Preparation of Gelatin Fiber by Gel Spinning and Its Mechanical Properties

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ABSTRACT: A gel-spinning process was used in an attempt to prepare a gelatin fiber with a high level of drawability. A gel fiber prepared by extrusion of 15 wt % gelatin in dimethyl sulfoxide into methanol at −20°C was drawn to sixteen times the original length. After extraction of the dispersion medium, the mechanical strength of the fiber increased markedly with the draw ratio, and the fiber exhibited a tensile strength of 146 MPa and a Young's modulus of 2.3 GPa when drawn to the maximum. A gelatin fiber with greater mechanical strength was obtained when ethylene glycol was used as the spinning solvent. The X-ray diffraction profile indicated the formation of triple-helical structures and their lateral association, which is responsible for the mechanical strength of the fiber. Heat-treatment improved the water-resistance of the prepared fiber. γ-Irradiation and treatment with glutaraldehyde improved the mechanical strength of the fiber. © 2008 Wiley Periodicals, Inc. J Appl Polym Sci 110: 4011–4015, 2008

Key words: gelatin; fibers; gel spinning; mechanical properties; gelation

INTRODUCTION

Gelatin is used in the form of powder or sheets in many products, such as foodstuffs, capsules, and photographic emulsion. Fibers and sponges made from collagen, which is the raw material for the production of gelatin, have attracted much attention as a useful matrix for tissue-engineering applications as well as conventional use in medical supplies.[1–4] Gelatin fiber offers an alternative to collagen fiber, and its physical properties can be manipulated by changes to the production process. Gelatin fiber is expected to contribute to regenerative medicine and the development of new biodegradable material through the production of both woven and nonwoven fabric.[5–7]

There have been several studies on the preparation of gelatin fiber. Tokura et al. prepared gelatin fiber by wet spinning a solution of gelatin in LiCl-*N*,*N*-dimethylacetamide containing glutaraldehyde, and a concentrated aqueous solution of gelatin with a crosslinking agent.[8,9] Nagura et al. used wet spinning of a 20 wt % aqueous solution of gelatin into methanol to produce a gelatin fiber for use as sutures.[10–12] The spun fiber was immediately crosslinked by heat treatment under vacuum and immersion in various epoxy compounds, for example, ethylene glycol diglycidyl ether. They also prepared fibers by wet spinning an aqueous solution of gelatin and citric acid. These fibers were reported to exhibit considerable mechanical strength and water resistance. The strength and the retention of shape in the gelatin fibers referred to above are thought to be due mainly to chemical crosslinking rather than orientation of the chain along the fiber axis, although few details were given of the physical properties and drawability of the spun fiber before it was crosslinked.

It has been reported that drawing a gelatin film prepared by casting an aqueous solution of gelatin markedly improves the mechanical strength.[13,14] Drawing a gelatin film in a mixture of water and ethanol is reported to result in reconstitution of the collagen-fold structure, that is, a triple helix.[15] Drawing a spun fiber in the gel state may allow preparation of a gelatin fiber with useful mechanical strength due to reconstitution of the collagen-fold structure and its orientation. Moreover, the preparation of a fiber without chemical crosslinking is desirable in terms of the biodegradation and biocompatibility inherent in gelatin.

We previously reported that gelatin fiber in the gel state without chemical crosslinking has a high level of drawability and the orientation of the pseudocrystallite along the fiber axis.[16] In this work, we have examined the effect of the conditions used for preparing gelatin fiber on the formation of higher-order structure and clarified how the structure and

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TABLE I
Maximum Draw Ratio of Spun Fiber in the Gel State

Temperature of gelation (°C)	Temperature of drawing (°C)	Maximum draw ratio
−20	−20	5.3
	5	4.2
	20	16.1
−10	5	1.7
	20	4.6
0	5	2.7
	20	2.9

the mechanical properties of the fiber are interrelated. The effect of post-treatment on the properties of the fiber has also been examined with regard to the practical use of the gelatin fiber prepared by gel-spinning process.

EXPERIMENTAL

Materials and measurements

Type A gelatin (~ 300 bloom grade) prepared from porcine skin was obtained from Sigma-Aldrich. Dimethyl sulfoxide (DMSO, 99%), ethylene glycol (EG, 99.5%), glutaraldehyde (GA, 25% aqueous solution), and methanol (99%) were purchased from Wako Chemical Industries and used without further purification.

The tensile tests were carried out on 20 mm long fibers at a crosshead speed of 20 mm/min using a Tensilon Universal Tester RTC-1350A (Orientec). The tensile strength and Young's modulus were evaluated from stress–strain curves. Birefringence was measured with an Oympus BH-2 polarizing microscope equipped with a Berek compensator.

Water resistance was examined by soaking 20 mm long fibers in temperature-controlled water. Degree of swelling is expressed as the elongation ratio of the length after soaking to the original length. Wide-angle X-ray diffraction patterns were recorded on an imaging plate for 40 min using a Rigaku RAXIS-IV X-ray Diffractometer, which was operated at 50 kV and 100 mA with a graphite-monochromated CuK_{α}.

Fiber preparation and post-treatment

A solution of 15 wt % gelatin in DMSO at 55°C was extruded into a temperature-controlled methanol bath through a single nozzle of 0.4 mm internal diameter with an air gap of 5 mm. The rate of extrusion was 0.45 mL/min so that the spun fiber was soaked in the bath for 1 min. The fiber that had gelated in the bath was wound onto a bobbin and kept in a chamber at −20°C, 5°C, and 20°C for 10 min. Then the fiber was drawn in the chamber to predetermined multiples of its original length by controlling the speed of two winders. The dispersion medium was extracted by immersing the drawn fiber in methanol at room temperature for 10 days while keeping the length fixed.

Fibers were kept under constant tension and heat-treated under an inert atmosphere in an oven for 1 h. Chemical crosslinking was achieved by immersion of the fiber in a 2.5 wt % GA in sodium phosphate buffer at pH 7.4. Fibers were sealed in a glass tube under vacuum and irradiated with ^{60}Co γ-ray at Koga Isotope, Japan.

RESULTS AND DISCUSSION

Preparation and mechanical properties of gelatin fiber

Solutions of gelatin in DMSO are highly viscous and concentrations of more than ~ 8 wt % exhibit spinnability. Table I gives the maximum draw ratios of fibers in the gel state obtained when 15 wt % solutions of gelatin in DMSO at 55°C were extruded into a refrigerated methanol bath. The fiber had insufficient drawability after gelation at 0°C. The maximum draw ratio of the fiber gelated at −10°C was only 4.6, even when drawn at 20°C. The fiber gelated at −20°C was drawn to 4.2 times its original length at 5°C, and to 16.1 times its original length at 20°C. All of the fibers discussed below were prepared by extrusion into a refrigerated methanol bath at −20°C, followed by drawing at 20°C.

Figure 1 shows typical stress–strain curves recorded for gelatin fibers prepared by drawing the spun fiber followed by extraction of the dispersion medium. Table II gives the values of tensile strength (σ_b) and Young's modulus (E) for the fibers. When using DMSO as a spinning solvent, the values of σ_b and E of the fiber drawn to four times its original length (D4) are 91 MPa and 2.0 GPa, respectively.

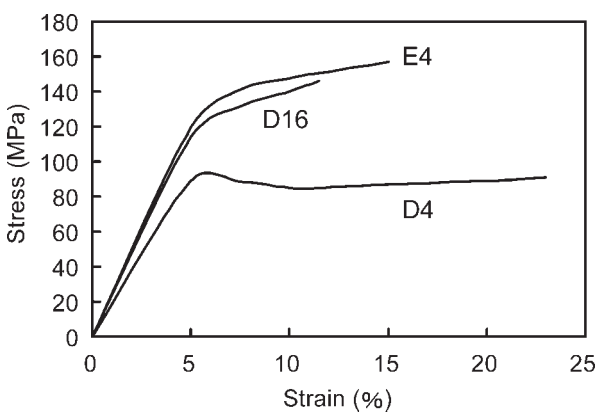

Figure 1 Typical stress-strain curves recorded for gelatin fibers: D4, fiber prepared using DMSO as a solvent and drawing to 4 times; D16, using DMSO and drawing to 16 times; E4, using EG and drawing to 4 times.

TABLE II
Mechanical Properties of Gelatin Fiber

Spinning solvent[a]	Draw ratio[b]	Tensile strength (MPa)	Young's modulus (GPa)
DMSO	–	32 ± 4	0.9 ± 0.1
	4	91 ± 4	2.0 ± 0.2
	16	146 ± 3	2.3 ± 0.2
EG	4	157 ± 6	2.5 ± 0.2
DMSO+glycerol[c]	4	118 ± 12	3.0 ± 0.4
	6	110 ± 8	3.1 ± 0.2

[a] Gelatin concentration; 15 wt %.
[b] Drawing temperature; 20°C.
[c] Glycerol to gelatin ratio; 1/5 (wt/wt).

The undrawn fiber (D1) had a σ_b value of 32 MPa and an E value of 0.9 GPa, confirming that the improvement of mechanical properties was due to drawing in the gel state. Moreover, σ_b and E of the fiber drawn to 16 times its original length (D16) were 146 MPa and 2.3 GPa, respectively. When EG was used as a solvent, the fiber (E4) had a higher σ_b of 157 MPa and a higher E of 2.5 GPa, despite the low draw ratio of four times its original length.

Figure 2 shows the X-ray diffraction patterns for gelatin fibers prepared using DMSO as the solvent. Undrawn fiber D1 showed an isotropic pattern, whereas the pattern of the D4 fiber showed a slight diffuseness, but the layer-lines were clearly observed and the lattice spots were located near the equatorial axial line. The D16 fiber pattern showed lattice spots located even closer to the line.

Okamoto et al. reported two distinct peaks at $2\theta = 8°$ and $2\theta = 31°$ in the X-ray diffraction pattern of a film prepared from an aqueous solution of gelatin in a gel state.[17–20] The peak at 31° was assigned to the diffraction arising from the repeat of an amino acid residue involved in a collagen fold structure (a triple helix). The peak at 8° was assigned to diffraction from the lateral association of fibrils composed of triple helices.[21] The area of the diffraction peak was reported to be approximately in proportion to the content of triple helix in a specimen.[15]

Figure 3 shows X-ray diffraction profiles of the gelatin fibers prepared in this work. The diffraction intensity observed at 31.5° increases in the order D1 < D4 < D16. The ratio of the peak area of D4 to D1 is 2.1, and the ratio of D16 to D1 is 2.7. There is some difference between D1 and D4 in the intensity at 7.8°, but the intensity for D16 is clearly stronger than that for either D1 or D4. The ratio of the peak area of D16 to D1 is 1.6. The effective drawing in the gel state in the preparation of gelatin fiber is considered to promote the reconstitution of the triple-helical structure and the formation of crystallite composed of triple-helical segments.

The value of birefringence was 1.1×10^{-3} for D4, and 1.6×10^{-3} for D16, suggesting that the orientation of amorphous and crystalline regions in the fiber is promoted by drawing, and we propose that both the increase and the orientation of crystallite along the fiber axis improved the fiber strength. On the other hand, the value of birefringence for the E4 fiber is 1.1×10^{-3}, which is equal to the value for the D4 fiber, and the diffraction intensities at bath 7.8° and 31.5° of E4 are stronger than those of D16. When compared with D4, which has the same draw ratio as E4, the ratio of peak area of E4 to D4 is 1.7 at 7.8° and 1.5 at 31.5°. These results show that using EG as the spinning solvent promotes formation of helical structures and their association even at a low draw ratio. The reason for the difference of higher-order structure of gelatin fibers formed with different spinning solvents is not known, but it may be due to the difference of their solvation forces.

We determined whether addition of glycerol to a spinning solution of gelatin in DMSO improved the drawability of the spun fiber. The addition of

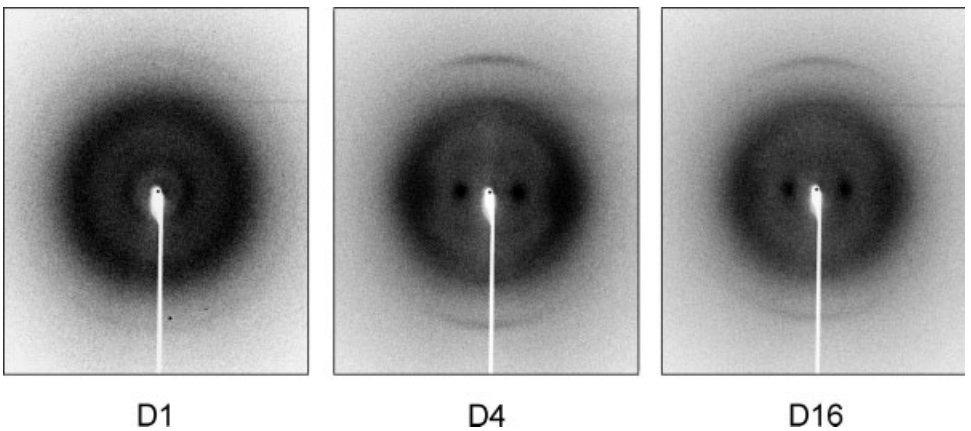

Figure 2 X-ray diffraction patterns for the gelatin fibers prepared using DMSO as a solvent: D1, undrawn fiber; D4, drawn to four times; D16, drawn to 16 times.

glycerol resulted in a change of the maximum draw ratio of spun fiber as follows: 6.6-fold for 1/10 parts by weight; 7.1-fold for 1/5 parts by weight; 6.4-fold for 1/2 parts by weight; and ~ 4-fold for the parts from 1 to 4 by weight. The addition of glycerol to the spinning solution caused the drawability of the fiber to decrease. Nevertheless, the increase of fiber strength after drawing and extraction of the dispersion medium was confirmed, as given in Table II. The fiber prepared using a spinning solution with a glycerol to gelatin ratio of 1/5 by weight, and drawing to six times the original length, had a σ_b value of 110 MPa and an E value of 3.1 GPa.

TABLE III
Mechanical Properties of Post-Treated Gelatin Fiber

Post treatment[a]	Tensile strength (MPa)	Young's modulus (GPa)
None	91	2.0
Heat treatment 120°C	89 ± 2	1.9 ± 0.1
180°C	82 ± 4	1.9 ± 0.1
220°C	84 ± 2	1.7 ± 0.2
GA treatment 4 h	184 ± 18	2.3 ± 0.2
24 h	117 ± 12	2.2 ± 0.2
72 h	56 ± 9	1.5 ± 0.4
γ-irradiation dose 50 kGy	151 ± 7	2.1 ± 0.1
100 kGy	74 ± 11	1.8 ± 0.2

[a] Using fiber drawn to four times the original length.

Post-treatment of gelatin fiber

Heat-treatment and γ-irradiation of gelatin fibers were carried out for the purpose of crosslinking in the amorphous region, which leads to improvement of mechanical strength and water-resistance.[17,22] The fibers were treated with GA to compare the effects of the treatments.

The effects of various post-treatments of gelatin fiber drawn to four times the original length are given in Table III. The mechanical strength of the fiber was hardly changed by heating at 120°C for 1 h under an inert atmosphere. The strength of fibers was decreased slightly by heating at 180°C and at 220°C, probably due to thermal decomposition of the gelatin chain.[23]

Treatment with GA for 4 h resulted in a marked increase of mechanical strength, which can be attributed to chemical crosslinking. However, treatment for 72 h resulted in a decrease of mechanical strength. The decrease of mechanical strength is probably due to disruption of the fiber structure through swelling in the reaction solution simultaneously with crosslinking.[24] Crosslinking of a fiber induced by 50 kGy γ-irradiation gave a high σ_b of 151 MPa. A dose of 100 kGy resulted in a decrease of strength that was probably due to parallel degradation by γ-irradiation.[25,26]

Fiber without any post-treatment swelled in water at 25°C, and dissolved at ~ 35°C. The fiber treated at 120°C swelled by 124% in water at 35°C, and the fiber treated at 180°C swelled by 50%. Heat-treatment at these temperatures provided the fiber with insufficient water-resistance. However, the fiber treated at 220°C showed hardly any swelling in water at temperatures below 40°C and remained insoluble even at ~ 80°C. The fiber treated with GA did not dissolve at 95°C, and the degree of swelling in water of even higher temperature was extremely low. The water-resistance of the fiber is improved due to crosslinking in the amorphous region through heat-treatment and treatment with GA.

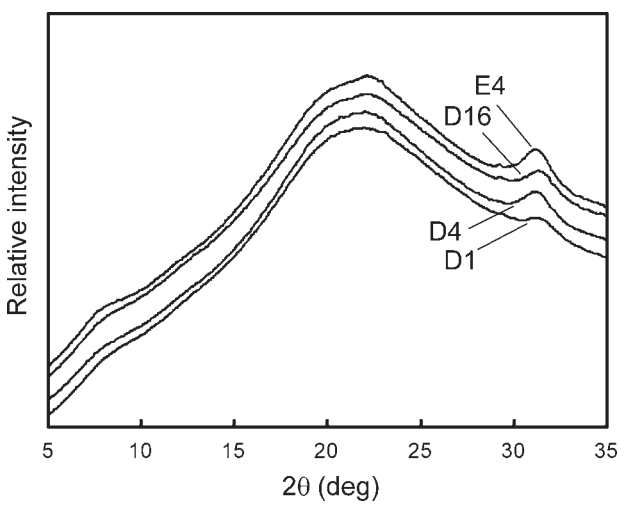

Figure 3 X-ray diffraction profiles of the gelatin fibers.

CONCLUSIONS

Gelatin fiber prepared by the extrusion of 15 wt % gelatin in DMSO into a refrigerated methanol bath at −20°C had a high level of drawability. The tensile strength and the Young's modulus of the fiber drawn to 16 times its original length were 146 MPa and 2.3 GPa, respectively. When EG was used as the spinning solvent, the resulting fiber had a tensile strength of 157 MPa and a Young's modulus of 2.5 GPa despite the low draw ratio of four. The addition of glycerol to the spinning solution improved the mechanical strength of the resulting fiber. The peaks of X-ray diffraction intensity observed at 7.8° and 31.5° increased with the draw ratio. The improvement in mechanical strength is due to the increase and the arrangement along the fiber axis of crystallite composed of the triple-helical segments as the result of drawing. In the absence of a crosslinking agent, the fiber heat-treated at 220°C was insoluble in water at temperatures up to 80°C. We demonstrated that

gel spinning, and drawing in the gel state, is useful for the preparation of gelatin fiber.

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