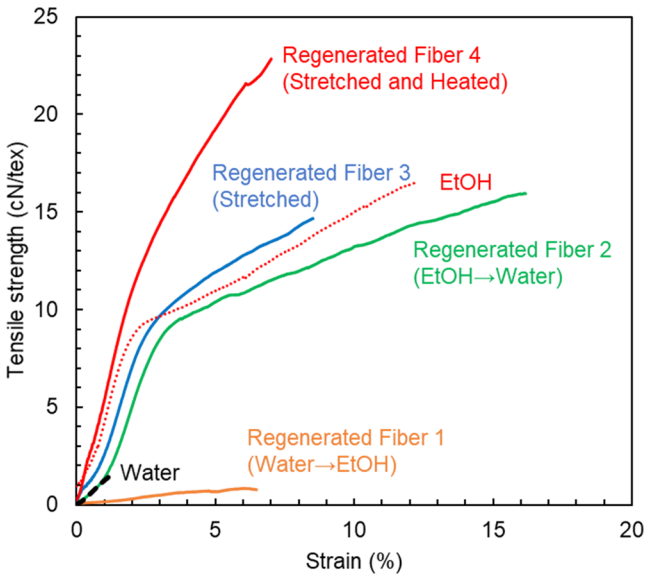

Figure 5. Stress−strain curves of the regenerated α-1,3-glucan fibers in the tensile tests.

Table 2. Mechanical Properties of Regenerated α-1,3-Glucan Fibers

Fiber	Tensile strength (cN/tex)	Elongation at break (%)	Young's Modulus (GPa)
Regenerated Fiber 1 (Water → EtOH)	0.6 ± 0.3	5.3 ± 1.9	0.5 ± 0.4
Regenerated Fiber 2 (EtOH → Water)	15 ± 6.2	14 ± 3.8	1.4 ± 0.1
Regenerated Fiber 3 (Stretched)	15 ± 3.1	9.2 ± 1.8	5.7 ± 1.8
Regenerated Fiber 4 (Stretched and Heated)	18 ± 6.8	9.5 ± 3.3	11 ± 4.3

The results in tensile tests of Regenerated Fiber 3 (Stretched) and Regenerated Fiber 4 (Stretched and Heated) are also shown in Figure 5 and Table 2. The strain in Regenerated Fiber 3 (Stretched) was lower than in Regenerated Fiber 2 (EtOH → Water), but the Young's modulus of Regenerated Fiber 3 (Stretched) was four times higher than that of Regenerated Fiber 2 (EtOH → Water). Therefore, it was suggested that increasing f_c and Δn improved the Young's modulus while reducing flexibility owing to the oriented amorphous region. This suggests that the mechanical properties of regenerated α-1,3-glucan fibers can be controlled by stretching them and immersing them in water. On the other hand, the Young's modulus of Regenerated Fiber 4 (Stretched and Heated) was significantly increased by drying it at 105 °C, and its tensile strength increased to 18 cN/tex (Table 3)—i.e., comparable to that of viscose rayon (22 cN/tex)[26]—although f_c decreased from 87% to 80% during heating. The enhanced tensile strength may be attributed to the high Δn, the high X_c (Table 1) and the difference in the elastic modulus in the crystalline regions. This mechanical strength of α-1,3-glucan fiber was still lower than that of lyocell fiber (34−36 cN/tex)[2] which is commercial cellulose regenerated fiber. Therefore, the optimization of spinning conditions such as draw ratio and the molecular weight of α-1,3-glucan should be investigated in the future. In addition, the elastic modulus of the crystalline regions of the anhydrous and hydrated forms has not yet been investigated, and it should be researched in the future.

3. EXPERIMENTAL SECTION

3.1. Materials. α-1,3-Glucan was prepared by *in vitro* enzymatic polymerization, as described in our previous paper.[8] The weight-average molecular weight (M_w) of the resulting polymer was 1.2 × 10^5 g/mol, and its number-average molecular weight (M_n) was 0.7 × 10^5 g/mol. The

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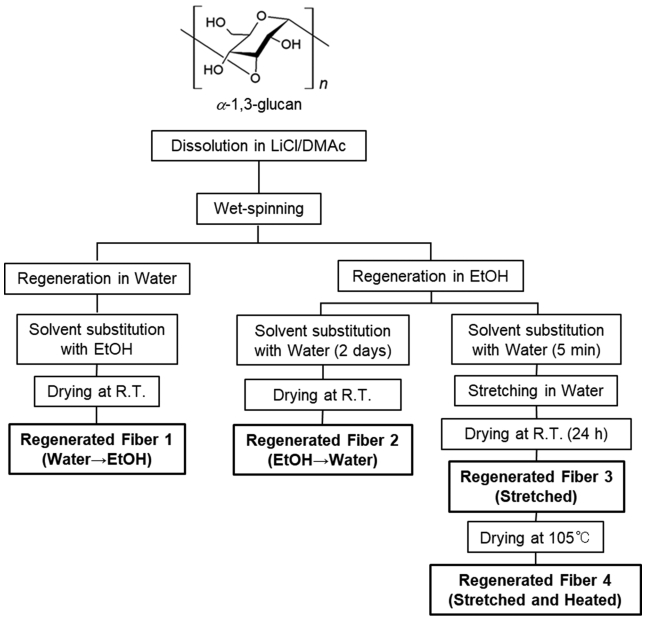
polydispersity (M_w/M_n) was 1.8. The prepared α-1,3-glucan powder was dried at 105 °C in a vacuum oven for 24 h before use. All other reagents were purchased from commercial sources and were used as received.

3.2. Preparation of Spinning Dope and Wet-Spinning of α-1,3-Glucan. The spinning dope was prepared using the LiCl/DMAc system depicted in Scheme 1. First, a solution of 8 wt % LiCl/DMAc was prepared: LiCl (2 g) was dissolved in DMAc (26 g) by stirring at 100 °C for 20 min. The dried α-1,3-glucan (3 g, 10 wt %) was added to the 8 wt % LiCl/DMAc solution (28 g), and the mixture was stirred at 100 °C for 24 h to obtain a clear viscous solution. The resulting α-1,3-glucan solution was transferred to a stainless-steel barrel equipped with a one-hole spinneret (hole-diameter, 1.0 mm), and spun using a lab-scale customized wet-spinning unit (AIKI RIOTECH Co., Ltd., Aichi, Japan) at room temperature. The filament was extruded through the spinneret equipped with four types of metal meshes (30, 100, 200, and 300 mesh) to remove the undissolved substrate. After the fluid filament had passed through an air gap of 30 mm, it was coagulated in distilled water or ethanol (EtOH) at room temperature. The extrusion velocity (V_e) was set to 2 cm^3/min (2.5 m/min), and the take-up velocity (V_t) of the godet roller was 17 m/min, resulting in a draw ratio (DR = V_t/V_e) of 6.8. The resulting fibers were washed in water or EtOH at room temperature for 6 days and air-dried for 24 h.

Scheme 1. Preparation of Regenerated α-1,3-Glucan Fiber



3.3. Solvent Substitution of the Regenerated α-1,3-Glucan Fibers. The obtained two types of regenerated fibers are referred to as Regenerated Fiber (EtOH) and Regenerated Fiber (Water). These regenerated fibers were subjected to solvent substitution with either EtOH or water. Precisely, the Regenerated Fiber (EtOH) was replaced with water over 2 days, and the water in Regenerated Fiber (Water) was replaced with EtOH over 2 days. After solvent substitution, the resultant fibers were dried and fixed at room temperature for 24 h and referred to as either Regenerated Fiber 2 (EtOH → Water) or Regenerated Fiber 1 (Water → EtOH) depending on the coagulation bath and substitution solvent.

3.4. Stretching the Regenerated α-1,3-Glucan Fibers in Water. Regenerated Fiber (EtOH) was cut into 10 mm-long pieces. The cut fibers were fixed to a stretching machine and immersed in water to allow them to swell for 5 min at room temperature. After immersion in water, the regenerated α-1,3-glucan fibers were stretched to 1.5 times their initial length. The stretched fibers were fixed in the stretching machine in water for 2 days and air-dried at room temperature. These stretched and air-dried fibers are referred to as Regenerated Fiber 3 (Stretched). Moreover, some of the Regenerated Fiber 3 (Stretched) was dried in an oven at 105 °C for 24 h to remove the water, completely. The oven-dried fiber is referred to as Regenerated Fiber 4 (Stretched and Heated).

3.5. Polarized Optical Microscope (POM). An ECRIPSE E600 polarized optical microscope (Nikon Co., Tokyo, Japan) equipped with a DFC 450 charge-coupled device camera (Leica Microsystems, Tokyo, Japan) was used to determine the orientation of the regenerated fibers. The polarizer and depolarizer were set at appropriate angles to obtain clear images. The average orientation of both the amorphous and crystalline parts in each fiber was determined by a POM equipped with a Berek compensator (Nichia Inc., Kyoto, Japan). The birefringence (Δn) values of the fibers were calculated from fiber diameters (nm) which were measured with micrographs. The total orientation factor (f_t) can be determined by dividing Δn by the maximum birefringence (Δn_{max}). However, the Δn_{max} of α-1,3-glucan has never been determined, and the value of Δn was considered as an analog factor for discussing the fiber orientation in this study.

3.6. Scanning Electron Microscope (SEM). SEM images of the regenerated fibers were obtained using a field emission scanning electron microscope (S-4800, Hitachi Ltd., Tokyo, Japan) operated at an accelerating voltage of 1.0 kV after the fiber samples had been coated with platinum for 30 s using ion sputtering (E-1030, Hitachi Ltd., Tokyo, Japan).

3.7. Wide-Angle X-ray Diffraction (WAXD). Two-dimensional WAXD (2D-WAXD) patterns of the regenerated fibers were obtained using a MicroMax-007HF system (Rigaku Co., Tokyo, Japan) operated at 40 kV and 30 mA with Cu Kα radiation (λ = 0.15418 nm) in transmission geometry mode and in a high vacuum. The X-ray diffraction pattern was recorded on an imaging plate (BAS-SR 127, 2540 × 2540 pixels, 50 × 50 mm^2/pixel; Fujifilm Co., Tokyo, Japan) and read with a RAXIA-D*i* system (Rigaku Co., Tokyo, Japan). The sample-to-detector distance was set at approximately 83 mm. The sample holder was maintained at room temperature, and the measurements were also obtained at room temperature. 2D-WAXD pattern analysis and conversion to one-dimensional (1D) profiles were performed using 2DP software (Rigaku Co. Tokyo, Japan). The degree of crystallinity (X_c) values of the fibers were determined from the corrected and normalized 1D-WAXD curves. The degree of crystalline chain orientation (f_c) was determined by an azimuthal scan of the meridional main interference taken from well-aligned fiber samples in the longitudinal direction. It is defined by (180° − WH)/180°, where WH represents the half width of the orientation peak from the intensity azimuthal profile.

3.8. Tensile Tests. The linear density (titer) of the regenerated fibers was determined by a precise gravimetric measurement of the fibers which were cut into 30 mm lengths. Tensile tests were performed on the cut fibers using an EZ test instrument (Shimadzu Co., Kyoto, Japan) at a tensile speed of

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1 mm/min at room temperature. The initial gauge length was set to 10 mm. The tensile test was conducted five times for each sample, and the tensile strength was taken as the average.

4. CONCLUSIONS

The mechanical properties of regenerated α-1,3-glucan fibers were successfully improved by the solvent substitution and the stretching in water. The anhydrous crystalline form of regenerated α-1,3-glucan fibers coagulated in EtOH was transformed to the hydrated form by solvent substitution with water. During this hydration process, the fibers were stretched to 1.5 times the initial length, and the hydrated form was retransformed into the anhydrous form by drying at 105 °C. The high-oriented regenerated α-1,3-glucan fiber was obtained by these post-treatments, and the fiber had higher tensile strength (18 cN/tex) than that of the regenerated α-1,3-glucan fiber without any post-treatment (11 cN/tex). The enhanced tensile strength was comparable to that of viscose rayon. Moreover, distinctive 2D-WAXD patterns of α-1,3-glucan polymorph II (the hydrated form) and polymorph III (the anhydrous form) were first obtained. The 2D-WAXD pattern of polymorph III was similar to that of another anhydrous form (polymorph IV). This similarity suggests that polymorph III and polymorph IV might have the same anhydrous unit cell. Therefore, this research is meaningful not only in material science but also in crystallography associated with α-1,3-glucan.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at https://pubs.acs.org/doi/10.1021/acsomega.1c02365.

d-spacings of the regenerated fibers (PDF)

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Notes

The authors declare no competing financial interest.

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