Structures and Physical Properties of Cross-Linked Gelatin Fibers

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ABSTRACT: This study aims to prepare a practical gelatin fiber suitable for use as a cross-linked suture. Various gelatin fibers were created in different ways: by cross-linking using heat (H); by cross-linking with carbonic acid (CA), namely citric acid; and by cross-linking with either of two epoxy compounds: ethylene glycol diglycidyl ether (E) and diglycerol triglycidyl ether (D). For each fiber type was produced at several different pH levels (7, 8, 10, and 12) in order to measure changes in the rate at which the amino group reacted with the epoxy group. E and D swelled more than H and CA did, and D swelled more than E and at every pH, D swelled more than E did. The cross-linking densities of E and D fibers reached a minimum at pH 8, corresponding to the degree of swelling. The stresses at a strain of 50% for E and D reached a minimum at pH 8. The toughest fiber, which has the largest surrounded area between stress and strain, was the cross-linked E at pH12.

KEY WORDS   Cross-Link / Gelatin / Fiber / Carbonic Acid / Diepoxide Compound / Swelling / Fracture /

Although collagen is expensive, it is a very important protein as a biomaterial, because it has a high rate of cell growth on its surface[1] and is biodegradable.[2] Collagen has therefore served as a cell culture substrate and as a tissue substitute.

Although low production cost makes it seem convenient to use gelatin regenerated from collagen as a biomaterial, there are three problems with the use of such gelatin: it dissolves at the temperature of the human body, it causes antigen-antibody reactions[3] and prion proteins can be contaminated by Bovine Spongiform Encephalopathy (BSE).[4]

The chemically cross-linked gelatin will not dissolve when heated, and is useful as a biomaterial. Murabayashi *et al.*[5] prepared a gelatin-coated polyurethane membrane that was grafted. This membrane showed excellent blood compatibility. Yoshizato *et al.*[6] made a porous gelatin membrane that was cross-linked with a compound, *i.e.*, with a cross-linker. This compound had two epoxy groups and showed excellent cell growth on the surface as well as biocompatibility. In a previous study we tried to prepare another membrane with a cross-linker containing two or three carbonyl groups. We also studied the growth of cultured L929 cells derived from mouse connective tissue fibroblast, and clarified that the rate of growth of those cells was similar to that on collagen-coated polystyrene film.[7,8] These results mean that chemically cross-linked gelatin is useful as a tissue substitute. It is considered that cross-linking using amino groups in gelatin molecules is also effective for preventing an

antigen-antibody reaction, by virtue of the presence of an amino group in the side chain. Furthermore, even if the prion protein contaminates the gelatin, the prion will be cross-linked by using amino groups such as lysine, asparagine and glutamine in the protein[9] with epoxy and carbonic acid compounds. This will prevent the protein from becoming an abnormal prion.

It is well known that gelatin is also useful as a biodegradable material, *e.g.*, as a food. Gelatin fibers are therefore expected to find use as biodegradable sutures that are biocompatible as described above. No one, however, has successfully prepared a practical gelatin fiber.
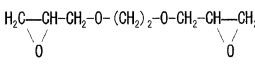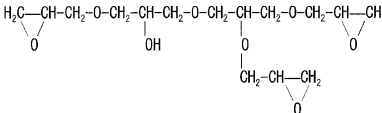
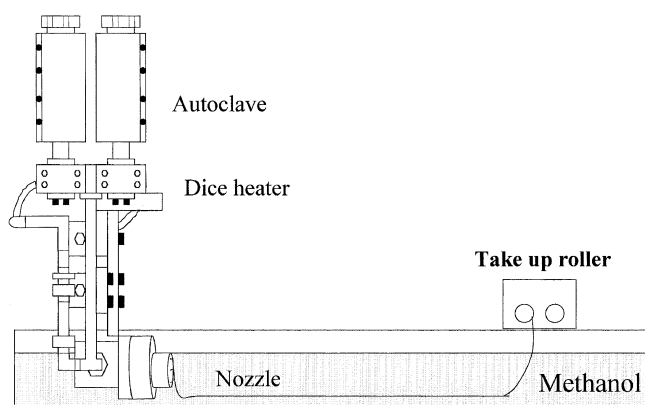This study aims to prepare a practical gelatin fiber as a cross-linked suture.

EXPERIMENTAL

The gelatin used in this study, kindly supplied by Nitta Gelatin Co. Ltd., Japan, was derived from cattle bones gelatinized with alkali. This study used two types of cross-linking agents, called cross-linkers. One type includes a tri-carbonic acid compound, namely citric acid, purchased from Wako Co., Japan, without any purification. The other type of cross-linker includes two multi-functional epoxy compounds: ethylene glycol diglycidyl ether (EGDE) and diglycerol triglycidyl ether (DTE), purchased from Nagase Chem. Tec. Co., Japan. The chemical structure of each cross-linker is shown in Table I. These cross-linkers will react with the amino group in the gelatin to carry out the objectives described in the introduction.

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Table I. Chemical structure of cross-linker

Citric acid (CA)	$HOOC-CH_2-CH(COOH)-CH_2-COOH$
Ethylene glycol diglycidyl ether (EGDE)	$H_2C-CH-CH_2-O-(CH_2)_2-O-CH_2-CH-CH_2$ with epoxide O groups
Diglycerol triglycigyl ether (DTE)	$H_2C-CH-CH_2-O-CH_2-CH-CH_2-O-CH_2-CH-CH_2-O-CH_2-CH-CH_2$ with OH and epoxide groups, and $CH_2-CH-CH_2$ branch

Figure 1. Schematic drawing of wet spinning apparatus.

Three types of cross-linked gelatin fibers were produced according to the following procedures.

① A 20 wt% aqueous solution of gelatin at 40 °C was spun in methanol at 20 °C by using the wet spinning apparatus shown in Figure 1, with a nozzle diameter 0.5 mm. The resulting fiber was heated in an oven in vacuum at 150 °C for 3 h to cross-link.[10] We named this fiber H.

② A 20 wt% aqueous solution of gelatin was mixed with citric acid in an amount equal to the mol of the amino group in the gelatin. The mixture at 40 °C was spun in the same way as described above. The resulting fiber was cured in an oven in vacuum at 150 °C for 3 h to cross-link. We named this fiber CA.

③ A 20 wt% aqueous solution of gelatin at 40 °C was spun under the same conditions as above, and then the gelatin fiber was immersed in the mixture {1/9 (w/w)} of the epoxy compound (as the cross-linker) and solvent {water/methanol(6/4)} for 3 h at 20 °C, at pH 7, 8, 10, and 12 to change the reaction rate of the amino group with the epoxy group.[11] After immersion, the cross-linked gelatin was dried in air atmosphere. We named the fibers cross-linked with EGDE and DTE E and D, respectively.

The reaction ratio of the amino group of gelatin with the epoxy group of the cross-linker was estimated by the TNBS method.[12] The reaction ratio (X) was calculated by using eq 1,

$$X = 100\,(S_u - S_r)/S_u \qquad (1)$$

where S_u and S_r are the absorption intensities at 345 nm of UV spectra before and after the cross-linkage, respectively. UV spectra were measured by using a Hitachi U-3500 at 300 to 450 nm, with a scanning speed of 300 nm min^{-1}.

The degree of swelling was calculated using eq 2 and assuming the swelling would be isotropic because the fiber was not oriented toward fiber axis.

$$S = (L_s/L_d)^3 \qquad (2)$$

where L_d and L_s are the length of the cross-linked fiber before and after swelling, respectively. Swelling was measured in water at 40 °C for 1 h.

Mechanical properties were measured by using a Tensilon, UTM-4L (Toyo Sokki Co., Ltd.), at a crosshead speed of 40 mm min^{-1}. The equilibrium stress was measured at strains of 20, 40, 60, 80, and 100%.

To obtain the storage modulus, dynamic mechanical thermal analysis (DMTA) was carried out with an ITK Co. DVA-225 in a stretching mode with a frequency of 10 Hz and at a heating rate of 5 °C min^{-1}.

RESULTS AND DISCUSSION

The degree of swelling depended heavily on the cross-linker used. Figure 2 shows the degree to which each of the cross-linked fibers swelled. E and D swelled more than H and CA did, indicating that E and D had relatively low cross-linking densities. In every pH case, D swelled to a greater degree than E did. This was because DTE contained a hydrophobic OH group. For both E and D fibers, the degree of swelling reached a maximum when the mixture of the cross-linker and the solvent was at pH 8. This means that the degree of cross-linking reached a minimum at pH 8. Therefore, it is necessary to know how the degree of swelling relates to cross-link density.

Figure 3 shows the reaction rates of amino groups in gelatin with the carboxyl and epoxy groups of the various cross-linkers. The reaction rate of CA is higher than
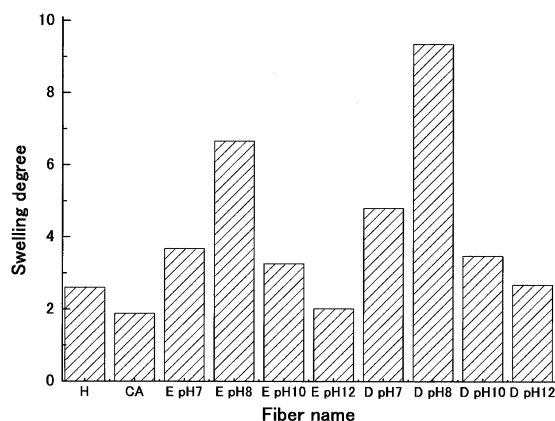
**Figure 2.** Swelling degree of cross-linked fiber.
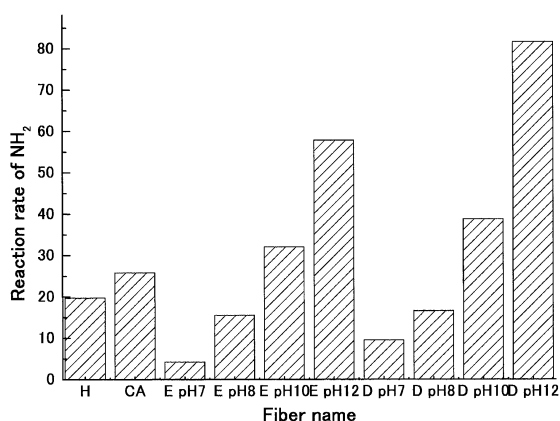
Figure 3. Reaction rate of amino groups of gelatin with carboxyl group and epoxy group of cross-linker.

that of H, because the mixed citric acid has three carbonic acids as functional groups, so these groups cross-link easily with the amino group in gelatin. The reaction rates for E and D increase as pH increases. There is a discrepancy between the reaction rate of the amino group and the degree of swelling, as seen Figure 2. This discrepancy occurs because stress depends directly on the entropy-elasticity of the molecules between cross-link points for the hydrogel. The reason for the discrepancy is probably clarified by the cross-linking density, which we evaluated by measuring the stress-strain relation of the cross-linked fibers swollen in water.

Figures 4a, 4b, and 4c show the dependence of stress, σ (Nm^{-2}), on $\lambda - \lambda^{-2}$, where λ is the elongation ratio. All data are linear at below 0.4 of $\lambda - \lambda^{-2}$, namely $\lambda = 1.15$. This indicates that every cross-linked fiber satisfies the equation of ideal rubber elasticity in this region. Therefore we can estimate the cross-linking density, n_c, (Number m^{-3}), by using eq 3 of ideal rubber elasticity,[13]
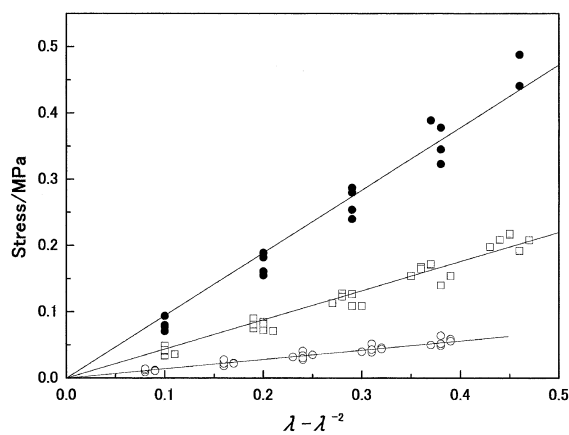
$$\sigma = n_c kT(\lambda - \lambda^{-2}) \qquad (3)$$

where k is Boltzman's constant, 1.38×10^{-23} (N m K^{-1}), T is absolute temperature (K), which in this study is 313 (K). The calculated cross-linking
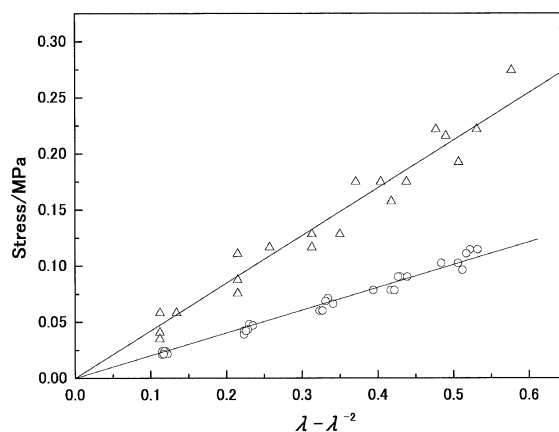

(a)
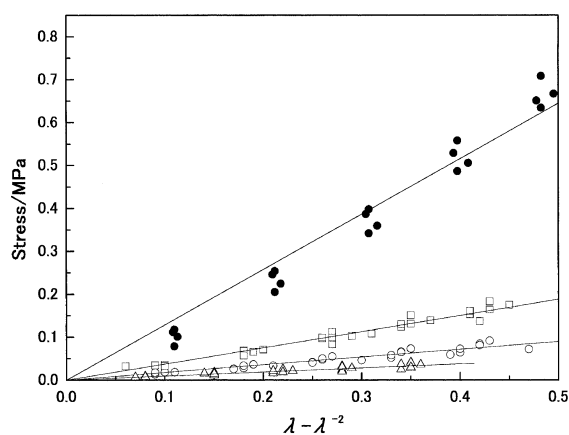
(b)

(c)

Figure 4. Dependence of stress, σ, on $\lambda - \lambda^{-2}$, (a) H; —○—, CA; —△—, (b) E: pH7; —○—, pH8; —△—, pH10; —□—, pH12; —●—, (c) D: pH7; —○—, pH10; —□—, pH12; —●—.

densities are shown in Figure 5. The cross-linking density of CA is larger than that of H. This corresponds to the differences in the reaction rate of the amino group with the carboxyl group as the cross-linking point, as seen in Figure 3. The cross-linking densities of E and D reach a minimum at pH 8, indicating correspondence with the swelling degree. It is known that the carbonyl group reacts easily with the epoxy group at low pH. This suggests that at pH 7, the epoxy
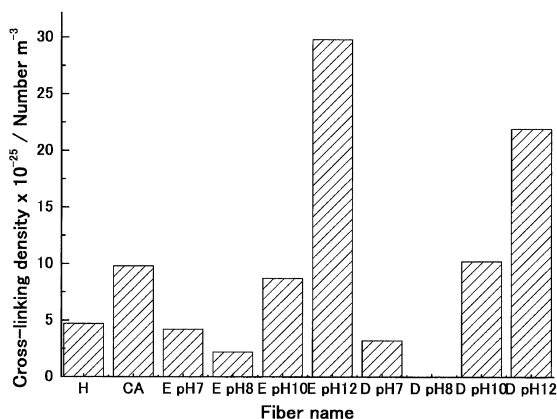
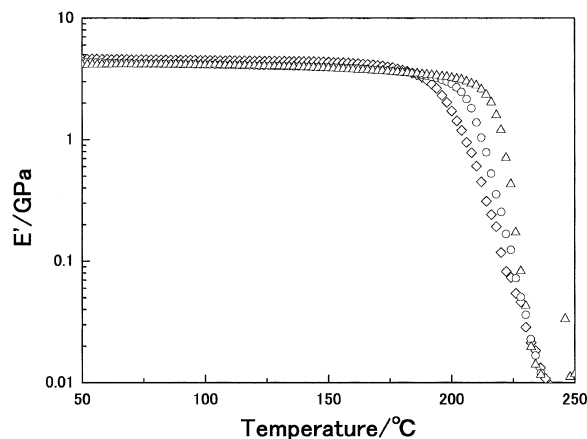**Figure 5.** Cross-linking density of cross-linked fibers.
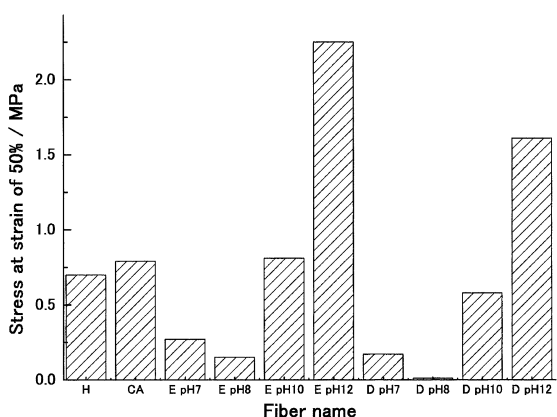
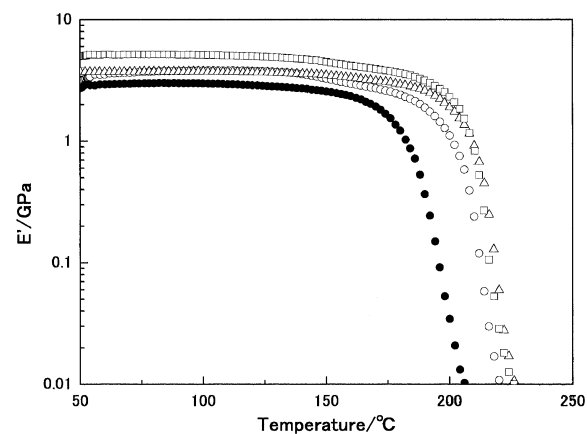**Figure 6.** Dependence of stress at strain of 50% for cross-linked fibers.
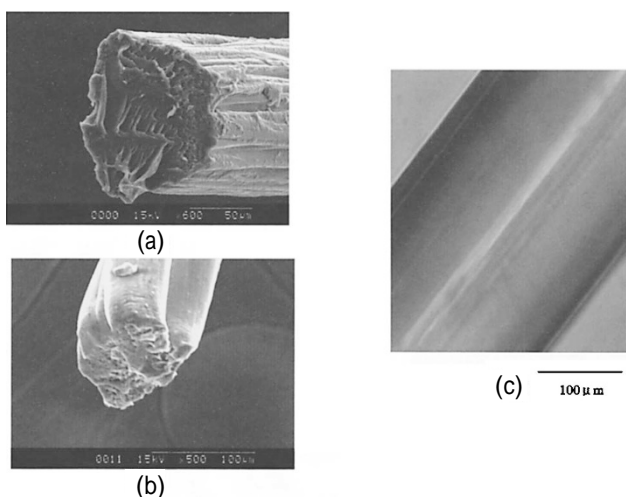
Figure 7. SEM photographs, (a) uncross–linked dried E, (b) cross–linked E at pH7, (c) wet fiber of cross–linked E at pH7.

Figure 8. Dependence of storage modulus on temperature, (a) Uncross-linked gelatin; —◇—, H; —○—, CA; —△—, (b) E: pH7; —○—, pH8; —△—, pH10; —□—, pH12; —●—, (c) D: pH7; —○—, pH8; —△—, pH10; —□—, pH12; —●—.

group will react not only with the carbonyl group but also with the amino group. This result of the cross-linking density will be related to the mechanical property of the cross-linked gelatin hydrogel fiber.
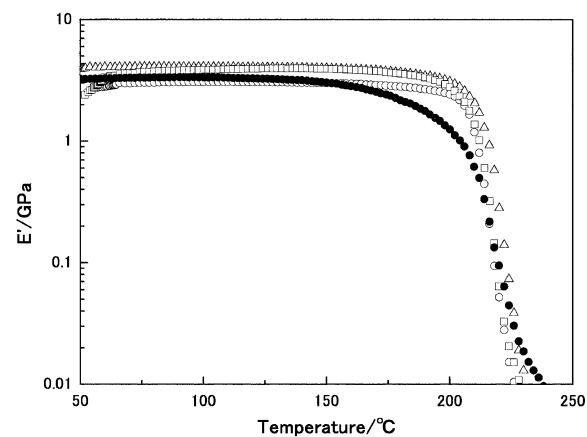
Figure 6 shows the stress at a strain of 50% for the cross-linked fibers. The stress of CA is larger than that of H because of the higher cross-linking density of the former. Furthermore, the stresses of H and CA are

lower than those of E and D. This is probably caused by the high temperatures, at which those fibers become thermally decomposed. The stresses of E and D fibers reach a minimum at pH 8. This result supports the above speculation. The stress of the swollen fiber cross-linked at pH 12 is probably enough to make this fiber suitable for practical application as a cell culture substrate of woven or unwoven cloths.

(a)

(b)

(c)
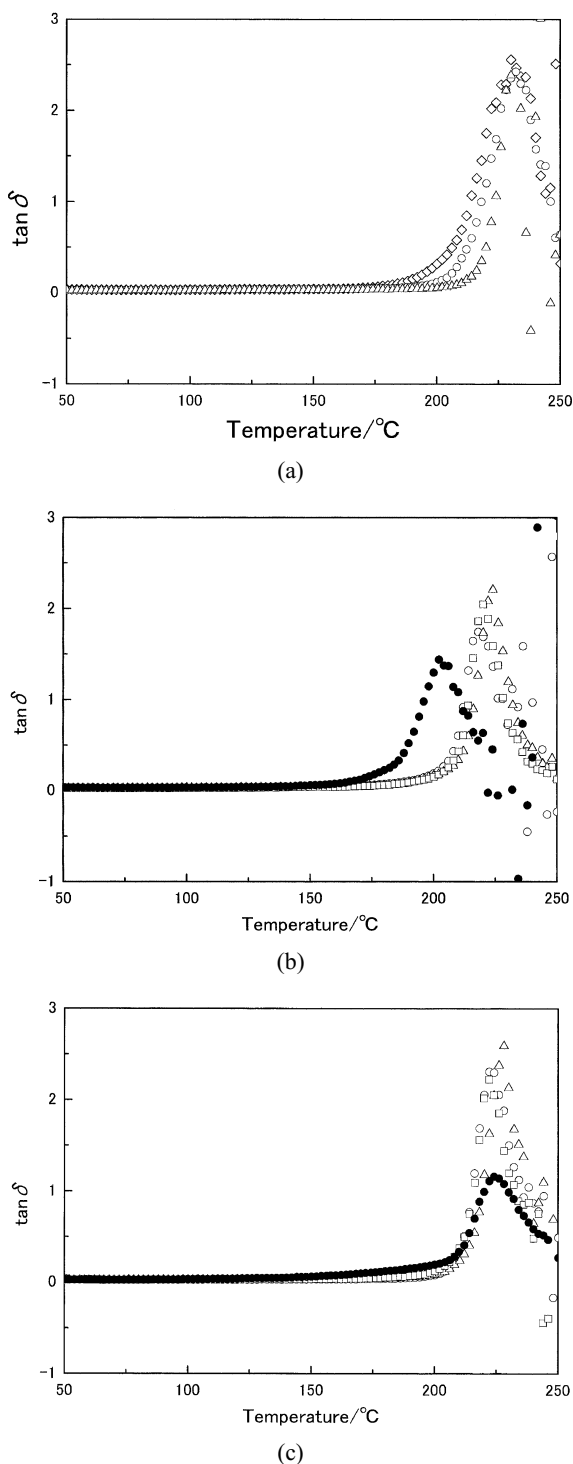
Figure 9. Dependence of tan δ on temperature, (a) Uncross-linked gelatin; —◇—, H; —○—, CA; —△—, (b) E: pH7; —○—, pH8; —△—, pH10; —□—, pH12; —●—, (c) D: pH7; —○—, pH8; —△—, pH10; —□—, pH12; —●—.

Figure 7 shows scanning electron microphotographs (SEM) of (A) dried non-cross-linked fiber, (B) dried cross-linked H, and an optical microscope photograph of (C) wet cross-linked H. The dried fiber has many grooves on the surface, caused by dehydration in spinning into methanol, while the wet fiber has no grooves, because of swelling. Every SEM for other cross-linked
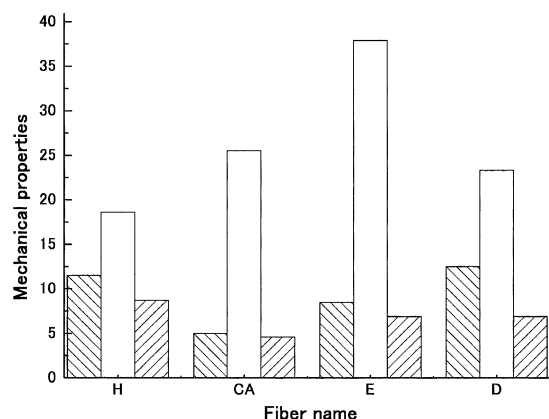
Figure 10. Mechanical properties of cross-linked fibers, ⊠; Fracture stress × 100/GPa, □; Fracture strain/%, ▨; Young's modulus/GPa.

fibers (not shown) looked like the SEMs in the figure.

Figures 8a, 8b, and 8c show the temperature dependence of the storage modulus, E′, for dried fibers. Above roughly 180 °C, every E′ of fiber decreases abruptly. In comparison with the uncross-linked gelatin fiber, H and CA decrease on the higher temperature side, while E and D with high cross-linking density decrease on the lower temperature side. This phenomenon is caused by lengthening the long-chain molecule or of the cross-linker; either of these would affect mobility more than they would affect the cross-linking density. Since gelatin has a glass transition temperature (T_g) at 196 °C, as determined by using a differential thermal analysis, this abrupt decrease in E′ change is due to the onset of the segmental motion of the amorphous chain between cross-linked points. The segmental motion can be observed as a peak in the dependence of tan δ on temperature in the cases of crystalline polymers and cross-linked polymers. Figures 9a, 9b, and 9c show the temperature dependence of the tan δ. Every fiber shows a peak around 230 °C, which is about 35 °C higher than in the case of T_g. This means that the cross-link in the fibers is tightly formed by heating, because the weight loss of gelatin is about 3 wt% at 220 °C. Meanwhile, the temperature at which tan δ increases is close to the decrease temperature of E′. Therefore, these results support that the molecular motion of cross-linkers occurs at lower temperatures than that of gelatin molecules in the case that E and D have high cross-linking as described above.

Figure 10 shows fracture stresses and strains as well as the Young's modulus of dried H, CA, E, and D. The toughest fiber, which has the largest surrounded area between stress and strain, was E cross-linked at pH 12, and it could be knotted by hand. Therefore, E can probably serve as a suture fiber.

It should be studied in future to what extent these

cross-linked gelatin fibers prevent the protein from becoming an abnormal prion.

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