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Cellulose–acidic glycosaminoglycan blend fibers releasing a portion of the glycosaminoglycans in water

S. Hirano*, M. Zhang

Chitin/Chitosan R&D, 445-Sakuradani, Tottori-shi, Tottori 680-0853, Japan

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Abstract

Novel artificial blend fibers of cellulose with each of hyaluronate, heparin, chondroitin 4-sulfate, chondroitin 6-sulfate, and a chitin–chondroitin 6-sulfate blend were prepared by using an aqueous 10% H_2SO_4 solution containing 40–43% ammonium sulfate as the coagulating solution. The tenacity values of the extruded cellulose–hyaluronate filaments did not decrease on blending with up to 22% hyaluronate, but those on blending with 9% sulfated glycosaminoglycans decreased significantly. A portion (55–75%) of the acidic glycosaminoglycans was released upon soaking the fibers in water at room temperature.

Keywords: Cellulose–glycosaminoglycan fibers; Chitin; Chondroitin sulfates; Heparin; Hyaluronate

1. Introduction

Both chitin and chitosan fibers have been used as dressing or covering materials for the healing acceleration of the wound tissues of humans (Kifune, 1995), animals (Shigemasa & Minami, 1995) and plants (Hirano, Kitaura, Sasaki, Sugiyama, Hashimoto & Tanatani, 1996). However, chitin and chitosan are not the structural components of animal and plant tissues, and it is essential to improve the biocompatibility in their tissues for this application. One of the ways for improvement is to prepare the blends of chitin and chitosan with an animal tissue component (e.g. glycosaminoglycans and collagen) for animals and with a plant tissue component (e.g. cellulose and lignin) for plants. The cellulose–chitin and rubber–chitin blend membranes have been demonstrated as biocompatible wound-dressing materials for plants (Hirano et al., 1996). Several artificial blend fibers of biopolymers have been reported: cellulose–chitin (Hirano, Usutani & Midorikawa, 1997), cellulose–chitosan (Lim, Nam & Ko, 1997), silk fibroin–chitin (Hirano, Nakahira, Nakagawa & Kim, 1999), silk fibroin–chitosan (Park, Oh, Yoo & Shin, 1999), and collagen–chitin (Hirano, Zhang, Nakagawa & Miyata, 2000). Any constituent biopolymers are not released from these blended fibers at pH 7 in water. Furthermore, no report has dealt with the blend fibers of the acidic glycosaminoglycans releasing their portion from the fibers.

The present report deals with the preparation of the novel fibers of cellulose–acidic glycosaminoglycan blends and a cellulose–acidic glycosaminoglycan–chitin blend, and the releasing properties of a portion of the glycosaminoglycans from their blend fibers in water.

2. Experimental

2.1. Materials

A solution of 9% sodium cellulose xanthate (viscose) in aqueous 5% NaOH was prepared by the conventional method from cellulose (Lot MBE 7547, Nakalai Tesque Company, Kyoto) via sodium cellulose salt (Hirano & Midorikawa, 1998). Sodium hyaluronate (HA) from umbilical cord, sodium chondroitin 4-sulfate (Ch4S) from whale nose cartilage, and sodium chondroitin 6-sulfate (Ch6S) from shark cartilage were isolated in our laboratory (Anno & Hasegawa, 1972). Sodium heparin (Hep) was a product of Sigma Company, USA.

2.2. Methods

For spinning, a viscose-type spinneret ($12.5 \times 18.0 \times 10.0\ mm^3$ in size with 300 holes at each exit and 0.10 mm in diameter, Japan Nozzle Company, Kobe, Japan) was used. The filament titer values were analyzed under the dry conditions on a Vibroscope Micro (Lenzing Technic Instrument Co., Ltd, Austria) and is expressed by the weight

* Corresponding author.

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Table 1
Some analytical data for the blend fibers of cellulose with each of the acidic glycosaminoglycans

Fiber	Glycosaminoglycan		N (%)	S (%)	Uronic acid (%)[a]	Titer (denier)	Tenacity (g/denier)	Elongation (%)
	Percentage[b]	Type[c]						
1	22	HA	0.66	0	8.9	4.61	1.35	5.51
2	9	HA	0.27	0	3.6	3.65	1.32	4.82
3	23	Hep	0.51	2.71	9	7.36	0.79	29.9
4	9	Hep	0.20	1.12	4	7.90	0.63	28.6
5	5	Hep	0.11	0.63	2	9.55	0.66	27.3
6	44	Ch4S	1.19	2.35	16	8.06	0.38	9.0
7	23	Ch4S	0.62	1.22	8	4.21	0.32	12.2
8	9	Ch4S	0.25	0.46	3	4.62	0.27	8.9
9	23	Ch6S	0.62	1.25	8	9.01	0.39	8.4
10	9	Ch6S	0.24	0.47	3	8.10	0.30	7.6
11	30/20	Chitin/Ch6S	nd[d]	1.05	7	4.61	0.38	8.6
12	0		0	0	0	4.10	1.24	29.2
13	10	HA	0.30	0	3.9	–[e]	–	–
14	9	Hep	0.21	1.10	3.0	–	–	–
15	11	Ch4S	0.30	0.57	4.3	–	–	–
16	7	Ch6S	0.19	0.33	2.5	–	–	–

[a] Analyzed by the carbazole reaction.
[b] Calculated on the basis of nitrogen contents.
[c] Abbreviations used: HA, sodium hyaluronate; Hep, sodium heparan sulfate; Ch4S, chondroitin 4-sulfate; Ch6S, chondroitin 6-sulfate.
[d] Not determined.
[e] Very weak but not breakable even in the dry state.

(g) of a filament (9000 m in length) as one denier, and the filament tenacity and elongation values were on a Vibrodyne 400 (Lenzing Technic Instrument Co., Ltd, Austria). FTIR spectra (KBr) were recorded on a Jasco FTIR 5300 spectrometer (Jasco Co., Ltd, Tokyo, Japan), and scanning electron microscopic (SEM) analyses were on a scanning electron microscope (JSM 6301F, Jeol Co., Ltd, Tokyo, Japan). Uronic acid was quantitatively analyzed by the carbazole reaction (Dische, 1947) on a spectrophotometer (Type 100-50, Hitachi, Tokyo, Japan). Nitrogen and sulfur analyses were performed at the micro-analytical center of Kyoto University, Kyoto, Japan. The amount of acidic glycosaminoglycans in the blend fibers was calculated on the basis of nitrogen contents: 3.0% for HA, 2.2% for Hep and 2.7% for Ch4S and Ch6S, respectively (Hasegawa, Suzuki, Seno & Hirano, 1968).

2.3. Fiber spinning

An acidic glycosaminoglycan solution in an aqueous 5% NaOH solution was mixed with a solution of sodium cellulose xanthate in aqueous 5% NaOH at several mixing ratios (Table 1). A mixed solution of sodium cellulose xanthate, Ch6S and sodium chitin salt (alkaline chitin) in aqueous 14% NaOH was also prepared. Each of the above mixed solutions was filtered through a glass filter, treated at a reduced pressure for a few minutes to remove air-bubbles, and then spun at room temperature through a viscose-type spinneret into a coagulating bath containing an aqueous 10% H_2SO_4 solution containing 40–43% $(NH_4)_2SO_4$ at room temperature. The extruded filaments were allowed to soak in the above coagulating solution at room temperature overnight. After washing several times with aqueous 40 and 60% methanol, the fiber was suspended in aqueous 80% methanol, and the suspension mixture was kept at room temperature overnight. The produced fibers were collected, pressed and air-dried (75–98% yields).

2.4. The release of acidic glycosaminoglycans from the blend fibers in water

Each portion (0.20 g) of the dry fibers (1, 3, 6 and 9) was suspended in water (60 ml), and the suspension mixture was allowed to stand at room temperature by occasional stirring. A portion (0.2 ml) was withdrawn from the outer solution at time intervals of 0.3, 1, 2, 3, 4, 8 and 24 h, and the released glycosaminoglycans from the blend fibers was analyzed by the carbazole reaction. After additional soaking in an aqueous 0.1% NaOH solution at room temperature overnight, the fiber was washed thoroughly with water, collected, pressed and air-dried. The released amount of the corresponding glycosaminoglycan was calculated on the basis of the fiber weight loss, the uronic acid content, and the nitrogen and sulfur contents.

3. Results and discussion

3.1. Spinning and coagulating solutions

The mixed solutions of sodium cellulose xanthate and

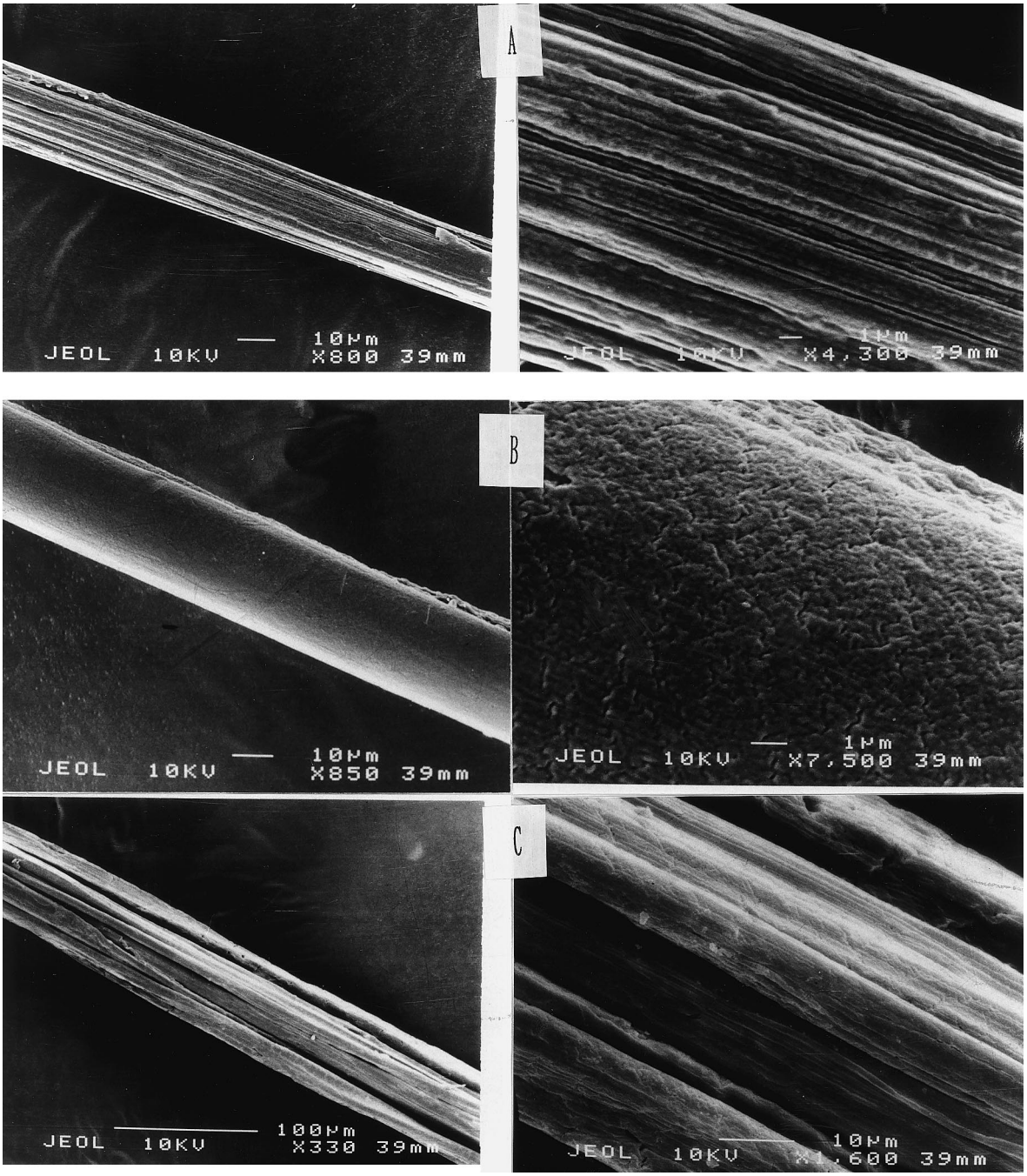

Fig. 1. A scanning electron microscopic (SEM) observation on the surface structure of some cellulose–acidic glycosaminoglycan blend filaments. (A) cellulose–HA blend filament (1). (B) cellulose–Hep blend filament (3). (C) Cellulose–Hep blend filament (14) obtained after the treatment of (3) in water and in aqueous 0.1% NaOH solution at room temperature (see text).

acidic glycosaminoglycans in aqueous 5% NaOH were used as the spinning solution. An aqueous 10% H_2SO_4 solution containing 40–43% $(NH_4)_2SO_4$ was used as the coagulating solution at room temperature. Cellulose, glycosaminoglycans and chitin were insoluble in this coagulating solution. The aqueous ammonium sulfate solutions at various concentrations are widely used for the salting-out and purification of native enzyme proteins (Green & Hughes, 1955).

3.2. Some properties of blend fibers and cellulose fiber

Five kinds of novel blend fibers were prepared in 75–98% yields. In addition, four kinds of the blend fibers were prepared by soaking the above blend fibers in water at room temperature. Sulfur and nitrogen analyses on the blend fibers indicated that almost no desulfation occurred during the spinning step as examined by the S analysis. All of these blend fibers were white and mechanically soft. In

the FTIR spectra, these blend fibers exhibited absorptions (cm^{-1}) at 3416–3426 (OH), 2922–2960 (CH), 1633–1639, 1400–1404, and 1070–1115 (CO), which were similar to those of cellulose fiber (Rayon). In addition, several absorptions appeared at 1735–1736 (CO_2H) in the spectra of 1 and 6, and an absorption at 1230 (S=0) in the spectrum of 6. However, S=0 and C–O–S absorptions in the regions of 1230–1250 and 800–900 cm^{-1} were too weak to be detected due to overlapping with cellulose absorptions.

The cellulose–hyaluronate blend filament had relatively good mechanical properties among these filaments. The cellulose–HA blend filaments (1 and 2) exhibited almost the same tenacity value as that of the cellulose filament, and the elongation value about 1/6 times that of the cellulose filament. The tenacity value of the cellulose–Hep blend filaments (3–5) was about 3/5 times that of the cellulose filament, and the elongation value was similar to that of the cellulose filament. The tenacity values of the blend filaments of cellulose–Ch4S (6–8), cellulose–Ch6S (9 and 10) and cellulose–chitin–Ch6S (11) was about 1/3 times that of the cellulose filament, and their elongation values were 1/4–1/3 times (Table 1). The mechanical properties of the extruded filaments were effected by the kinds of the glycosaminoglycans but were little effected by their blend ratio up to 44%. The tenacity values of the extruded cellulose–hyaluronate filaments did not decrease on blending with up to 22% hyaluronate, but those on blending with 9% sulfated glycosaminoglycans (e.g. Hep, Ch4S and Ch6S) decreased significantly although the elongation value did not decrease on blending with Hep. These data indicate that the sulfate groups interfere with the arrangement of cellulose fibrils in the filaments. The filaments (13–16) obtained after the soaking treatment in water were mechanically weak, and their mechanical parameters could not be analyzed, but these filaments were not breakable even at the dry state.

3.3. The release of the acidic glycosaminoglycans from their blend fibers in water

A portion of the glycosaminoglycans was released from the fibers in water at room temperature within 4 h for 1 and 3, and within 2 h for 6 and 9. The thus treated fibers were further soaked in a solution in aqueous 0.1% NaOH at room temperature for 8 h, and were washed thoroughly with water, pressed and air-dried to give rise to 13–16 in 55–75% yields. As shown in Table 1, the main portion of the glycosaminoglycans was released from the blend fibers by this treatment, and a portion (25–45%) of the glycosaminoglycans retained in the fibers. No significant swelling of these blend fibers appeared during their soaking in water. These data indicate that there are no strong molecular interactions between cellulose and each of HA, Hep, Ch4S and Ch6S in the blend filaments. A portion of the glycosaminoglycans was solubilized into water, and the cellulose portion remained as a filament. The releasing properties of the glycosaminoglycans from the blend fibers will be usable as an improvement step towards biocompatibility.

3.4. SEM analyses

As shown in Fig. 1, photograph A shows a striped surface pattern of cellulose–HA blend filament (1; 19 μm in diameter), and photograph B shows a smooth porous surface pattern of cellulose–Hep blend filament (3; 24 μm in diameter). On releasing a portion of Hep in water and aqueous 0.1% NaOH solution, filament 3 shrinks to give rise to a thin filament (14) 6.7 μm in diameter. Filament 14 consists of cellulose (about 91%) and Hep (about 9%), and on the filament surface (photograph C) a striped pattern appears, which is similar to that of the cellulose filament.

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