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Cytocompatible cellulose hydrogels containing trace lignin

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ABSTRACT

Sugarcane bagasse was used as a cellulose resource to prepare transparent and flexible cellulose hydrogel films. On the purification process from bagasse to cellulose, the effect of lignin residues in the cellulose was examined for the properties and cytocompatibility of the resultant hydrogel films. The cellulose was dissolved in lithium chloride/*N*,*N*-dimethylacetamide solution and converted to hydrogel films by phase inversion. In the purification process, sodium hydroxide (NaOH) treatment time was changed from 1 to 12 h. This resulted in cellulose hydrogel films having small amounts of lignin from 1.62 to 0.68%. The remaining lignin greatly affected hydrogel properties. Water content of the hydrogel films was increased from 1153 to 1525% with a decrease of lignin content. Moreover, lower lignin content caused weakening of tensile strength from 0.80 to 0.43 N/mm^2 and elongation from 45.2 to 26.5%. Also, similar tendency was observed in viscoelastic behavior of the cellulose hydrogel films. Evidence was shown that the lignin residue was effective for the high strength of the hydrogel films. In addition, scanning probe microscopy in the morphological observation was suggested that the trace lignin in the cellulose hydrogel affected the cellulose fiber aggregation in the hydrogel network. The trace of lignin in the hydrogels also influenced fibroblast cell culture on the hydrogel films. The hydrogel film containing 1.68% lignin showed better fibroblast compatibility as compared to cell culture polystyrene dish used as reference.

1. Introduction

Naturally occurring polymers such as collagen, hyaluronic acid, chitin and chitosan are favorable candidates to develop novel hydrogels for biomedical application because of their biocompatibility and biodegradability [1–3]. Synthetic polymers also have been employed to fabricate hydrogels for biomedical application due to their advantages in controllable structure and good mechanical strength. However, they offered limited biocompatibility as implantable or injectable materials [4,5]. For this reason, naturally occurring polymers have attracted much attention of many researchers for development of hydrogels mimicking body tissues as scaffold for tissue regeneration [6,7]. Sugarcane bagasse is one of the largest cellulosic waste materials, which is generated as a fibrous residue in an extraction process of sugar juice from the sugarcane [8,9]. Although it was utilized as manure, feed stock and fuel in sugar industry, remaining bagasse still continues to be an environmental problem [10]. Therefore, efficient utilizations of the sugarcane bagasse residue have been studied as a potentially renewable cellulose resource for functional materials. It is well known that cellulose is a natural polymer and the most abundant one consisting of β-glucose in the nature and forms about half to one-third of plant tissues [11]. Also, the biocompatibility of cellulose and its derivatives is well established [12]. Recently, our research group studied cellulose hydrogel films regenerated from cellulosic waste materials including agave bagasse [13] and bamboo fibers [14]. These hydrogel films were applied to cell scaffold material for tissue regeneration and exhibited fibroblast compatibility [15]. In addition, biocompatibility of the cellulose hydrogel regenerated from sugarcane bagasse was evaluated [16]. In this study, it was revealed that in vivo behavior of the cellulose hydrogel in mice was excellent in the biocompatibility. Also, the effect of pretreatment of the sugarcane bagasse waste on the hydrogel properties was investigated for hydrophilicity, viscoelasticity, cellulose crystallinity, and the hydrogel morphology [17]. The One of potential application is to use in scaffold of skin regeneration. It is noted that cellulose hydrogel films obtained from waste biomass shows good cytocompatibility for fibroblast which is an important cell involved in wound healing. As such skin regeneration, it is known that hydrogel scaffold can provide a moist covering for skin repairing and protect the wound from infection [18,19]. However, still little is known about the cellulose hydrogels derived from waste bagasse materials, especially in the hydrogels containing trace lignin.

In the present work, the waste sugarcane bagasse produced in Okinawa Island (Japan) was used as cellulosic resource for regenerated cellulose hydrogel films. Usually, about 40 − 50% of cellulose is contained in the bagasse and another 25 − 35% is hemicellulose. The remains are mostly lignin and lesser amount of mineral and wax [20]. Sugarcane bagasse was treated by a sequential treatment of sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), and sodium hypochlorite (NaOCl) to remove lignin and hemicellulose [13,21]. After these processes for regenerated cellulose, cellulose hydrogels were obtained by phase inversion of dissolved cellulose in lithium chloride/*N*,*N*-

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dimethylacetamide solution. In their processes, small amount of lignin was incompletely removed in the cellulose fibers depending on treatment condition. Our previous research revealed that bleaching of agave bagasse was effective for lignin removal in the case of increased NaOCl concentration and the resultant cellulose hydrogel films enhanced fibroblast compatibility [21]. However, little is known about the effect of lignin in the cellulose hydrogel films on cytocompatibility, yet. It was reported that lignin obtained from various sources showed cytotoxicity at high concentration in the range of 400 − 1200 μg/mL [22] Also, composite of gelatin films with lignosulphonate was cytotoxic, when the range of lignin was 1480 − 1745 μg/mL [23]. Therefore, the present work described the influence of the trace lignin in the cellulose hydrogels, when the resultant hydrogel films were characterized in the properties of hydrogel films and their cytocompatibility.

2. Experimental procedures

2.1. Materials

Sugarcane bagasse was obtained from local sugar factory (Okinawa, Japan). Sulfuric acid (H_2SO_4), sodium hydroxide (NaOH), sodium hypochlorite (NaOCl), *N*,*N*-dimethylacetamide (DMAc), lithium chloride (LiCl), and ethanol (EtOH) were purchased from Nacalai Tesque, Inc. (Tokyo, Japan). DMAc was stored with potassium hydroxide for over 3 days and LiCl was dried at 80 °C in a vacuum oven before using. Polystyrene standards for SEC calibration were obtained from Tosoh Corp. (Japan). Phosphate buffered saline (PBS, DS Pharma Biomedical Co., Ltd.) was used as received. Bicinchonic acid (BCA) kit, fetal bovine serum (FBS), and bovine serum albumin (BSA) were purchased from Sigma-Aldrich. Dulbecco's modified eagle medium (DMEM), penicillin streptomycin from Gibco were used as cell culture medium. NIH 3T3 mouse embryonic fibroblast cells were obtained from BioResource Center, Japan.

2.2. Sugarcane bagasse fiber treatment

Purification of cellulose fiber from the sugarcane bagasse was performed by following procedure. The bagasse was firstly washed in hot water (80 °C) five times to remove the remaining sugar and then dried in an oven at 50 °C. In 300 mL of 4 vol.% H_2SO_4 aqueous solution, 10 g of bagasse was stirred at 90 °C for 1.5 h and then was treated in 300 mL of 10 wt.% NaOH aqueous solution at 90 °C for different time of 1, 6, and 12 h. In each step, the residue of fibers was washed with excess distilled water until neutral pH. After this process was finished, fibers were bleached in 300 mL of 10 vol.% NaOCl at 40 °C for 3 h. They were washed with excess water and then dried in vacuum oven at room temperature overnight. These cellulose fibers purified at different NaOH treatment time and then bleached were labeled as N1B, N6B and N12B for 1, 6, and 12 h. Also, the cellulose fibers treated by NaOH without bleaching were named as N12 for the comparison to above three samples. These are listed in Table 1.

2.3. Preparation of cellulose solution and hydrogel films

The treated bagasse cellulose fibers (1 g) were stirred in distilled water at room temperature for 24 h and the swollen fibers were obtained. After filtration, the swollen fibers were stirred in EtOH and then DMAc at room temperature for 24 h for the solvent exchange [24]. Both dried DMAc (93 g) and LiCl (6 g) were added to dissolve cellulose fibers and stirred at room temperature for 1 day or more. The resultant viscous solution was centrifuged at 9000 rpm by centrifugal (H-201FR, KOKUSAN Co. Ltd., Japan) for 20 min to remove insoluble residues. Eventually, LiCl/DMAc solution containing 6 wt.% LiCl and 1 wt.% of the purified cellulose was obtained. Fig. 1 shows pictures of purified cellulose fibers (bottom) and the LiCl/DMAc solutions (top) containing cellulose treated with different NaOH condition and also without and with NaOCl treatment.

By using each cellulose in LiCl/DMAc solution, phase inversion process was made to prepare hydrogel films according to our previous reports [13,15,17]. The detailed procedure was followed. For the preparation of cellulose hydrogel films, 8 g of cellulose solution was poured into glass dish (91 mm diameter) and kept for 24 h in a plastic container which was filled with 40 mL of EtOH and the vapor atmosphere. In this step, coagulation of cellulose was gradually progressed in the vapor at room temperature. Finally, the cellulose hydrogel film was obtained by phase inversion process from liquid to solid hydrogel. The resultant hydrogel films were washed with excess water and then placed in distilled water for 24 h to remove DMAc and LiCl. The obtained hydrogel films were kept in plastic container filled with distilled water until further experiments.

2.4. Evaluation of cellulose hydrogel films

FT-IR spectra of hydrogel films were recorded by using FT-IR spectrophotometer (IR-prestige 21, Shimadzu, Japan), as potassium bromide (KBr) tablet method was applied with 8 cm^{-1} resolution and 16 scans in the range of 500–4000 cm^{-1}. In order to evaluate lignin content in the hydrogel films, a small piece of swollen film (20 mm × 20 mm) was sandwiched with two quartz glasses (25 mm × 25 mm × 0.1 mm) and then UV–Vis spectrum was measured by UV–Vis spectrometer (V-570, JASCO, Japan). Equation which was used in the National Renewable Energy Laboratory (NREL) Laboratory Analytical Procedures (LAP) [25] to determine concentration of acid soluble lignin, was modified to calculate lignin content as following equation: Lignin (wt.%) = $(A \cdot V)/(\varepsilon \cdot \ell \cdot M) \times 100$, where A and V were absorbance at 240 nm and volume of the swollen hydrogel film in liter, respectively. Absorption coefficient ε was 25 L/g·cm which was determined in acid soluble lignin of bagasse [25]. The light path length ℓ was thickness of the film in cm and M was the dry weight of hydrogel film in g. To determine molecular weight distribution of cellulose, size exclusion chromatography (SEC) was performed by the SEC system which was consisted of an online degasser (DGU-20A, Shimadzu, Japan), high-pressure pump (LC-20AD, Shimadzu), manual injector (7725i, Rheodyne), SEC column (KD-806M, Shodex) and refractive index (RI) detector (RID-10A, Shimadzu) [26,27]. The chromatogram was recorded by Chromatpac integrator (C-R8A, Shimadzu). The column temperature and the RI detector cell were kept at 50 and 40 °C, respectively. As an eluent, 1 g of LiCl/100 mL of DMAc was used. The SEC system was calibrated by narrow distribution polystyrene standards. The cellulose fibers were dissolved in 8% LiCl/DMAc and then diluted to 1% concentration of the LiCl/DMAc. Final sample concentration of 1 mg/mL was used for the SEC measurement. Before injection, the

Table 1
Treatment conditions of sugarcane bagasse and hydrogel properties: lignin content, waster content, tensile strength, and elongation of swollen cellulose hydrogel films.

Sample	NaOH treatment time[1)] (h)	NaOCl bleaching[2)]	Lignin content (wt.%)	Water content (%)	Tensile strength (N/mm^2)	Elongation (%)	Swelling ratio (%)
N12	12	None	1.62	1153 ± 56	0.80 ± 0.14	45.2 ± 4.0	547 ± 43
N1B	1	Bleached	0.88	1390 ± 20	0.77 ± 0.20	43.8 ± 6.8	795 ± 79
N6B	6	Bleached	0.73	1445 ± 87	0.44 ± 0.15	31.5 ± 7.9	928 ± 75
N12B	12	Bleached	0.68	1525 ± 73	0.43 ± 0.11	26.5 ± 7.4	940 ± 60

[1] Different treatment times were done in 10 wt.% NaOH solution.
[2] The bleaching in the aqueous 10 vol.% NaOCl was done.