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Doctoral Dissertation

Development of Bacterial Cellulose-Based Materials  
Utilizing its Unique Properties

(バクテリアセルロースの特徴を活かした材料の開発)

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January 2016

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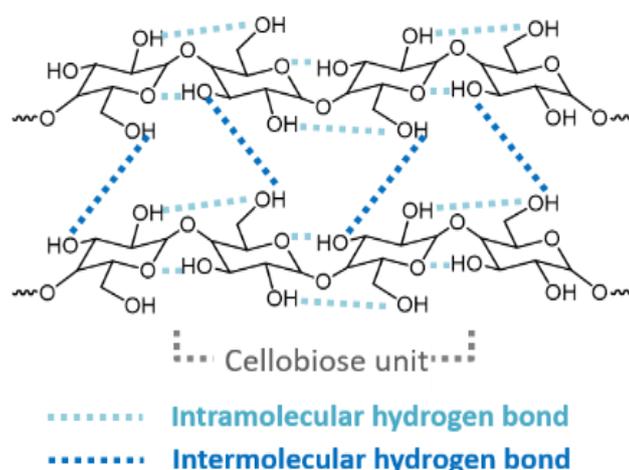
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## General Introduction

Cellulose is one of the most abundant natural polymers on earth to replace non-renewable resources<sup>1-5</sup>. This ubiquitous biopolymer is found in plants, marine animals, algae, fungi, amoeba and bacteria. In most them, cellulose acts as the structural component and reinforcement phase in cell walls. Cellulose was firstly described in 1838 by the French chemist Anselme Payen<sup>6</sup>. He isolated it from plant tissues and determined its chemical formula to be  $C_6H_{10}O_5$ . The term of cellulose was first recorded in 1839 in a report of the French academy on his work<sup>7</sup>. Its polymeric structure was postulated and determined by Hermann Staudinger in 1920<sup>8</sup>. However, as a chemical raw material, it has been used to produce the first successful thermoplastic polymer called celluloid by Hyatt Manufacturing Company from 1870. Commercial production of rayon as an artificial silk from cellulose began in the 1890s and cellophane made of regenerated cellulose was invented in 1912<sup>3,4</sup>.



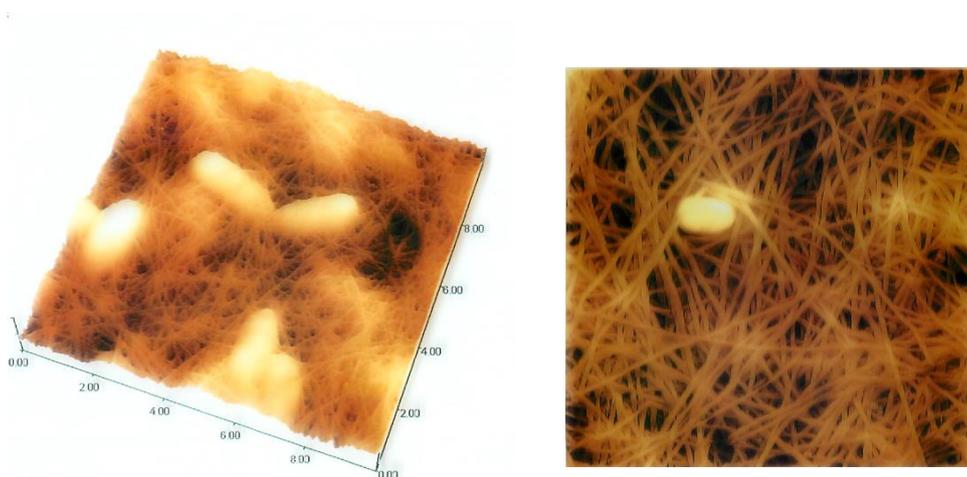
**Figure 1.** Molecular structure of cellulose with intra- and inter-chain hydrogen bonds.

The enchantment of the cellulose is a result of its specific structure. Cellulose is a carbohydrate polymer which composed of a disaccharide repeat unit called cellobiose, and these two D-glucoses linked via a  $\beta$ -1,4 glycosidic bond (Figure 1). Cellulose chains having abundant hydroxyl groups are attached together by intra- and intermolecular hydrogen bonds and van der Waals interactions<sup>1,3</sup>. As a result, it is able to form a crystalline core that is covered by para crystalline chains. Due to its hydrophilicity, chirality, biodegradability and chemical modifying ability, various functional materials from cellulose have been developed over a broad range of applications such as foods, housing and paper, fibers and clothes, cosmetic and pharmaceutical industries<sup>1</sup>.

The isolation and purification from plant sources need mechanical and chemical processes. In spite of those processes, the residues such as hemicellulose, lignin, pectin and other substances are hard to remove from the plant-derived cellulose, and sometimes products such as beached wood pulp contain carbonyl and carboxyl groups<sup>2</sup>. Additionally, the study of nanocellulose as a reinforcing material in nanocomposites started 20 years ago<sup>9</sup>. Since then a large number of studies on nanocellulose have been reported, and it is becoming an increasingly noticeable issue. Because this material with one dimension in the nanometer range has the very large surface area. The nanocelluloses include nanofibrillated cellulose and nanocrystalline cellulose<sup>4,10,11</sup>. These nano-scale materials are prepared by various physical, chemical, and enzymatic methods; they are largely depends on their biological sources<sup>4</sup>. Cellulosic material is usually derived from plant sources such as cotton, wood and algae, but also, bacteria<sup>3,4</sup>.

Bacterial cellulose (BC) is formed by acetic acid bacteria of the genus

*Gluconacetobacter*, and they are especially suitable for the production and investigation of cellulosic material<sup>12-16</sup>. These rod shaped, gram-negative and strictly aerobic bacteria are able to form biofilm composed of pure nanocellulose at the interface between liquid and air (Figure 2). BC was initially reported by A.J. Brown in 1886; it was described as vinegar plant, jelly-like translucent mass on the surface of the culture fluid<sup>17</sup>. After then, a number of studies had reported, however virtually no attention had been paid to BC as a functional material. In the recent decades, investigation and utilization of BC in various functional materials have grown rapidly<sup>14,16</sup>. BC based functional nanomaterials are especially a fascinating topic in various fields due to its improved and new properties by preparation of composites<sup>4,5,18,19</sup>. Indeed, BC is a traditional food component in Southeast Asian countries such as Philippine, Indonesia and Thailand. In 1990s, especially it was considered as a popular dessert in Japan. In these countries, it is called *nata-de-coco* which is fermentation of coconut water using *Gluconacetobacter xylinus* produces gelatinous BC<sup>14,21,22</sup>.

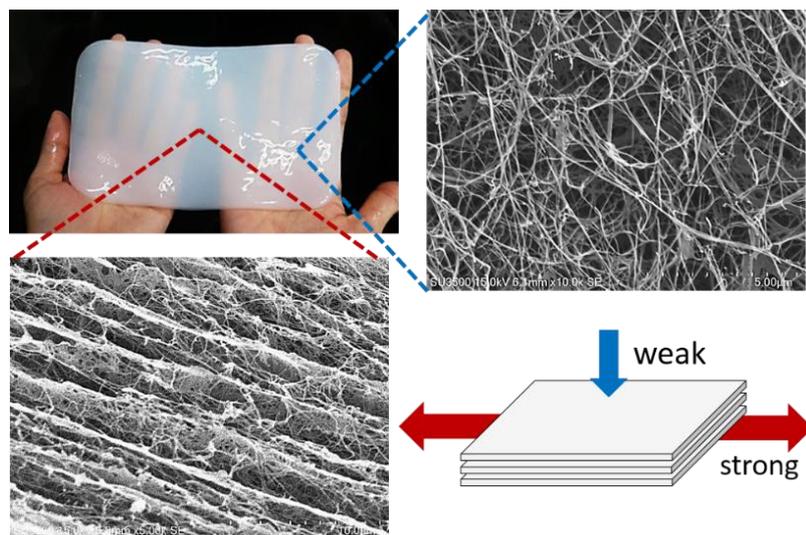


**Figure 2.** AFM images of *Gluconacetobacter* bacteria.

Synthesis of BC by *Gluconacetobacter xylinus* is a precisely and specifically regulated multi-step process. It involves a large number of individual enzymes and complex of catalytic and regulatory proteins. Its supramolecular structure has not been characterized. The biosynthesis process includes the formation of uridine diphosphoglucose (UDP-glucose) which is a nucleotide sugar and a precursor of cellulose<sup>12-15</sup>. However, molecular mechanisms of glucose polymerization are still not clear. The reason why *Gluconacetobacter xylinus* generates cellulose has been studied. One consideration is that the aerobic bacteria produce cellulose sheet to maintain their position close to the surface of culture solution. Another consideration is that the bacteria form cellulose hydrogel to guard themselves from ultraviolet or enemies. BC productivity depends on the incubating conditions including the cultivation method, carbon and nitrogen sources, temperature, pH, surface area and dissolved oxygen, however culture volume and depth had no effect on production rate<sup>13-16</sup>. *Gluconacetobacter xylinus* has the capability to grow and produce nanocellulose on a variety of substrates, and the main source substance of BC is saccharides such as sugar and D-glucose. It leads to many investigations of optimal composition for incubation medium. The standard medium for BC cultivation was suggested by S. Hestrin and M. Schramm in 1954<sup>23</sup>; it is composed of glucose, peptone, yeast extract, disodium phosphate and citric acid, and pH was adjusted to 6.0 using hydrochloric acid aqueous solution. However, the Hestrin-Schramm medium still required additional supplements for effective cultivation. Additionally, optimal incubating condition depends on origin of *Gluconacetobacter xylinus*, medium composition and pH need modification to grow bacteria and produce cellulose<sup>13</sup>. Culture of BC is generally carried out at around 27-30 °C, and its thickness increases with incubating time in static condition<sup>12-14</sup>.

Several attempts to produce BC with high productivity have been reported using the submerged and rotated method<sup>12-14</sup>. In most cases, BC is obtained as a hydrogel form and it is free of contaminant molecules including hemicellulose, lignin, pectin, and other substances. Its purification method is simple; bacteria and residues from the culture medium can be easily removed by using aqueous sodium hydroxide with low energy consumed.

In comparison to plant-derived cellulose, BC has several interesting characteristics such as nano-sized network structure, high purity, high crystallinity and high moisture content (around 99%)<sup>14,21,22</sup>. Additionally, BC with layered structure is formed under static culture conditions<sup>20,22</sup>. On the basis of the characteristic structure<sup>21,22,24</sup>, BC hydrogel shows mechanical anisotropy<sup>24</sup> (Figure 3); BC hydrogel sheet has high tensile strength<sup>20-22</sup>; whereas it is easy to deform and lose the moisture under compression<sup>24-26</sup>.



**Figure 3.** Photograph of bacterial cellulose and its vertical (left) and horizontal (right) cross-section SEM images.

Generally, cellulose has highly polar surface and wettable fibril property because of abundant hydroxyl groups and its extensive usage depend largely on functional derivatization to prepare composite materials<sup>27</sup>. For this purpose, delicate surface modification<sup>11,28,29</sup> or derivatization after homogeneous solubilization<sup>30</sup> of cellulose fibers is usually carried out. Chemical modification of cellulose surface is a popular topic; many categories of reactions has been carried out (Table 1)<sup>11,28,29</sup>.

**Table 1.** Categories of chemical modification of cellulose surface.

<b>Categories of chemical modification</b>	<b>Reaction examples</b>
Acid hydrolysis	Sulfuric acid hydrolysis, hydrochloric acid hydrolysis and phosphoric acid hydrolysis
Oxidation	TEMPO (2,2,6,6-Tetramethylpiperidinyloxy radical) oxidation, periodate oxidation and iodate oxidation
Esterification	Acetylation, tosylation
Amidation	Various types of amine products
Carbamation	Isocyanate and isothiocyanate
Etherification	Glycidyltrimethylammonium chloride, alkyltrimethylsilyl chlorides and epichlorohydrin

Homogeneous solubilization of cellulose can be prepared by using non-derivatizing solvents (aqueous inorganic complexes (Cuprammonium hydroxide solution, cupriethylenediamine hydroxide solution), 10 % NaOH aqueous solution,

ammonia/ammonium salt ( $\text{NH}_3/\text{NH}_4\text{SCN}$ ), mineral acids, melts of inorganic salt hydrates, *N,N*-dimethylacetamide/LiCl, dimethyl sulfoxide/ $\text{SO}_2$ /diethylamine, *N*-methylmorpholine-*N*-oxide, so on.) and derivatizing solvents ( $\text{CF}_3\text{COOH}$ ,  $\text{HCOOH}$ , *N,N*-dimethylformamide/ $\text{N}_2\text{O}_4$ , DMSO/paraformaldehyde,  $\text{NaOH}/\text{CS}_2$  so on.)<sup>2</sup>. In recent years, various new solvents has developed and reported to dissolve cellulose effectively; there are  $\text{NaOH}/\text{urea}$ ,  $\text{NaOH}/\text{thiourea}$ ,  $\text{LiOH}/\text{urea}$  and ionic liquids. If cellulose dissolves in a solvent by splitting inter- and intra-chain hydrogen bonds, its characteristics based on the highly ordered structure are lost. Furthermore, the unique structure of BC also could not maintain. The risks involved in using hazardous organic solvents for dissolving cellulose prompted us to use aqueous media for its functionalization.

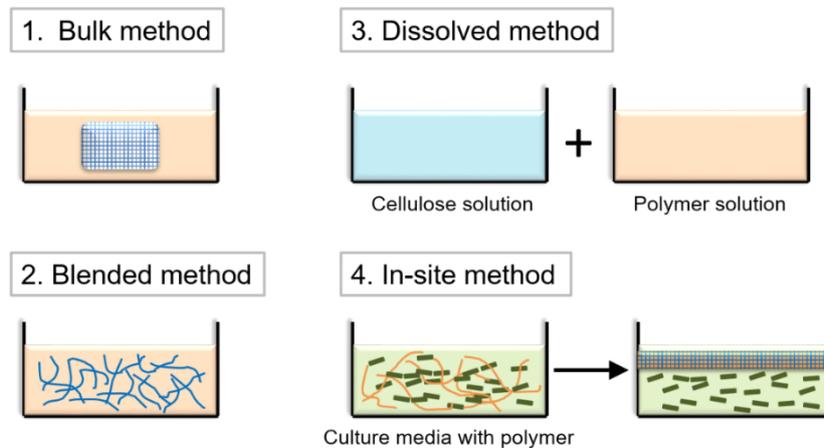
Dialdehyde cellulose is one of candidates<sup>31,32</sup> for a line of investigation of surface modification of cellulose. Preparation of dialdehyde cellulose of plant origin is usually carried out by using cellulose powders as raw materials<sup>26</sup> and no attempt has yet been made to prepare selectively surface modified cellulose sheets. Based on this background, nano-sized network structure and layered structure of BC were more focused on further investigation. Utilizing its interesting structure, it is easy to prepare the high density hydrogel sheet under compression. In Chapter 1, the semi-dried BC sheet was intended for a one-sided modification by limited periodate oxidation. This semi-dried BC sheet also has high reactivity for the modification, which may due to the high surface area with the wet state.

From result of chapter 1, it was assumed that one of the most important effects on one-sided modification of BC is its subtle network structure. This structure prompted to design a new polymer composite using BC as a matrix. Since BC has been

considered as one of the most attractive functional materials, various composites of BC have been prepared to overcome its limitations and increase its applications (Table 2). Chapter 2 deals with the composite using never-dried BC. So far, preparation of BC-containing polymer composites by impregnation<sup>33-36</sup>, blend<sup>33,34,37</sup>, dissolved<sup>33</sup> and in-situ methods<sup>33,34</sup> have been reported (Figure 4), and the characteristics of BC unfortunately did not remain in some cases.

**Table 2.** Classification of bacterial cellulose composites and its examples.

<b>Composite materials</b>	<b>Examples</b>
Organic materials (polymers and nanomaterials)	Polyethylene glycol, poly(L-lactic acid), polyacrylamide, poly(acrylic acid), poly (3-hydroxybutyrate), poly (3-hydroxybutyrate-co-4-hydroxybutyrate), poly(vinyl alcohol), polyaniline, chitosan, gelatin, graphene oxide, carbon nanotubes, epoxidized soybean oil, collagen, aloe vera, pectin, xyloglucan, starch, sisal fibers, alginate and so on
Inorganic materials (metals, metal oxides and solid particles)	Silica, silver nanoparticle, gold nanoparticle, palladium, hydroxyapatite, sodium triphosphate, montmorillonite and so on



**Figure 4.** Preparation of bacterial cellulose and polymer composites by impregnation, blend, dissolved and in-situ methods.

Hydrophilic polymers are suitable candidates for composites with hydrophilic BC. Poly(vinyl alcohol) (PVA) is one of the most popular hydrophilic polymers and it has attracted a lot of attentions because of its biocompatible and non-toxic properties<sup>36</sup>. Chemical or physical crosslinking of PVA is often used to improve its mechanical strength and a freeze-thaw cycle method for preparation of the physically crosslinked PVA has been of specific interest due to a simple process<sup>38</sup>. Another strategy for reinforcement of PVA is the combination of cellulose. Although cellulose is highly hydrophilic, the uniform dispersal with PVA is challenging owing to the low processability<sup>39</sup>. Using BC as matrix will provide good dispersibility of cellulose in a PVA aqueous solution. BC-PVA composite prepared by a bulk method, in which gelation of PVA was conducted by cyclic freezing-thawing technique in the presence of BC sheet. The resulting composite is expected to have the network structure from both

materials. Interestingly, the thermostability of the composite was greatly improved in spite of immiscibility of cellulose and PVA chains. On the other hand, there are some limitations in BC-hydrophobic polymer composite. For example, delamination of BC in the composite with poly(L-lactic acid) has been reported<sup>40</sup>. Consequently, the reinforcement of the BC layered structure is required to expand the applications.

In general, dried cellulose sheet is not able to swell or dissolve in common solvents including water, because of the strong hydrogen bondings between cellulose chains<sup>3,30,41</sup>. BC also hardly swells in water after once drying. On the other hand, cellulose solvents can break the hydrogen bondings of cellulose. Solubilization of BC in cellulose solvents was examined for analysis, regeneration, and homogeneous modification of the cellulose<sup>41</sup>; however, the characteristics of BC did not remain in some cases.

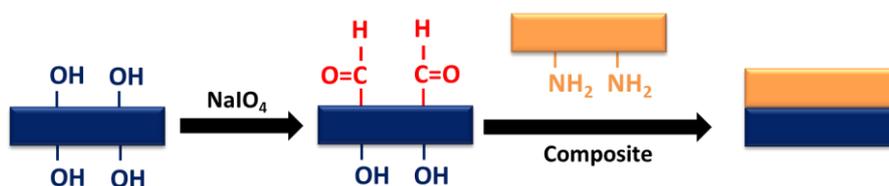
Chapter 3 focused on the layered structure of BC, and it demonstrated the unique one-dimensional shrinkage-swelling behaviors of the crosslinked BC gel. Interestingly, the BC hydrogel was subjected to the solvent exchange with various organic solvents by a convenient immersion procedure, in which shrinkage, deformation, and destroy of the gel did not take place by choosing the conditions. Herein the solvent of the BC gel exchanged from water to acetone and the crosslinking with diisocyanate was conducted in acetone. By using acetone as gel medium, isocyanate compounds with high reactivity could be used under mild and anhydrous conditions. If the crosslinking of BC proceeds in its gel form, the network formation of BC may take place mainly in the layer, leading to the efficient reinforcement of the BC sheet.

This thesis is composed of 3 chapters, and it describes new functional BC based materials which are developed utilizing its unique properties. If BC lose its structure, it is hard to find differences with plant-derived cellulose. Therefore, utilizing and keeping of its unique properties are the most important ideas in this study. The content of each chapter is briefly described below.

### Chapter 1. One-sided Surface Modification of Bacterial Cellulose Sheet as 2,3-Dialdehyde

In this chapter, one-sided surface modification of BC was prepared by the introduction of reactive 2,3-dialdehyde derivatives (DABC) preferentially with limited periodate oxidation. The results indicate that the best reaction condition for one-sided modification was 3 h at 50 °C with a periodate solution (cellulose/sodium periodate near 1.21, w/w) in the dark. Semi-dried BC sheets (thickness 20 μm, moisture content 96.7%) have high reactivity for the modification.

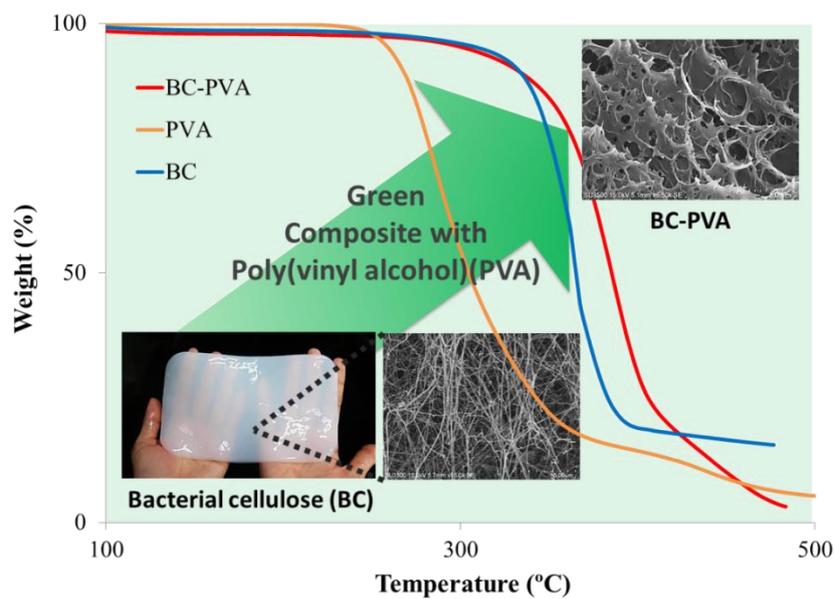
**Scheme 1.** One-sided surface modification of bacterial cellulose sheet and its laminated composition.



## Chapter 2. Unique Enhancement of Thermostability in a Green Composite of Bacterial Cellulose and Poly(vinyl alcohol)

This chapter deals with a green composite of never-dried BC and poly(vinyl alcohol) (PVA) showing enhanced thermostability. The BC-PVA composite was prepared by a bulk method to keep unique properties of BC; gelation of PVA was conducted by a cyclic freezing-thawing method in the presence of BC. The resulting composite maintained the network structure and the thermal stability much improved despite of the immiscibility between BC and PVA in the molecular level.

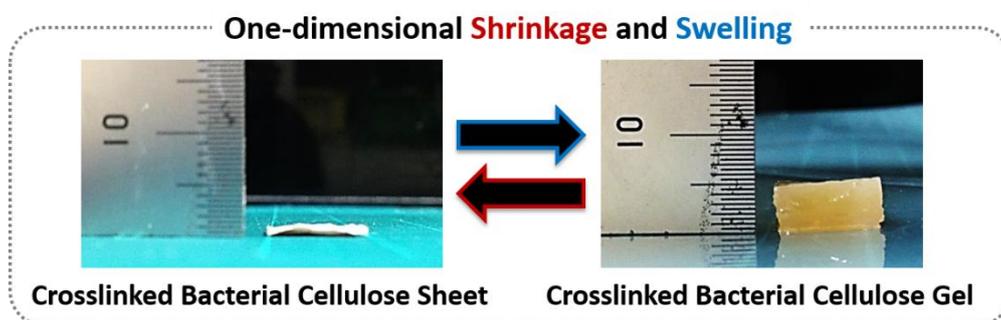
**Scheme 2.** Green composite of bacterial cellulose and poly(vinyl alcohol) without any chemical gelation agent and its enhancement of thermostability.



### Chapter 3. One-dimensional Shrinkage and Swelling of Crosslinked Bacterial Cellulose Gel

This chapter deals with crosslinked BC showing unique one-dimensional shrinkage-swelling behaviors. The crosslinked BC was prepared by the solvent exchange of the BC gel from water to acetone, followed by the reaction with methylenediphenyl 4,4'-diisocyanate (MDI). The resulting cuboid-shaped crosslinked BC organogel one-dimensionally shrank by drying under vacuum to form the thin sheet. It also one-dimensionally swelled in LiCl/DMAc solution at 25 °C, followed by the solvent replacement with water to recover the original shape.

**Scheme 3.** One-dimensional shrinkage and swelling of crosslinked bacterial cellulose gel



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## **Chapter 1.**

### **One-sided Surface Modification of Bacterial Cellulose Sheet as 2,3-Dialdehyde**

#### **Introduction**

Cellulose is one of the most abundant natural polymer on the earth to replace non-renewable resources<sup>1</sup>. Cellulose chains are attached together by hydrogen bonds and van der Waals interactions to form a crystalline core that is covered by paracrystalline chains. Because of its highly polar surface and wettable fibril property, its extensive usage depend largely on functional derivatization to prepare composite materials<sup>2,3</sup>. For this purpose, delicate surface modification<sup>4,5</sup> or derivatization after homogeneous solubilization<sup>6,7</sup> of cellulose fibers is usually carried out. If cellulose dissolves in a solvent by splitting inter- and intra-chain hydrogen bonds, its characteristics based on the highly ordered structure are lost. The risks involved in using hazardous organic solvents for dissolving cellulose prompted us to use aqueous media for its functionalization. Dialdehyde cellulose is one of candidates<sup>8,9</sup> for a line of investigation of surface modification of cellulose. Preparation of dialdehyde cellulose of plant origin is usually carried out by using cellulose powders as raw materials<sup>2</sup> and no attempt has yet been made to prepare selectively surface modified cellulose sheets.

Bacterial cellulose (BC) produced by *Gluconacetobacter xylinus* is unique in its highly swollen gel sheet shape, and in having a crystalline allomorph I<sub>α</sub> with high purity<sup>10</sup>. This gel pellicle property lends itself to the modification of its surface to make one-sided surface modified cellulose sheets as a source for production of

environmental-friendly biomaterials. This modification will open a new field of cellulose film utilization.

This chapter was intended to convert surface cellulose of BC sheet to reactive 2,3-dialdehyde derivatives (DABC) preferentially by limited periodate oxidation. Here, a one-sided modification of BC could be achieved with the reaction condition of 3 h at 50 °C with periodate solution.

## Experimental section

### Materials

BC Sheets were prepared in Research Centre for Physics, Indonesian Institute of Sciences (LIPI), Bandung, Indonesia. BC gel (about 10 mm in thickness) was produced by fermenting the inoculum containing coconut water, supplemented with sucrose, ammonium sulfate and acetic acid and keeping *Gluconacetobacter xylinus* in LIPI (purchased from home factory in Cianjur, Indonesia) under static condition for several days at room temperature, and cleaned up by washing with water and boiling in 2% aqueous NaOH solution. After thorough washing with water until neutral pH was achieved, BCs were dried to make semi-dried (thickness 20  $\mu\text{m}$ , moisture content 96.7% at 23 °C) and dried sheets<sup>10</sup>.

Sodium periodate and sodium disulfite were purchased from Wako Pure Chemical Industry, Ltd., Japan. Hydroxylamine hydrochloride and basic fuchsin were obtained from Sigma-Aldrich Co. All chemicals were used without further purification.

### Measurements

Fourier transform infrared (FT-IR) measurement was carried out in an attenuated total reflectance (ATR) mode by a Nicolet iS5 Spectrometer, Thermo Fisher Scientific Inc., Waltham, MA, USA. The OMNIC software equipped with a diamond ATR crystal was used for measurements (penetration depth  $\sim 1.1 \mu\text{m}$  at  $1744 \text{ cm}^{-1}$ ). Scanning electron microscopy (SEM) images were recorded on a HITACHI S-3500 instrument operated at 15 kV. Thermogravimetric analysis (TGA) was performed with an EXSTAR TG/DTA 7200 thermogravimetric analyzer (Hitachi High-Tech Science Co., Tokyo, Japan) from 40 to 500 °C at a heating rate of 20 °C/min under nitrogen.

Mechanical properties were measured by a tensile testing (TT) apparatus (Tack Tester TA-500, UBM Co., Kyoto, Japan). Rectangular uniform segments 5 x 15 mm in size were clipped with stainless clamps and deformed at a constant rate ( $0.01 \text{ mm s}^{-1}$ ) at room temperature ( $23 \text{ }^{\circ}\text{C}$ ). Data of strain and tensile force were collected twice per second. Elastic modulus ( $E$ , MPa) was obtained from a linear region at the initial part of the S-S curve. Maximum stress ( $\sigma_{\text{max}}$ , MPa) and maximum strain ( $\varepsilon_{\text{max}}$ , %) were determined at the point of failure of the sheets, and the difference was evaluated using Turkey's  $t$ -test.

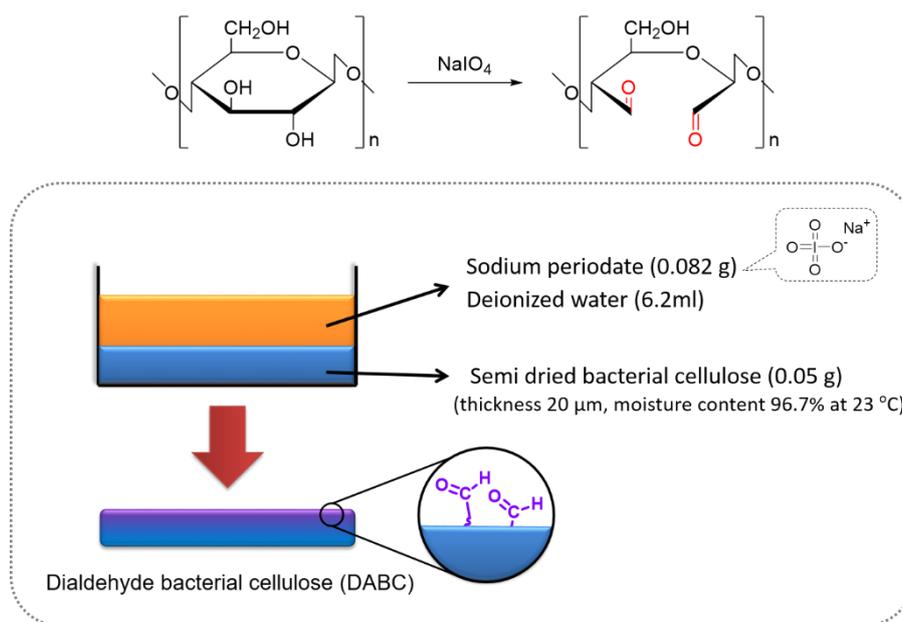
Wide-angle X-ray diffraction (WAXD) measurements under reflection mode were performed at  $25 \text{ }^{\circ}\text{C}$  using a Rigaku Ultima-IV diffractometer (Rigaku Co., Tokyo, Japan). Nickel-filtered  $\text{CuK}\alpha$  radiation ( $\lambda = 0.1542 \text{ nm}$ ) was used at 40 kV and 40 mA. The diffraction intensity profiles were collected in the range of  $2\theta = 5$  to  $40^{\circ}$  with a  $0.02^{\circ} \text{ min}^{-1}$  step.

### **Selective one-sided surface modification of bacterial cellulose sheet**

For selective modification of the one side surface of the BC sheets, a simple equipment for the reaction was used as follows (Scheme 1). Semi-dried BC sheets ( $\text{Ø} = 5.7 \text{ cm}$ , thickness  $20 \text{ }\mu\text{m}$  and  $10 \text{ mm}$ , moisture content 96.7% at  $23 \text{ }^{\circ}\text{C}$ ) and dried BC sheet ( $\text{Ø} = 5.7 \text{ cm}$ , thickness about  $10 \text{ }\mu\text{m}$ ) were separately set and fixed on glass Erlenmeyer flask (commercial plastic containers is also useable) and treated with periodate solution under dark for 0-3 h at  $30 \text{ }^{\circ}\text{C}$  to  $70 \text{ }^{\circ}\text{C}$ . The concentration of periodate was set at cellulose/sodium periodate near 1.21 (w/w) according to Sirviöa et al. (2011)<sup>9</sup>. After thorough washing with water until neutral pH, dialdehyde bacterial cellulose (DABC) was obtained and further used for derivatization under never-dried condition.

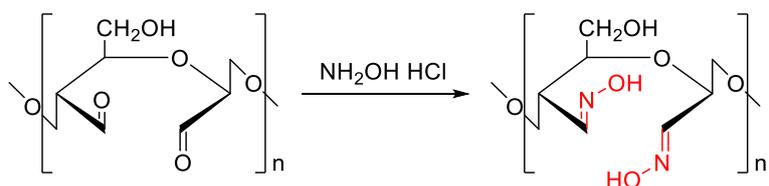
For ATR measurement, DABCs were dried overnight at 90 °C.

**Scheme 1-1.** Surface modification of bacterial cellulose.



### Determination of the aldehyde content

Degree of conversion to aldehyde was calculated based on the measurement of nitrogen content after oxime reaction between aldehyde group and hydroxylamine hydrochloride (Figure 1-1). A never-dried DABC gel sheet was soaked in 100 mL of 0.1 M acetate buffer (pH 4.5) and reacted with 1.39 g of hydroxylamine hydrochloride under dark for 48 h at room temperature. The product was washed thoroughly with Mili-Q water and dried. Elemental nitrogen content of the derivatized DABC was determined by using CHN Corder at Elemental Analysis Laboratory, Graduate School of Engineering, Osaka University.



**Figure 1-1.** Formation of oximes between aldehyde group and hydroxylamine hydrochloride.

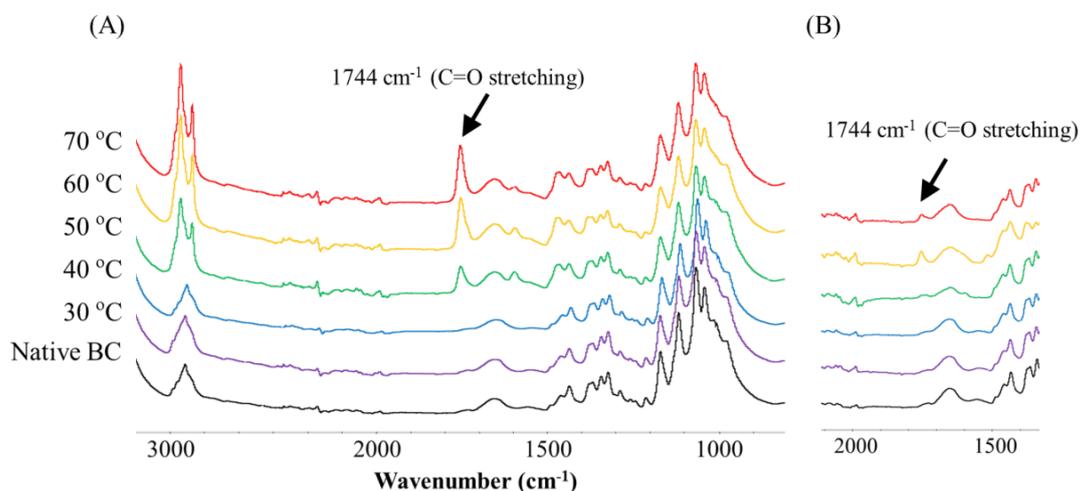
### Detection of aldehyde group on bacterial cellulose

Aldehyde groups were formed magenta colored materials by the Schiff's reagent. The Schiff's reagent was produced using the conventional protocol<sup>11</sup>. First, 2.5 g of basic fuchsin dissolved in 500 ml of deionized water, then 5g of sodium disulfite and 50 ml of 1 N HCl were added. The solution was stirred for several minutes, and then it was decolorized with about 2 g of activated charcoal. DABC was soaked into the Schiff's reagent and left overnight then transferred to bath containing 0.1% sodium disulfite, 0.01 N HCl for several hours, rinse with deionized water until rinse solution failed to change the color.

## Results and discussion

### Modification of one-sided surface of bacterial cellulose sheet by 2,3-dialdehyde

BC sheets were reacted with sodium periodate by using the reaction vessel devised as described in the experimental section. ATR spectroscopic analysis was found to be an indispensable for monitoring the dialdehyde conversion process by using the dried reacted BC sheet. When the surfaces of the reacted BC were analyzed, a new absorption peak at around  $1744\text{ cm}^{-1}$  assignable to the aldehyde group could be easily detected on the spectra with the progression of the reaction. The results indicated that the reaction was largely depended on the reaction temperature and duration of the reaction time. The representative ATR spectra of both the upper and lower surfaces of the BC sheets having a thickness of  $20\text{ }\mu\text{m}$  after reaction for 3 h at five different temperature conditions are shown in Figure 1-2. The results clearly showed that the reaction could have occurred exclusively at the upper surface BC sheets up to  $50\text{ }^{\circ}\text{C}$ , and no extension of the absorption at  $1744\text{ cm}^{-1}$  could be detected on the lower surface of the BC sheet. Beyond this temperature, however, although progression of the reaction was evident by the strengthening of this absorption at the upper surface, the same absorption also became evident at the lower surface, indicating diminishing surface selectivity.



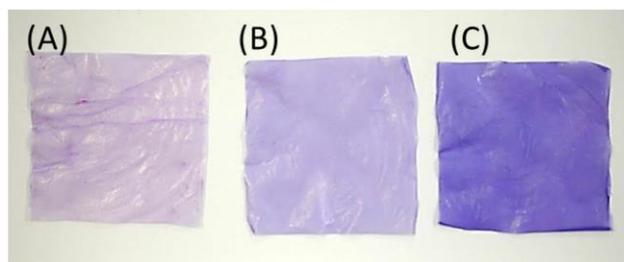
**Figure 1-2.** ATR spectra of (A) upper surface and (B) lower surface.

The progression of this conversion was estimated by measurement of the degree of aldehyde conversion per glucose unit based on the elemental nitrogen content, and the degree of conversion became higher with increase in reaction temperature as shown in the Table 1-1. The effect of the reaction time was also estimated by the same method, and the results (Table 1-1) also showed the progression of the reaction by extension of the reaction time. However, extending the reaction time longer than 3 h at 50 °C showed progression of the reaction even at the lower surface of the sheet similar to the results obtained by the ATR measurement. Lowering the degree of aldehyde conversion at 70 °C may have occurred by solubilization of the over-reaction products as previously shown by Sirviö et al.<sup>9</sup>. In addition, the use of the thick BC gel (never dried BC) was inadequate for selective surface modification because of the penetration of periodate inside the gel as evidenced by the appearance of gradation of purple color produced by reaction with Schiff's reagent from the upper surface to the lower surface

(Figure 1-3). In addition, It was also found that dried BC sheet was not suitable for the present purpose, because of low activity, in accordance with the previous observation at the time of enzymatic saccharification<sup>12</sup> and solubilization<sup>7</sup>. Based on the results presented above, it may be sufficient to show preferential surface modification of semi-dried BC sheet having thickness of 20  $\mu\text{m}$  under the reaction condition of 3 h at 50 °C.

**Table 1-1.** Aldehyde content of DABC produced under various condition.

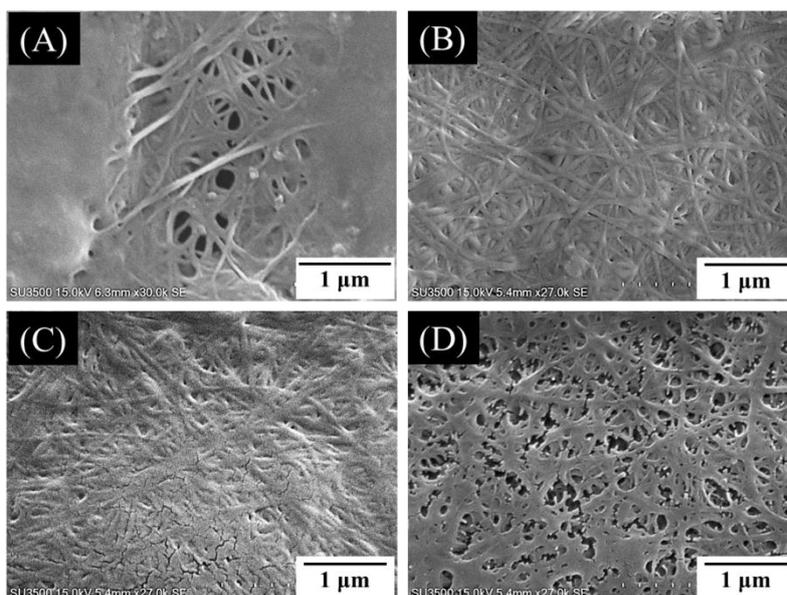
<b>Reaction condition</b>	<b>Aldehyde /Glc</b>	<b>Reaction condition</b>	<b>Aldehyde /Glc</b>
Native BC	0	4 h at 50 °C	0.134
3 h at 40 °C	0.109	3 h at 60 °C	0.228
1 h at 50 °C	0.068	3 h at 70 °C	0.179
2 h at 50 °C	0.098	3 h at 60 °C (thick sheet)	0.152
3 h at 50 °C	0.164	3 h at 60 °C (dried sheet)	0.121



**Figure 1-3.** Detection of aldehyde group on bacterial cellulose by using by the Schiff's reagent. (Reaction condition : (A) 3h at 30 °C, (B) 3h at 40 °C, (B) 3h at 50 °C)

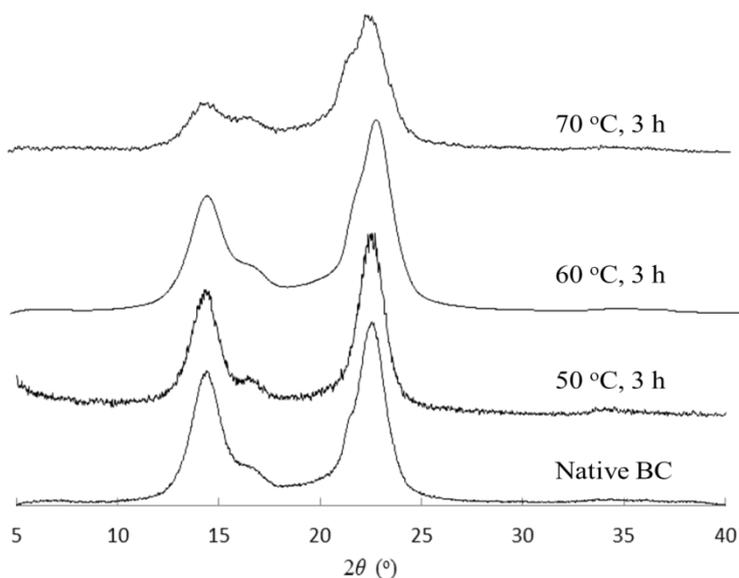
### Characterization of dialdehyde bacteria cellulose sheet

Properties of the DABCs were further analyzed by SEM, WAXD, TGA and TT. Figure 1-4 shows the SEM images of the native BC and DABC sheets. The nano-network structure originally presented by the native BC was still preserved in the DABC sheets. However, some cleaved fiber images probably formed by irradiation of the electron beam began to appear at 60 °C and became more evident at 70 °C, indicating the weakening of mechanical strength of the DABC sheets.

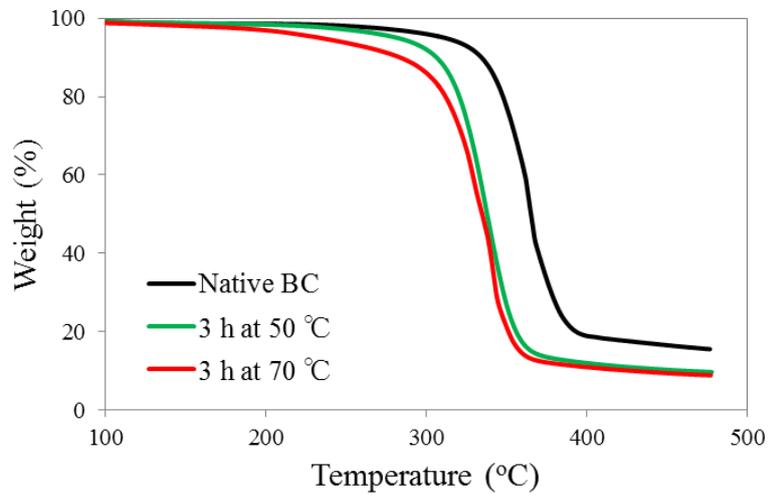


**Figure 1-4.** SEM images of (A) native BC, (B) DABC modified at 50 °C for 3 h, (C) DABC modified at 60 °C for 3 h and (D) DABC modified at 70 °C for 3 h.

The X-ray diffraction profiles shown in Figure 1-5 indicate the remaining of crystalline allomorph  $I\alpha$  after all reactions, supporting the inertness of the majority of cellulose crystalline structure with surface modification. The diameter of cellulose microfibrils (20-50 nm) did not change after reactions. The TGA curves (Figure 1-6) show that the onset temperature for degradation of DABC is lower than that of the native one. In addition TT analysis (Table 1-2) indicated that maximum stress, maximum strain, and elastic modulus dropped with the progression of dialdehyde conversion. At the optimum condition for one-sided surface modification of BC sheets (3 h at 50 °C), 32.8%, 29.0% and 64.7% of maximum stress, maximum strain and elastic modulus remained after the modification. This result suggests the importance of the surface structure of BC sheets for their mechanical strength.



**Figure 1-5.** X-Ray diffraction profiles of the native BC and DABCs.



**Figure 1-6.** TGA curves of native BC and its derivatives.

**Table 1-2.** Mechanical properties of native BC and DABCs

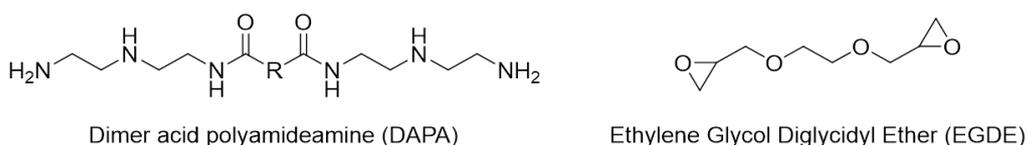
	Maximum stress (MPa)	Maximum strain (%)	Elastic modulus (MPa)
<b>Native BC</b>	192.0 ± 14.3 <sup>a</sup>	3.1 ± 0.4 <sup>a</sup>	9,369.1 ± 298.7 <sup>a</sup>
<b>3 h at 40 °C</b>	108.2 ± 10.2 <sup>be</sup>	1.8 ± 0.1 <sup>bef</sup>	7,892.9 ± 540.5 <sup>b</sup>
<b>3 h at 50 °C</b>	79.7 ± 5.5 <sup>ce</sup>	1.3 ± 0.5 <sup>cef</sup>	6,261.9 ± 372.3 <sup>ce</sup>
<b>3 h at 60 °C</b>	63.0 ± 6.7 <sup>d</sup>	0.9 ± 0.2 <sup>df</sup>	6,058.9 ± 241.4 <sup>de</sup>

Data are presented as mean ± SD (n=6).

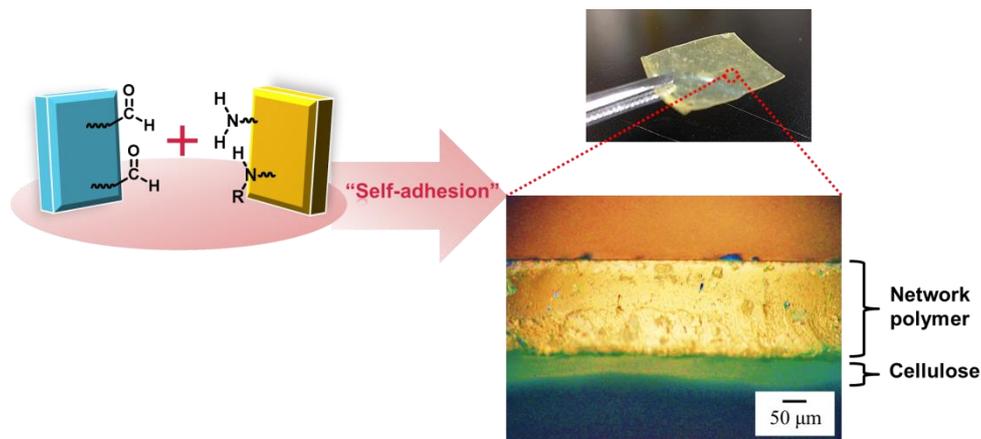
Significant differences ( $P < 0.05$ ) are indicated by different superscript letters.

### Laminated biobased composite using dialdehyde bacteria cellulose sheet

A laminated biocomposite consisting of a plant oil-based network polymer (OBP) and BC aiming at artificial plant cuticle was developed. OBP was obtained from an amine-terminated dimer acid derivative from pine oil and an aliphatic epoxide (Figure 1-7). An excess of the dimer acid derivative was used to produce an amine-containing OBP. The OBP sheet and BC-based 2,3-dialdehyde cellulose sheet strongly adhered without any external forces, whereas the adhesion between sheets of OBP and untreated BC did not take place (Figure 1-8). This interesting self-adhesion is probably due to the reaction between the amino group of OBP and the aldehyde group of DABC. The present results will open new direction of practical usage of surface-modified cellulose sheets for functional materials by combination of oil-based elastic network polymers.



**Figure 1-7.** Molecular structures of amine-terminated dimer acid derivative from pine oil and an aliphatic epoxide



**Figure 1-8.** Photograph of laminated OBP-DABC composite sheet and its cross-section: upper layer, OBP; under layer, DAC.

## Conclusions

By controlling the reaction condition for periodate oxidation at 50 °C for 3 h, selective modification of the one-sided surface of BC sheets was optimally achieved. The present results is the starting point for preparing practical surface modified cellulose sheets.

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## **Chapter 2.**

### Unique Enhancement of Thermostability in a Green Composite of Bacterial Cellulose and Poly(vinyl alcohol)

#### **Introduction**

Bacterial cellulose (BC) is a popular food component in Asian countries such as Philippines, Indonesia and Japan. Fermentation of coconut water using *Gluconacetobacter xylinus* produces gelatinous BC<sup>1-4</sup>, which is called nata-de-coco in these countries. Different from plant-derived cellulose, there are several characteristics such as nano-sized network structure, high purity, high crystallinity, high tensile strength and high water-holding capacity<sup>1,2,4</sup>. Interestingly, the moisture content is around 99%<sup>2</sup>. These features prompted us to design a new polymer composite using BC as a matrix. So far, preparation of BC-containing polymer composites by impregnation<sup>5-7</sup>, blend<sup>5,8-12</sup>, dissolved<sup>5</sup> and in-situ methods<sup>5,13,14</sup> have been reported, and the characteristics of BC unfortunately did not remain in some cases.

Hydrophilic polymers are suitable candidates for composites with hydrophilic BC. Poly(vinyl alcohol) (PVA) is one of the most popular hydrophilic polymers and it has attracted a lot of attentions because of its biocompatible and non-toxic properties<sup>7-10,15</sup>. Chemical or physical crosslinking of PVA is often used to improve its mechanical strength and a freeze-thaw cycle method for preparation of the physically crosslinked PVA has been of specific interest due to a simple process<sup>8,15-19</sup>.

Another strategy for reinforcement of PVA is the combination of cellulose. Although cellulose is highly hydrophilic, the uniform dispersal with PVA is challenging owing to

the low processability<sup>20,21</sup>. Using gelations BC as matrix will provide good dispersibility of cellulose in a PVA aqueous solution.

This chapter deals with a BC-PVA composite prepared by a bulk method, in which gelation of PVA was conducted by cyclic freezing-thawing technique in the presence of BC sheet. The resulting composite is expected to have the network structure from both materials. Interestingly, the thermostability of the composite was greatly improved in spite of immiscibility of cellulose and PVA chains. Relevant to this study, the similar composite hydrogel of BC and PVA was prepared and the mechanical properties were extensively investigated; however, only the effect of water state and polymer chain motion in the composite was mentioned<sup>22</sup>.

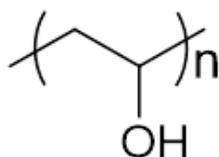
## **Experimental section**

### **Preparation of BC**

BC hydrogel was prepared as follows. *Gluconacetobacter xylinus* (NBRC 13693) was purchased from National Institute of Technology and Evaluation (NITE), Japan. The medium for NBRC 13693 contained 0.5 w/v % polypepton, 0.5 w/v % yeast extract, 0.5 w/v % glucose, 0.5 w/v % mannitol, 0.1 w/v %  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.5 v/v % ethanol, and pH of the medium was adjusted at 6.6 by acetic acid. Autoclaved medium was inoculated and incubated under static condition at 30 °C for 2 weeks. The resulting BC hydrogel sheet was washed with deionized water for 3 days and immersed in boiling 2% aqueous NaOH solution. Finally, it was rinsed with deionized water pH became neutral. The obtained sheet had 7.3 mm thickness and contained around 99 w% of moisture.

### **Preparation of the BC-PVA composite**

PVA (0.50 g,  $M_n = 88000$ , degree of hydrolysis = 98 %, Figure 2-1) powder was dissolved in 2.5 ml of deionized water at 85 °C for 6 h. Subsequently BC hydrogel sheet ( $\text{Ø} = 2$  cm) was added to the PVA solution, which was kept at 85 °C. After 1 day, it froze at -20 °C for 20 h and thawed at 25 °C for 2 h. The BC-PVA composite hydrogel was prepared by this freeze/thaw protocol with different cycle numbers. PVA hydrogel as a control sample was prepared by the same protocol without BC.



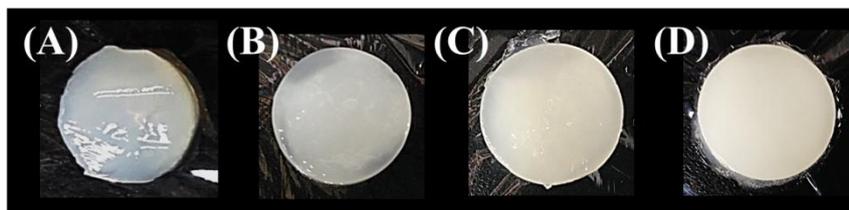
**Figure 2-1.** Molecular structure of of PVA.

### **Characterization**

Fourier transform infrared (FTIR) measurement was carried out in an attenuated total reflectance (ATR) mode by a Nicolet iS5 Spectrometer (Thermo Fisher Scientific Inc., USA). Scanning electron microscopy (SEM) images were obtained on a HITACHI S-3500 instrument (Hitachi Co., Japan). The samples were lyophilized before the observation. Thermal properties of the BC-PVA composite with 3 cycles were analyzed by differential scanning calorimetry (DSC) and thermogravimetric analysis (TGA). The samples were freeze dried prior to these measurements. DSC data were obtained by using Seiko DSC6020 (Hitachi High-Tech Science Co., Japan) at a heating rate of 10 °C/min in the range of 40 - 300 °C under a flowing nitrogen atmosphere. TGA was performed with an EXSTAR TG/DTA 7200 thermogravimetric analyzer (Hitachi High-Tech Science Co., Japan) from 40 to 500 °C at a heating rate of 10 °C/min under a flowing nitrogen atmosphere. Dynamic mechanical analysis (DMA) of the hydrogel composites were evaluated by using HAAKE RheoStress 6000 (Thermo Fisher Scientific Inc., USA) at 25 °C.

## Results and Discussion

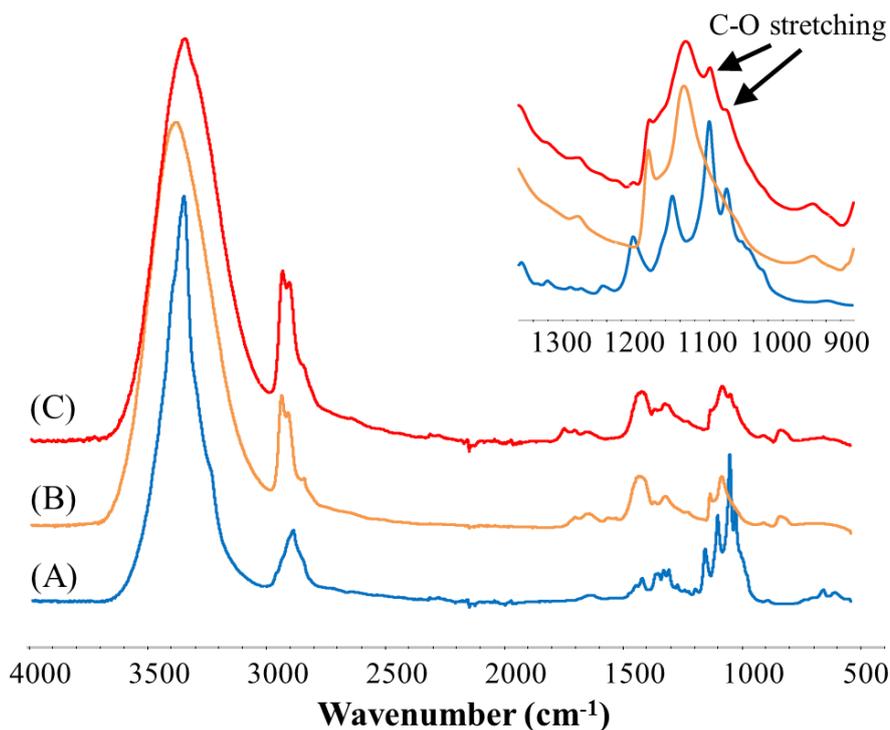
In this chapter, the BC-PVA composite hydrogel samples were prepared by different freezing and thawing cycles<sup>23</sup>. Based on the weight change before and after the freezing/thaw process, the weight ratio of BC and PVA in the composite was calculated as 1:8. The first-cycle hydrogel was transparent, and the hydrogel became opaque white with increase of the cycle number (Figure 2-2), which may be due to the growing of PVA crystallites<sup>15,16</sup>.



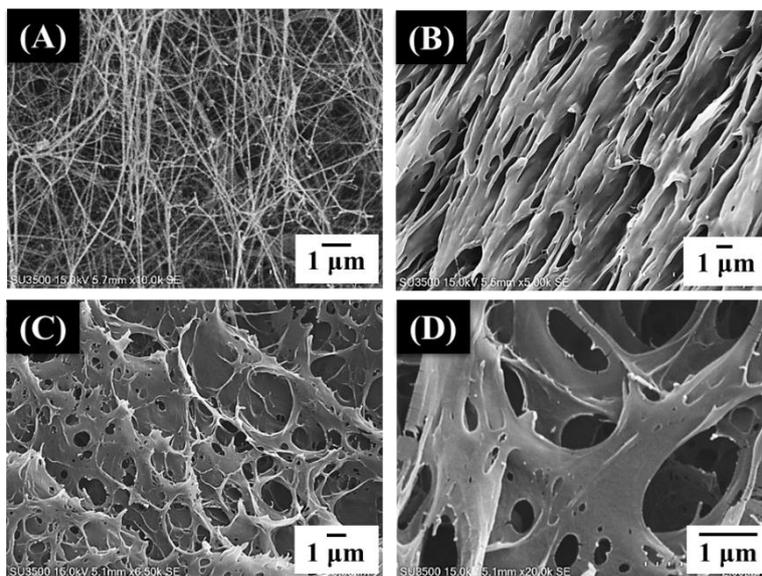
**Figure 2-2.** Images of (A) native BC, (B) BC-PVA composite with 1 cycle, (C) BC-PVA composite with 2 cycles and (D) BC-PVA composite with 3 cycles.

The composite formation was confirmed by ATR-FTIR; characteristics peaks of BC and PVA were found in the spectrum of the composite with 3 cycles (Figure 2-3). In the spectrum of the BC-PVA composite, almost of the all peaks were similar with those of PVA due to the much larger ratio of PVA to BC. However, small peaks at 1060 and 1036  $\text{cm}^{-1}$  ascribed to C-O stretching of cellulose were also observed (expanded chart in Figure 2-3)<sup>11,20</sup>. The presence of these bands indicates the existence of BC in the present composite. In addition, spectra of the several portions of the composite were the same, supporting the homogeneity of the composite. SEM images of BC, PVA

hydrogel, and the BC-PVA composite are shown in Figure 2-4. The samples were lyophilized before the observation. Native BC is well known to have three-dimensional nano-scale fibrous network structure. The morphology of the composite was somewhat different from that of the PVA hydrogel. Additionally, the nanofibrous structure of cellulose could be hardly found in the SEM image of the composite. These data strongly suggest that PVA covers the BC fibers in the dried composite and the network structure of BC is maintained.



**Figure 2-3.** ATR-FTIR spectra of (A) native BC, (B) PVA and (C) BC-PVA composite (weight ratio of BC and PVA = 1:8) with 3 cycles.



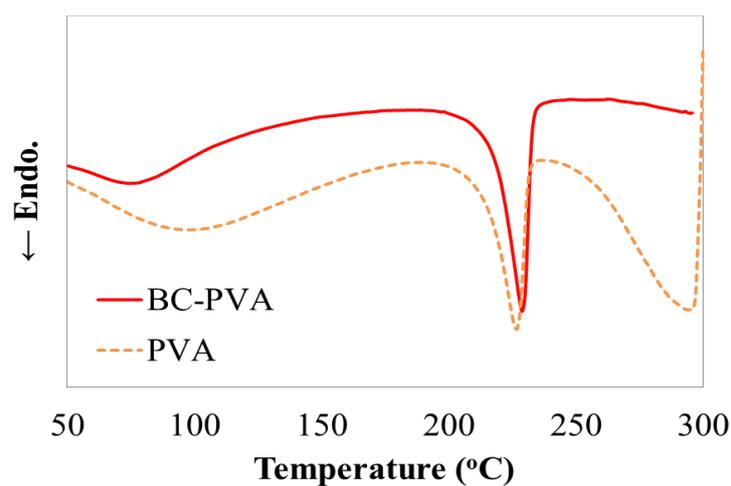
**Figure 2-4.** SEM images of lyophilized samples of (A) native BC, (B) PVA hydrogel and (C, D) BC-PVA hydrogel composite with 3 cycles (weight ratio of BC and PVA = 1:8).

Thermal properties of the BC-PVA composite with 3 cycles were analysed by DSC and TGA. Table 2-1 summarizes DSC results (first heating scan) of the PVA hydrogel and the composite (after lyophilization). The DSC curves are shown in Figure 2-5. Both had the melting point ( $T_m$ ) ascribed to PVA, and  $T_m$  of the composite was very close to that of PVA, implying that PVA and BC are immiscible in the composite. The enthalpy of crystallization ( $\Delta H$ ) of the composite was higher than that of PVA. This is probably because of the rapid formation of crystallites of PVA in the composite, in which the BC fibril works as a nucleating agent. This result conflicts with earlier study using cellulose nanocrystals (CNC) and PVA; CNC interrupted the crystallization of PVA<sup>20,21</sup>. It suggests that BC has high affinity with PVA under the wet condition. Thermal degradation of dried samples of BC, the PVA hydrogel and the BC-PVA hydrogel composite was examined by TGA (Figure 2-6). Interestingly, the initial weight

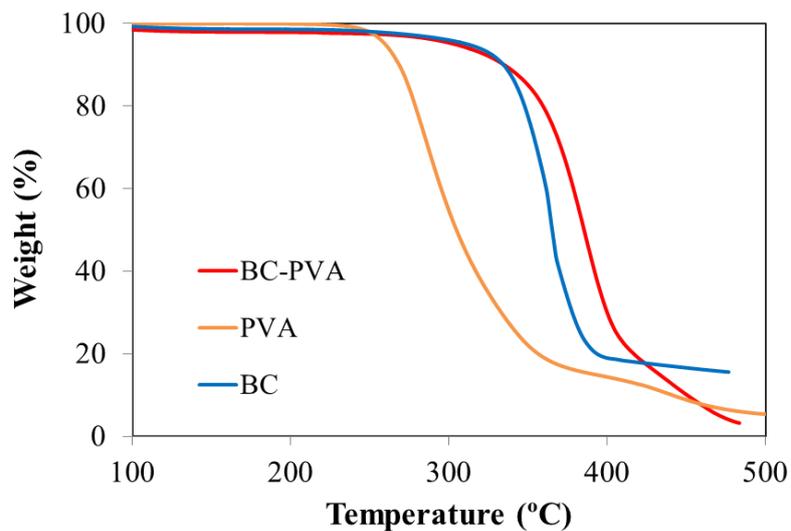
loss of the composite occurred at around 350 °C, although the composite contained only less than 7 wt% of BC. This value was higher than that of native BC or PVA, indicating the significant improvement of the thermal stability of both components by the present method without any crosslinking agents. It could be attributed to the formation of the physical crosslinking via hydrogen bond between PVA and cellulose chains.

**Table 2-1.** DSC results of the first heating process for PVA hydrogel and BC-PVA hydrogel composite with 3 cycles.

	$T_m$ (°C)	$\Delta H_m$ (J/g)	Crystallinity (%)
PVA	226	55	39
BC-PVA	229	73	53



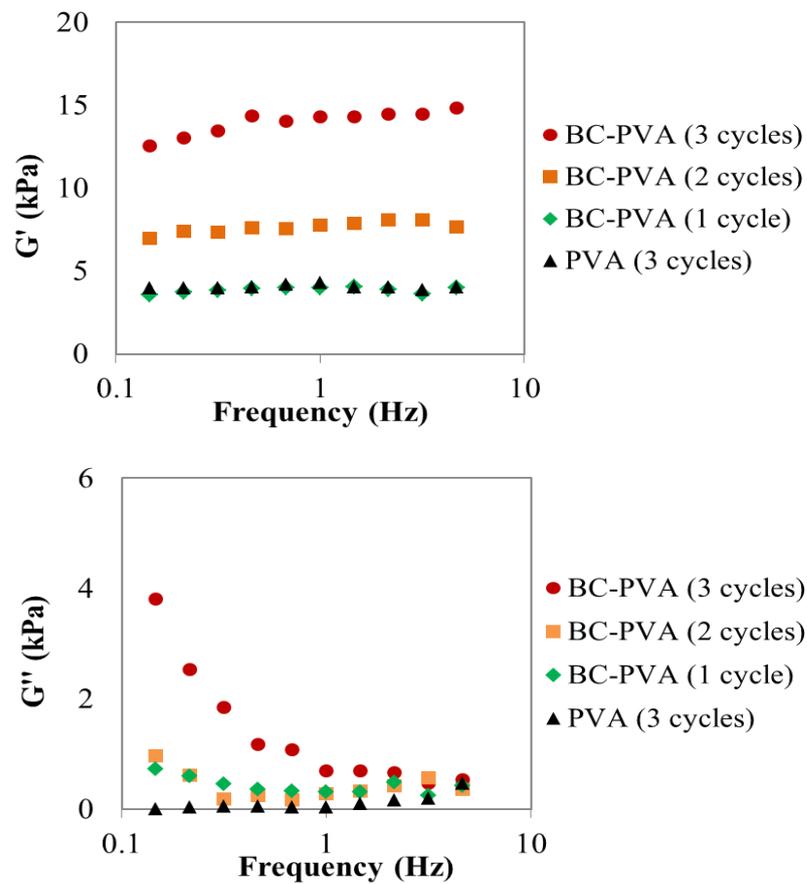
**Figure 2-5.** DSC charts (first heating) of PVA and BC-PVA composite (weight ratio of BC and PVA = 1:8) with 3 cycles.



**Figure 2-6.** TGA curves of BC, PVA hydrogel and BC-PVA hydrogel composite with 3 cycles.

The viscoelasticity properties of the hydrogels were evaluated by using DMA. In most samples, the values of the loss moduli  $G''$  were lower than that of the storage moduli  $G'$  (Figure 2-7), confirming that the composite is a hydrogel. The dependence of the storage modulus ( $G'$ ) on frequency was very small, suggesting the formation of the homogeneous hydrogel. The  $G'$  value of the composite significantly increased as a function of the freezing and thawing cycles and, after 3 cycles the  $G'$  value was almost constant. This may be because the network structure of both components via the hydrogen bonding is formed during the freezing and thawing cycles. The  $G'$  value of the PVA hydrogel with 3 cycles was close to that of the composite with 1 cycle, implying the efficient reinforcement effect of the BC fibril. These results show that the network structure between BC and PVA grows by the freeze-thawing method to reinforce the

composite. The improvement of the thermal stability might be owing to this unique network structure of the composite.



**Figure 2-7.** Dynamic frequency sweep of the storage moduli ( $G'$ ) and loss moduli ( $G''$ ) of BC, PVA and BC-PVA composite with 3 cycles (weight ratio of BC and PVA = 1:8).

## **Conclusions**

A strong hydrogel composite of biocompatible components was successfully prepared by the facile freeze-thaw method from a combination of BC and PVA without any crosslinking reagents. The PVA solution penetrated the network of BC and PVA covered the nanofibrous BC matrix in the dried state. Despite of the immiscibility between BC and PVA in the molecular level, the thermal stability and mechanical strength of the composite were much improved in comparison with those of PVA. These characteristics would be due to the strong interaction of both components in the network structure as well as high crystallinity of PVA. The present composite has large potential for various applications such as biomedical and cosmetic matrix.

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## **Chapter 3.**

### **One-dimensional Shrinkage and Swelling of Crosslinked Bacterial Cellulose Gel**

#### **Introduction**

Bacterial cellulose (BC) is a fermentation product using a medium containing glucose and *Gluconacetobacter xylinus*<sup>1-4</sup>. In most cases, BC is obtained as a hydrogel form. In comparison to plant-derived cellulose, BC has several interesting characteristics such as nano-sized network structure, high purity, high crystallinity and high moisture content (around 99%)<sup>1-4</sup>. Additionally, BC with layered structure is formed under static culture conditions<sup>1,3,5</sup>. On the basis of the characteristic structure<sup>1,4-6</sup>, BC hydrogel shows mechanical anisotropy<sup>6</sup>; BC hydrogel sheet has high tensile strength<sup>1-4</sup>; whereas it is easy to deform and lose the moisture under compression<sup>6-8</sup>. These unique properties lend itself to the surface modification and the preparation of composites with various polymers<sup>9-11</sup>. On the other hand, delamination of BC in the composite with poly(L-lactic acid) has been reported<sup>11</sup>. Consequently, the reinforcement of the BC layered structure is required to expand the applications.

Generally, dried cellulose sheet can not swell or dissolve in common solvents including water, because of the strong hydrogen bondings between cellulose chains<sup>12-14</sup>. BC also hardly swells in water after once drying. On the other hand, cellulose solvents can break the hydrogen bondings of cellulose. Solubilization of BC in cellulose solvents was examined for analysis<sup>15</sup>, regeneration<sup>9</sup>, and homogeneous modification of the cellulose<sup>9,16</sup>; however, the characteristics of BC did not remain in some cases. In this

chapter, we focused on the layered structure of BC and demonstrated the unique one-dimensional shrinkage-swelling behaviors of the crosslinked BC gel. Interestingly, the BC hydrogel was subjected to the solvent exchange with various organic solvents by a convenient immersion procedure, in which shrinkage, deformation, and destroy of the gel did not take place by choosing the conditions. Here, the solvent of the BC gel exchanged from water to acetone and the crosslinking with diisocyanate was conducted in acetone.

Modifications of cellulose are often performed in a heterogeneous system; cellulose powders are used as a suspension or cellulose fiber mats are immersed in a reaction solvent. Many studies on the crosslinking of cellulose by glutaraldehyde<sup>17</sup>, glyoxal<sup>18</sup>, divinyl sulfone<sup>19</sup>, epichlorohydrin<sup>20</sup>, diisocyanate<sup>21,22</sup>, and citric acid<sup>23</sup> were reported. Homogeneous modifications are often limited due to the range of solvents which can solubilize cellulose. If the crosslinking of BC proceeds in its gel form, the network formation of BC may take place mainly in the layer, leading to the efficient reinforcement of the BC sheet. In this chapter, methylenediphenyl 4,4'-diisocyanate (MDI) was used as crosslinking agent. By using acetone as gel medium, isocyanate compounds with high reactivity could be used under mild and anhydrous conditions.

## Experimental section

### Materials

Methylenediphenyl 4,4'-diisocyanate (MDI), 3,5-dimethyl phenyl isocyanates (DMPI) and dimethylacetamide (DMAc) were purchased from Tokyo Chemical Industry Co., Ltd., Japan. Triethylamine (TEA) and acetone were purchased from Nacalai Tesque, Inc., Japan. LiCl was purchased from Wako Pure Chemical Industries, Ltd., Japan.

Water was deionized by using a G-10C cartridge water purification equipment (Organo co., Japan). For preparation of the medium, water was freed from salt using Milli-Q water system (Nihon Millipore, Japan).

### Preparation of BC

BC hydrogel was prepared as follows. *Gluconacetobacter xylinus* (NBRC 13693) was purchased from National Institute of Technology and Evaluation, Japan. The medium for NBRC 13693 contained 0.5 w/v % polypepton, 0.5 w/v % yeast extract, 0.5 w/v % glucose, 0.5 w/v % mannitol, 0.1 w/v %  $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ , and 0.5 v/v % ethanol, and pH of the medium was adjusted at 6.6 by acetic acid. Autoclaved medium was inoculated and incubated under static condition at 30 °C for 2 weeks. The resulting BC hydrogel sheet was washed with deionized water for 3 days and immersed in boiling 2% aqueous NaOH solution. Finally, it was rinsed with deionized water until pH became neutral. The obtained sheet had 7.3 mm thickness and contained around 99 weight% of moisture.

### **Crosslinking of BC with diisocyanate**

Water in BC hydrogel was replaced with dehydrated acetone by immersion of the BC hydrogel sheet ( $\varnothing = 2$  cm) into a large amount of dehydrated acetone for 8 h under gentle shaking. The solvent replacement was carried out more than 3 times. MDI was added to the solution in the presence of the BC gel sheet. Subsequently it was agitated under shaking at 25 °C for 24 h. TEA was added in the solution as a catalyst and then the solution with BC was kept at 50 °C. After 48 h, the final product (BC-MDI) was thoroughly washed with acetone, and then a mixture of acetone and deionized water. The modification by DMPI was conducted similarly.

### **Swelling of BC-MDI**

Native BC gel and the BC-MDI gel were dried at room temperature under reduced pressure. The dried sheet samples were immersed in 8 wt% LiCl/DMAc solution at 25 °C under gentle shaking. The swollen samples were weighed; swelling ratio (%) was calculated using the following equation:  $(\text{BC-MDI gel weight} - \text{dried BC-MDI sheet weight}) * 100 / \text{dried BC-MDI sheet weight}$ .

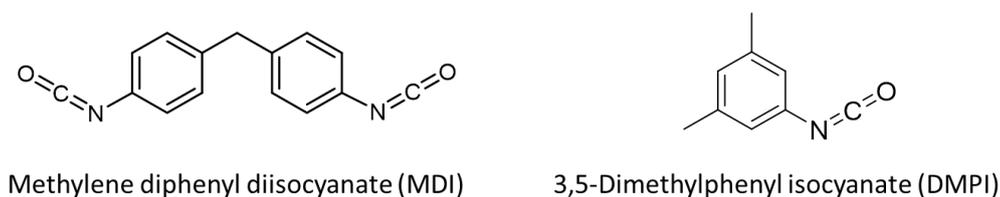
### **Characterization**

Fourier transform infrared (FTIR) measurement was carried out in an attenuated total reflectance (ATR) mode by a Nicolet iS5 Spectrometer (Thermo Fisher Scientific Inc., USA). Elemental analysis was conducted by using CHN Corder. Scanning electron microscopy (SEM) images were obtained on a HITACHI S-3500 instrument (Hitachi Co., Japan). The samples were lyophilized before the observation. Thermal properties of the BC-MDI samples were analyzed by thermogravimetric

analysis (TGA). The samples were freeze-dried prior to these measurements. TGA was performed with an EXSTAR TG/DTA 7200 thermogravimetric analyzer (Hitachi High-Tech Science Co., Japan) from 40 to 500 °C at a heating rate of 10 °C/min under a flowing nitrogen atmosphere.

## Results and discussion

To keep the unique structure of BC, the modification in the gel form is essential for the present study. At first, a model reaction of the BC acetone gel with isocyanate (3,5-dimethyl phenyl isocyanates, DMPI) was performed (Figure 3-1), since there are no reports on the modification of BC in its organogel form. An excess of DMPI (30 fold per glucose unit) was used. As reference, a commercial filter paper was modified under the same conditions. For the BC acetone gel, the introduced ratio of DMPI for BC determined by elemental analysis was around 0.4 per the glucose unit, and that for the filter paper was around 0.06. These data clearly show that the nanofibrous organogel is a good precursor for chemical modifications, which may be due to the high surface area with the wet state in the organogel. Additionally, it was confirmed that the elemental composition of the surface and internal parts was very close to each other.



**Figure 3-1.** Molecular structures of MDI and DMPI.

The crosslinking of BC with MDI, one of the most popular diisocyanates for preparation of industrial polyurethanes<sup>21,24</sup>, was conducted in the acetone gel form. Two samples with different MDI feed ratios (0.2 and 3 per glucose unit of BC) were prepared, which are abbreviated as BC-MDI-0.2 and BC-MDI-3, respectively. For BC-MDI-0.2, little significant difference of the sample color and weight was observed before and

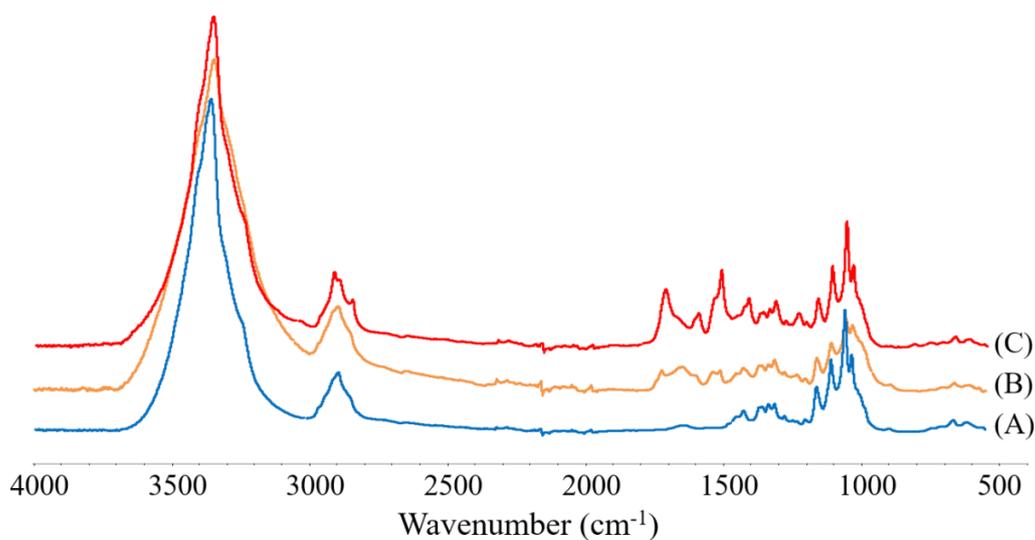
after the reaction (Figure 3-2). On the other hand, the sample color changed white to light orange and the weight of the dried sample slightly increased for BC-MDI-3 (approximately 11 % for dried BC). The introduced ratio of MDI (per glucose) of BC-MDI-0.2 determined by elemental analysis was almost the same as for the model sample (BC-DMPI), and the nitrogen content of BC-MDI-3 (H: 5.45%, C: 52.51%, N: 4.57%) was 3.3 times higher than that of BC-MDI-0.2 (H: 5.95%, C: 44.54%, N: 1.38%). These data indicate that the modification by MDI in the BC acetone gel efficiently proceeded and the introduced ratio of MDI depended on the feed ratio.



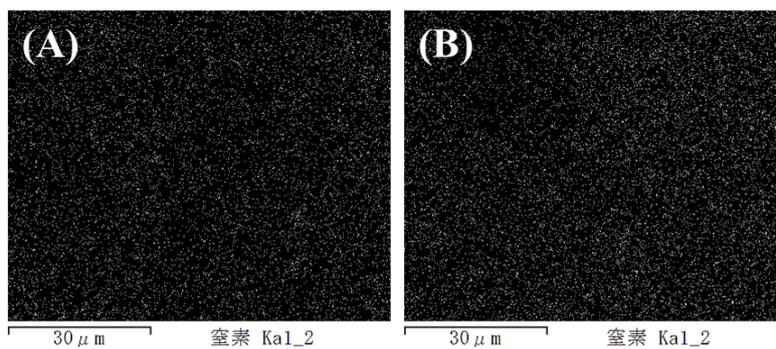
**Figure 3-2.** Images of (A) native BC, (B) BC-MDI-0.2 and (C) BC-MDI-3.

The modification of BC by MDI was confirmed by FT-IR (Figure 3-3). A characteristic peak around  $1720\text{ cm}^{-1}$  ascribed to C=O stretching of urethane linkage was found in the spectra of the BC-MDI samples. For BC-MDI-3, however, it was slightly shifted to  $1715\text{ cm}^{-1}$  in comparison with BC-MDI-0.2. This is probably because of the hydrogen bonded C=O band of the urethane group<sup>21</sup>. The peaks at  $1600\text{ cm}^{-1}$  and  $1537\text{ cm}^{-1}$  due to a C=C stretching of aromatic moiety of MDI also appeared. No absorption band at  $2270\text{ cm}^{-1}$ , which corresponds to the free isocyanate group, was recognized in the BC-MDI spectra, suggesting that the isocyanate group was completely

consumed<sup>21,24</sup>. The spectra of the several portions of the BC-MDI samples were almost the same, supporting the homogeneity of the reaction in the BC acetone gel. Nitrogen atoms were uniformly dispersed in the SEM/EDX images (Figure 3-4).

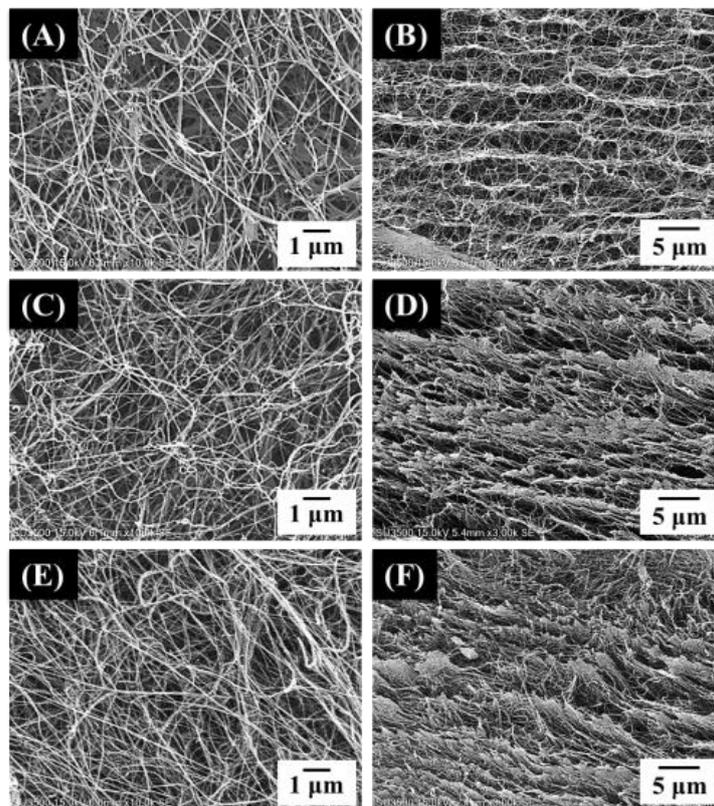


**Figure 3-3.** ATR-FTIR spectra of (A) native BC, (B) BC-MDI-0.2, and (C) BC-MDI-3.



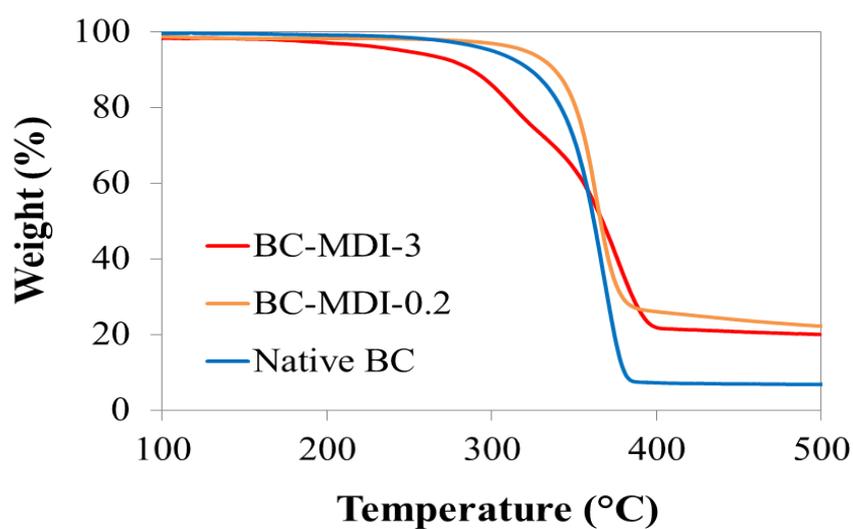
**Figure 3-4.** SEM/EDX images of (A) BC-MDI-0.2 and (B) BC-MDI-3.

Figure 3-5 shows SEM images of lyophilized BC and BC-MDI. The three-dimensional network structure was observed in the horizontal cross section image and the morphology of BC-MDI with different MDI feed ratios was very similar to that of the BC. In the vertical cross-sectional observation, all of the crosslinked samples maintained the layered structure. These data indicate that the morphology of BC hardly changed by the crosslinking by MDI, which is probably because the reaction of the nanofibrous cellulose by MDI occurs in the layer of BC. This little morphological change may be strongly related to the unique shrinkage-swelling behavior of the crosslinked BC gel.



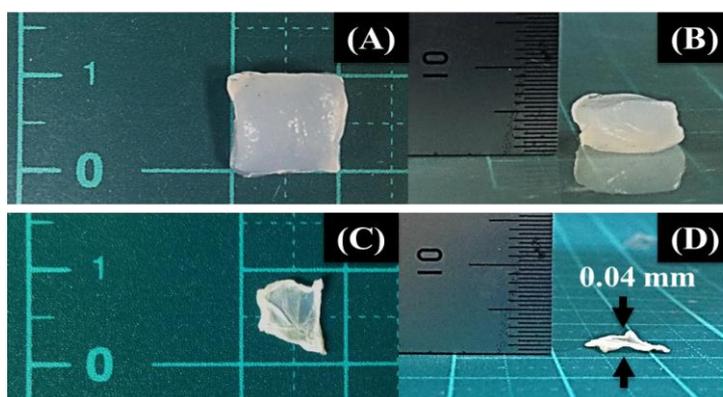
**Figure 3-5.** SEM images of lyophilized samples of (A, B) native BC, (C, D) BC-MDI-0.2 and (E, F) BC-MDI-3. Horizontal and vertical cross-section images are on the left and right columns, respectively.

Thermal degradation of the dried samples of BC and BC-MDI was examined by TGA (Figure 3-6). Previous studies showed the improvement of thermal stability due to the crosslinked structure<sup>25</sup>. The initial weight loss of BC-MDI-0.2 occurred at slightly higher temperature than that of BC probably because of the covalent bond formation between BC and MDI. However, the initial decomposition temperature of BC-MDI-3 was lower than BC-MDI-0.2 as well as BC. These results suggest the interruption of the intramolecular hydrogen bonding in case of the higher content of MDI, supporting the introduction of MDI on the BC surface.

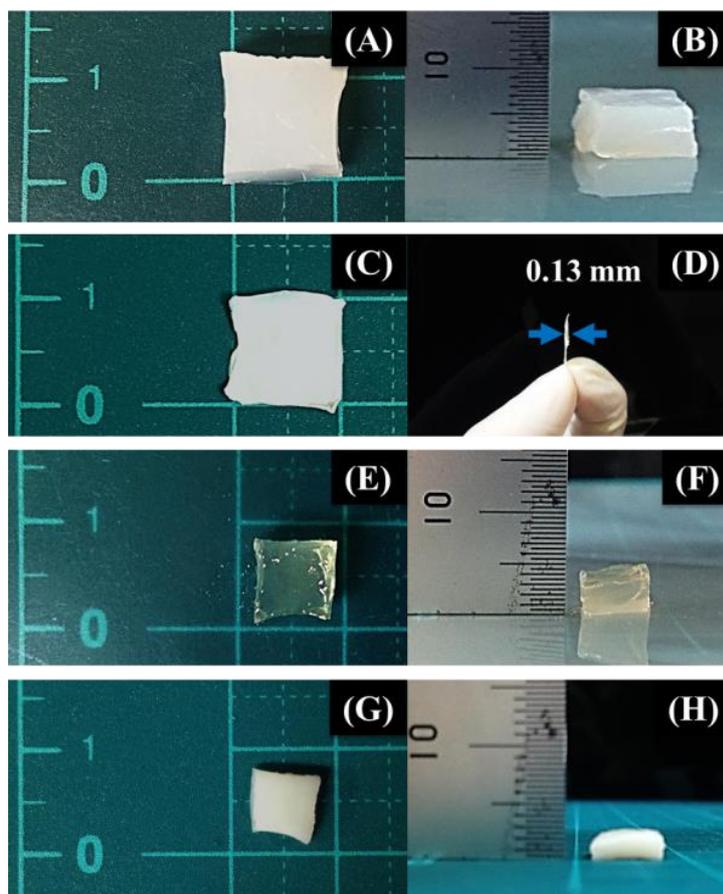


**Figure 3-6.** TGA curves of native BC, BC-MDI-0.2, and BC-MDI-3.

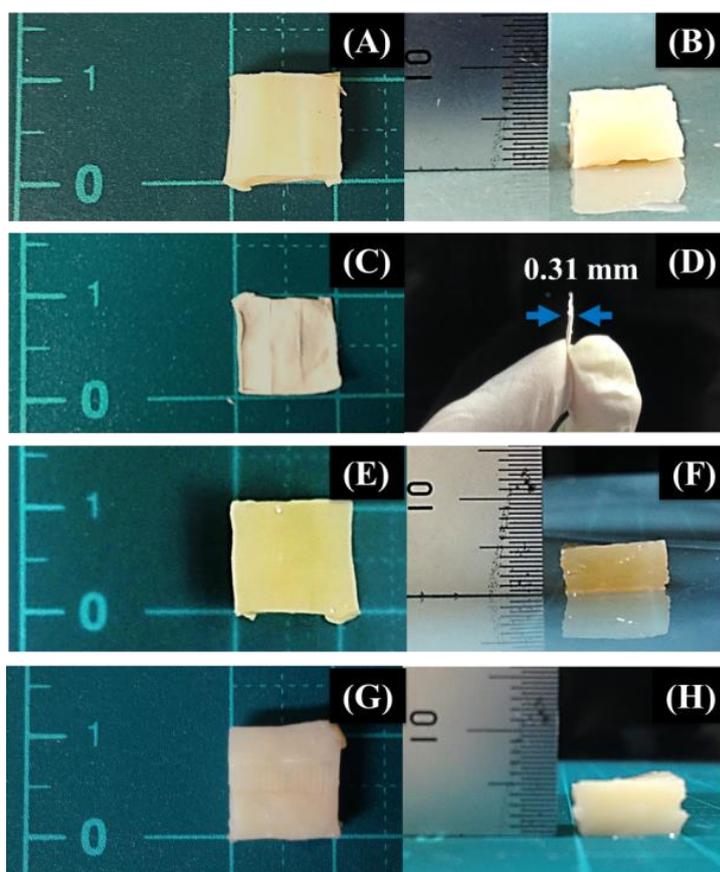
For shrinkage and swelling tests, native BC and BC-MDI samples were first immersed in acetone. Afterwards, the resulting acetone gel was dried under reduced pressure. All the samples one-dimensionally shrank to form the thin sheet. For BC, the extensively deformation took place and the crinkled sheet was obtained (Figure 3-7). On the other hand, such deformation was hardly observed for two BC-MDI samples (Figures 3-8(C) and 3-9(C)). These data clearly show the reinforcement effect of the crosslinked layered structure of BC by MDI. The thickness of the dried sheet of BC-MDI was slightly larger than that of BC, which may be because the introduced MDI group interrupts the stacking of the BC layers.



**Figure 3-7.** Images of (A, B) native BC and (C, D) dried BC thin sheet.



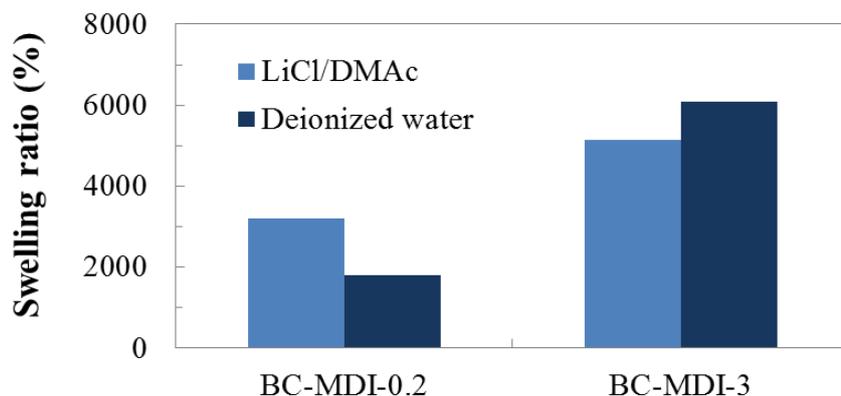
**Figure 3-8.** Images of BC-MDI-0.2 samples: (A, B) original BC-MDI-0.2 hydrogel; (C, D) dried BC-MDI-0.2; (E, F) swollen BC-MDI-0.2 gel in LiCl/DMAc solution for 48 h; (G, H) hydrogel after swelling in deionized water for 48 h.



**Figure 3-9.** Images of BC-MDI-3 samples: (A, B) original BC-MDI-3 hydrogel; (C, D) dried BC-MDI-3 sheet; (E, F) swollen BC-MDI-3 gel in LiCl/DMAc solution for 48 h; (G, H) hydrogel after swelling in deionized water for 48 h.

Next, the dried BC and BC-MDI samples were immersed in 8% LiCl/DMAc which is well known to solubilize cellulose<sup>26</sup>. The dried BC sheet was completely soluble in the LiCl/DMAc solution, whereas BC-MDI samples swelled in this solvent and interestingly, their thickness extensively changed in comparison with their horizontal size (Figures 3-8(E, F) and 3-9(E, F)). For BC-MDI-0.2, the swollen gel was transparent and the slight shrinkage of the horizontal plane was found, whereas the color and size of the horizontal hardly changed for the swollen gel of BC-MDI-3, strongly suggesting the one-dimensional swelling behavior.

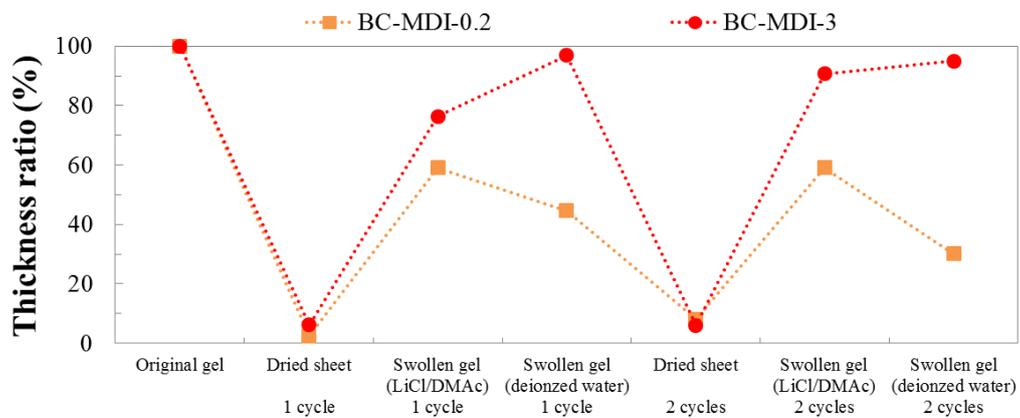
The dried samples between before and after immersing in LiCl/DMAc had almost same weight. These results strongly suggest that the internal crosslinked BC was successfully prepared by using MDI. Figure 3-10 shows the swelling ratio of BC-MDI samples in LiCl/DMAc. Both BC-MDI samples had high swelling ratio and BC-MDI-3 more highly swelled than BC-MDI-0.2. This result is closely related to the data of TGA (Figure 3-6); the interruption of the intramolecular hydrogen bonding by the MDI group on the fiber surface may result in the high accessibility of the solvent molecules.



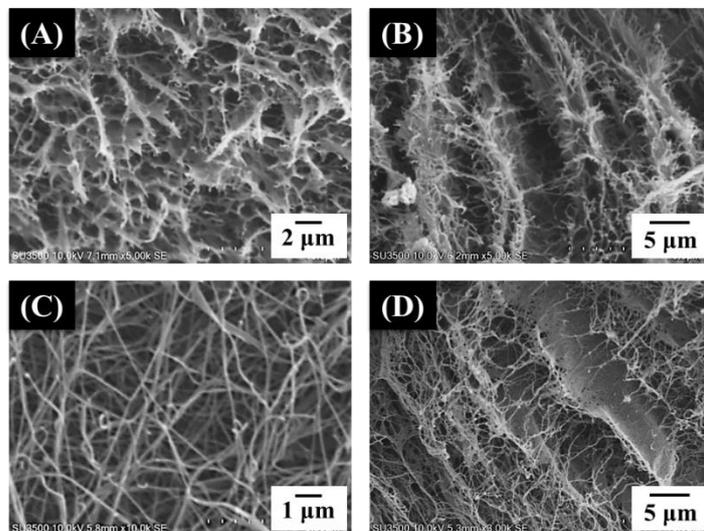
**Figure 3-10.** Swelling ratio of BC-MDI samples after immersing in LiCl/DMAc solvent for 48 h and subsequently the solvent displacement with deionized water for 48 h. Swelling ratio (%) was calculated using the following equation:  $(\text{BC-MDI gel weight} - \text{dried BC-MDI sheet weight}) \times 100 / \text{dried BC-MDI sheet weight}$ .

The swollen gel in LiCl/DMAc was immersed in an excess of water. For BC-MDI-0.2, the gel became white and the slight shrinkage took place (Figure 3-8(G, H), and Figure 3-10). This may be due to the formation of the strong hydrogen-bonding networks<sup>14</sup>. Interestingly, BC-MDI-3 further swelled only in the vertical direction; the horizontal size was almost the same (Figure 3-9(G, H), and Figure 3-10). The final thickness of BC-MDI-3 was close to the original BC gel (Figure 3-11). Furthermore, this unique one-dimensional shrinkage-swelling behavior could be repeatedly achieved for BC-MDI-3. In these repeating shrinkage-swelling processes, the nanofibrous morphology of BC-MDI-3 was hardly changed (Figure 3-12). On the basis of these data, we consider the mechanism of the present unique one-dimensional swelling behavior as follows. As described above, the crosslinking of BC by MDI mainly took place in the

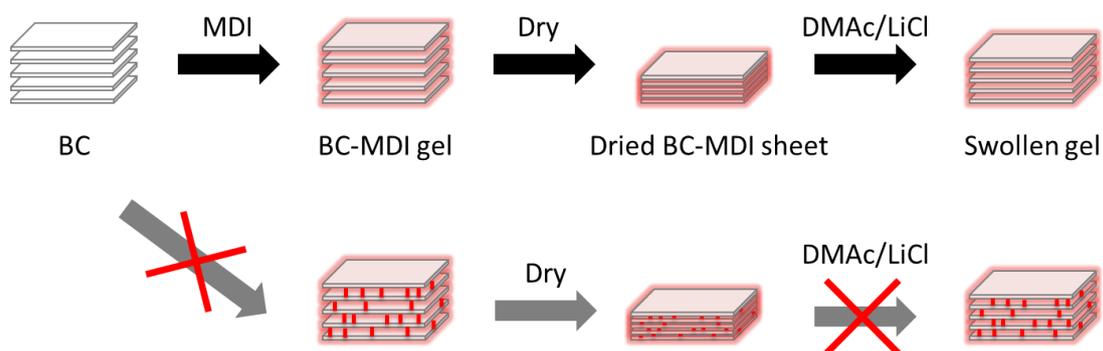
layer of BC. The cellulose solvent (DMAc/LiCl) mainly exfoliates the dried BC-MDI sheet and the swelling toward the horizontal plane is restricted by the crosslinking structure in the BC layer. If the crosslinking occurs between the layers of BC, the dried sheet does not swell in the vertical direction (Figure 3-13).



**Figure 3-11.** Repeated shrinkage-swelling cycles of BC-MDI samples for thickness change.



**Figure 3-12.** SEM images of lyophilized samples of (A, B) BC-MDI-0.2 and (C, D) BC-MDI-3 after 4 shrinkage-swelling cycles. Horizontal and vertical cross-section images are on the left and right columns, respectively.



**Figure 3-13.** The reinforcement of the crosslinked BC layered structure by MDI.

## Conclusions

The present chapter demonstrated unique shrinkage-swelling behaviors of the crosslinked BC (BC-MDI-3). The solvent exchange of BC hydrogel with acetone and subsequent crosslinking by MDI produced the reinforced BC organogel. This gel one-dimensionally shrank by drying under vacuum to give the thin sheet, which also one-dimensionally swelled in LiCl/DMAc. Additionally, the solvent of the swollen gel could be converted to the hydrogel by immersion in water. During this shrinkage-swelling processes, the size of the horizontal plane was almost the constant. This regeneration cycle could be repeatedly conducted. These unique behaviors are probably owing to the layered structure of BC and the crosslinking by MDI mainly in the layer enables the unique shrinkage-swelling behaviors. To our best knowledge, this is the first example on the one-dimensional shrinkage-swelling of polymer gels. Further studies including composite preparations and applications of the present crosslinked BC gel are under progress in our laboratory.

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## ***Concluding Remarks***

In this doctoral thesis, new functional bacterial cellulose-based materials utilizing its unique properties were developed.

In Chapter 1, semi-dried BC hydrogel sheet which has high reactivity was prepared by utilizing nano-sized network structure and layered structure of BC. By controlling the reaction condition for periodate oxidation at 50 °C for 3 h, selective modification of the one-sided surface of BC sheets was optimally achieved. The present results may be the starting point for preparing practical surface-modified cellulose sheets.

In Chapter 2, a strong hydrogel composite of biocompatible components was successfully prepared by the facile freeze-thaw method from a combination of BC and PVA without any crosslinking reagents. The PVA solution penetrated the network of BC and PVA covered the nanofibrous BC matrix in the dried state. Despite of the immiscibility between BC and PVA in the molecular level, the thermal stability and mechanical strength of the composite were much improved in comparison with those of PVA. These characteristics would be due to the strong interaction of both components in the network structure as well as high crystallinity of PVA. The present composite has great potential for various applications such as biomedical and cosmetic matrix.

In Chapter 3, unique shrinkage-swelling behaviors BC based material was prepared by internal crosslink reaction. The solvent exchange of BC hydrogel with

acetone and subsequent crosslinking by MDI produced the reinforced BC organogel. This gel one-dimensionally shrank by drying under vacuum to give the thin sheet, which also one-dimensionally swelled in LiCl/DMAc. Additionally, the solvent of the swollen gel could be converted to the hydrogel by immersion in water. This regeneration cycle could be repeatedly conducted. These unique behaviors are probably due to the crosslinked layered structure of BC. To my best knowledge, this is the first attempt on the one-dimensional shrinkage-swelling of polymer gels.

## ***List of Publications***

1. One-sided Surface Modification of Bacterial Cellulose Sheet as 2,3-Dialdehyde

Hyunhee Shim, Myrtha Karina, Rike Yudianti, Lucia Indrarti, Jun-ichi Azuma,  
Hiroshi Uyama

*Polym. Plast. Technol. Eng.* **2015**, *54*, 305-309.

2. Unique Enhancement of Thermostability in a Green Composite of Bacterial Cellulose and Poly(vinyl alcohol)

Hyunhee Shim, Ayumi Dobashi, Hiroshi Uyama

*Chem. Lett.* **2016**, *45*, 51-53.

3. One-dimensional Shrinkage and Swelling of Crosslinked Bacterial Cellulose Gel

Hyunhee Shim, Xingyu Xiang, Myrtha Karina, Lucia Indrarti, Rike Yudianti,  
Hiroshi Uyama

*Chem. Lett.*, doi:10.1246/cl.151100

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