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Elastic properties of reconstituted collagen hollow fibre membranes

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Tensile stress–strain curves of reconstituted collagen hollow fibres were measured along the fibre axis as a function of relative humidity. At low strains the stress–strain curves show a small "toe", a region of positive curvature at moderate relative humidity, which is expanded greatly when the fibre is immersed in water. The water-swollen fibre can be modelled as a cross-linked network of ~ 60 Å diameter collagen fibrils, which are distended and crimped on a microscale to accept water between them in ~ 160 Å diameter pores. The elastica analysis of Diamant *et al.* provides a good representation of the toe of the stress–strain curve as well as predicting crimped angles and fibril lengths between cross-links in reasonable agreement with those inferred from small-angle X-ray scattering results. The fibril elastic modulus E, defined as the slope of the linear region of the stress–strain curve past the toe, decreases continuously as a function of relative humidity and the modulus of the wet fibrils increases with increasing ultraviolet irradiation dose used for cross-linking. These changes probably reflect the water-induced denaturation of portions of the triple helix of the tropocollagen molecules to constrained random coils, evidence for which is observed in the wide-angle X-ray scattering pattern of the wet fibre. Achieving better strength properties in the hollow fibres may hinge on preventing or minimizing this partial denaturation of the reconstituted tropocollagen molecules.

1. Introduction

The technology for large-scale production of inexpensive reconstituted collagen products (chiefly edible sausage casing) has developed rapidly in recent years. Native collagen (from steer hide) can be made soluble in acid solution by treatment with proteolytic enzymes which remove from the rod-like, triple-helical tropocollagen molecule small non-helical appendages called "telopeptides" [1, 2]. An acid solution of tropocollagen molecules then can be reconstituted into solid collagen by extruding it into a coagulating bath of high ionic strength or high pH. This reconstituted product is subsequently cross-linked with ultraviolet irradiation to render it insoluble in acid and neutral media and to improve the wet strength [3]. In addition to its main use as sausage casing such reconstituted collagen has good prospects for various biomedical applications [4], especially as a membrane for artificial kidney dialysis.

Unfortunately the mechanical strength of such reconstituted membranes in water is only a small fraction of that of native collagen fibres and this weakness presently limits its application to membrane geometries, e.g. hollow fibres of small radius, which give rise to only small stresses in the membrane. As part of a programme to improve these strength properties, we have investigated the structure and mechanical properties of reconstituted collagen hollow fibres. Wide-angle and small-angle X-ray (WAXS and SAXS) measurements [5] suggest a model for the structure of the wet hollow fibre which is shown in Fig. 1. Collagen fibrils, approximately 60 Å in diameter and with the long axis of the tropocollagen molecule approximately aligned along the fibril axis, are

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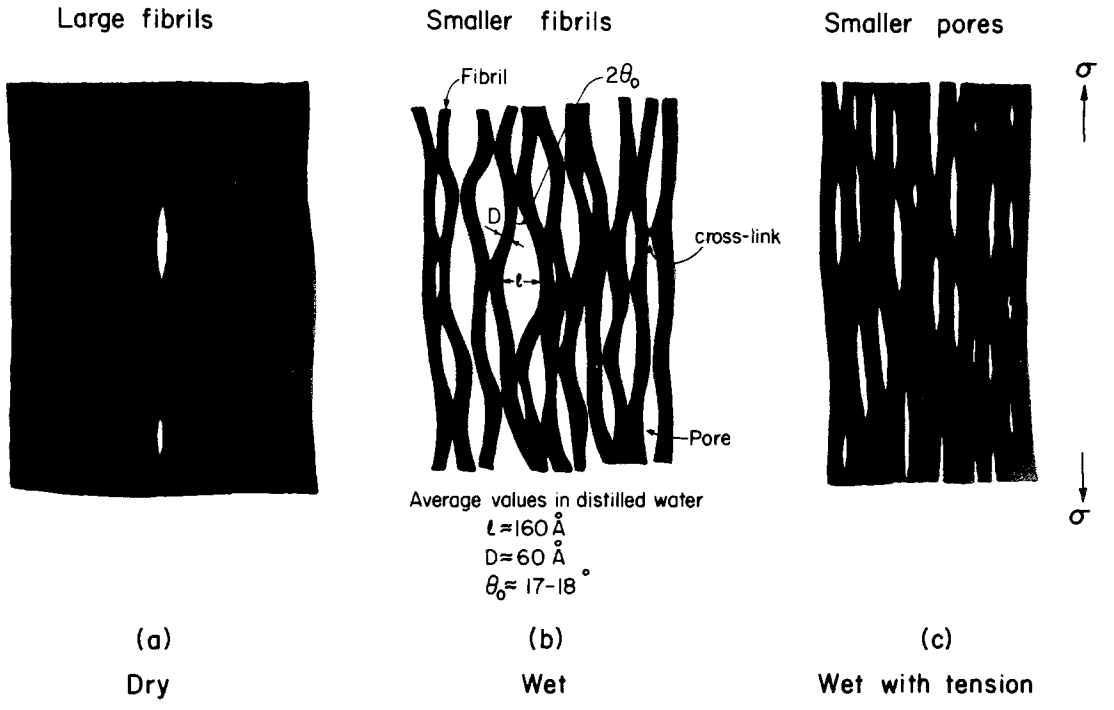

Figure 1 Schematic drawing of the model proposed in [5] to represent the structural changes in reconstituted collagen hollow fibres upon entry of water.

distended to accept water between them in pores approximately 160 Å in diameter. In the dry state, there is a marked increase in the fibril diameter and in the tropocollagen molecular orientation along the axis of the hollow fibre as the pores all but disappear. The WAXS pattern also indicates a marked increase in crystalline perfection (including the helical content of the molecule) of the fibrils as water is removed.

These structural changes induced by water, should produce marked changes in the mechanical properties. In particular the stress–strain curve of the wet fibre should reflect the fact that tensile strain can be produced by a straightening of the fibrils "crimped" by the pore structure before it is necessary to stretch the fibrils themselves in tension. One also expects the differences in crystal perfection of the fibrils with water content to product substantial changes in the Young's modulus of the fibre. These expectations are borne out by our results on the elastic properties of these hollow fibres, which we report below.

2. Materials and methods

The hollow fibres used in this study were 15 to 20 μm thick and 400 μm o.d. (dry dimensions) and were made by the Japan Leather Company. The reconstitution process, and subsequent chemical processing of the fibres are discussed in detail elsewhere [5].

All tension tests were carried out on an Instron testing machine with a special environmental chamber in which the relative humidity could be controlled at a constant value or in which the sample could be immersed directly in distilled water. Bouyancy corrections were made in the case of immersed tests. The strain-rates for testing varied from about 3×10^{-4} to 6×10^{-6} sec^{-1}. The cross-sectional area of the hollow fibres were determined by taking an average of the area measured under the microscope and the area calculated from density and weight measurements. All stress values are calculated on the basis of dry cross-sectional area* although the actual area of the hollow fibres increases several fold upon wetting. The gauge length was typically about 5 cm.

3. Experimental results and discussion

3.1. The stress–strain behaviour

The tensile stress–strain curves of the hollow fibres as a function of relative humidity are shown in Fig. 2. It can be seen that at relative humidities

* For comparison this is a preferred quantity since only the fibrils can bear load.

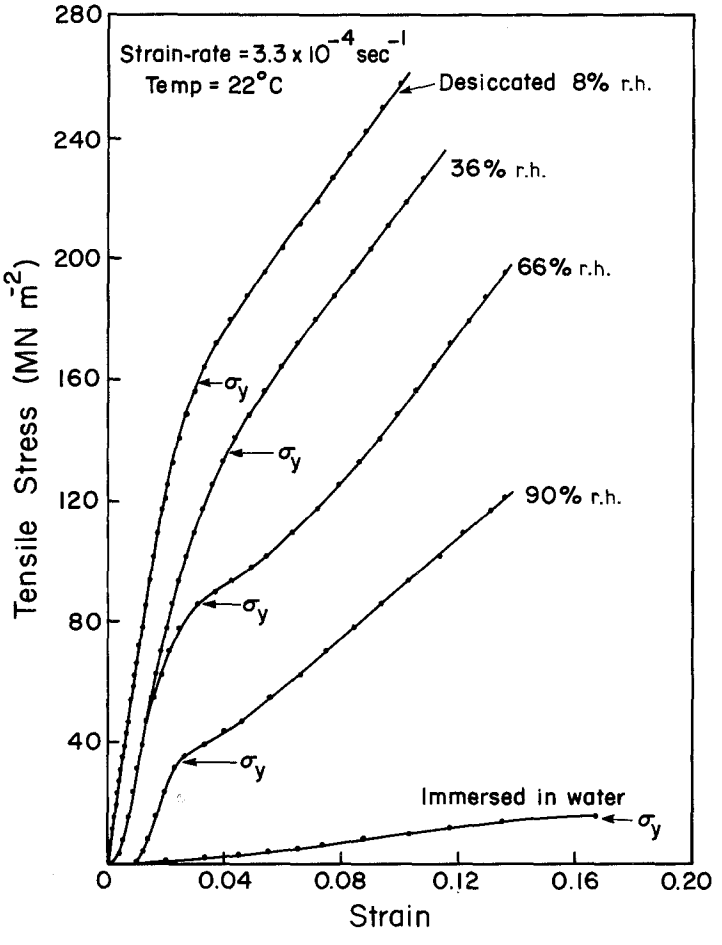

Figure 2 Tensile stress–strain curves of the hollow fibres as a function of relative humidity.

(RH) of ~ 60% or more a small "toe" (a region of upward curvature at low strains) develops and increases with increase in humidity. For the wet hollow fibres (immersed in water) this "toe" region is very pronounced. It is significant that this "toe" region only develops at RH's greater than that at which the volume fractions of water determined by wide-angle X-ray measurements and weight change measurements start to deviate from each other indicating the presence of "free water" in pores between the fibrils [5].

The toe region and the linear region immediately following it represent elastic deformation of the fibrils in the sense that the deformation is recoverable. The slope of the linear region will be referred to as the elastic modulus E. At still higher strains the stress begins to fall below the extension of the linear elastic line. Past this point some non-recoverable (plastic) strain is produced and correspondingly the stress at this point is called by us the "yield stress", σ_y. The values of σ_y are indicated on the various curves in Fig. 2.

However, this does not mean that all the strain beyond that corresponding to the yield stress is non-recoverable. Upon fracture it is found that a major portion (~ 90%) of the total strain is in fact recoverable. The yield stress then is simply that stress above which some permanent deformation begins to set in. In dry samples the yield stress is rather ill-defined but at both 60% and 90% RH well-defined yield behaviour is observed.

A magnified version of the tensile stress–strain curve of the wet hollow fibre at two temperatures is shown in Fig. 3. The "toe" region can be clearly distinguished. A similar feature is also present in the stress–strain curves of native tendon (a composite of collagen and various muco-polysaccharides) [6, 7]. This has been interpreted as due to the straightening of a macroscopic crimp of the tendon with increasing strain, which can be easily followed under a polarizing microscope [8]. In the hollow fibres such macroscopic crimping is not present as the fibres are uniformly birefringent. However, the structural model proposed in [5]

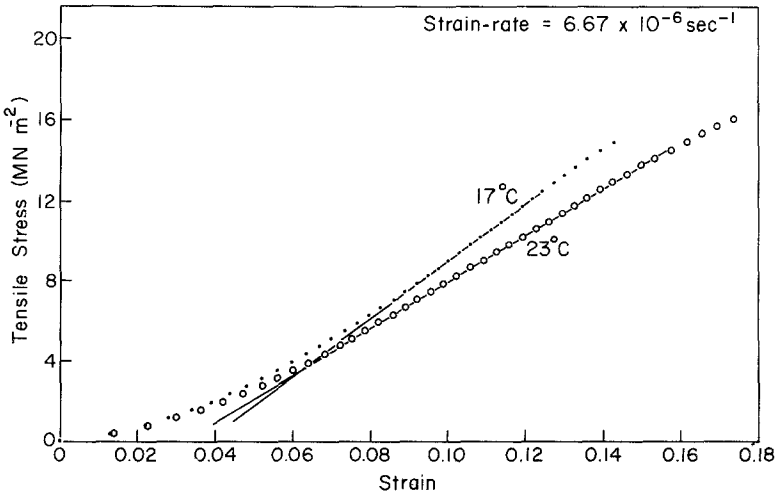

Figure 3 A magnified version of the tensile stress–strain curves of wet hollow fibres at two different temperatures.

predicts crimping on a microscopic scale, due to the distention of the fibrils caused by water absorption (see Fig. 1) between cross-links and this microcrimp should lead by the same mechanism to a toe in the stress–strain curves. In the following section, we examine this microcrimp hypothesis quantitatively and show it to be a reasonable explanation of the elastic behaviour of the membrane.

3.2. Elastica analysis

Diamant *et al.* have quantitatively analysed the stress–strain curves of native tendon using an "elastica" analysis [8]. Certain basic parameters involved in the analysis will be discussed with the aid of Fig. 4a and c. A typical stress–strain curve of the wet hollow fibre is shown in Fig. 4a. In the "toe" region the tangent modulus $d\sigma/d\epsilon$ increases steadily until it reaches a constant value. Following the elastica analysis [8] we propose that the toe region corresponds to straightening out of the crimp in the fibril structure introduced by water and the linear region to elastic extension of the fibrils. The slope of the linear portion will be referred to as the fibril elastic modulus E and the intercept of the linear portion with the strain axis will be denoted by ϵ'_∞. Fig. 4b shows a highly-idealized linear zig-zag crimp of the DKBLA model in which θ_0 is the angle and l_0 the half-period of the crimp along the axis of the hollow fibre. The length of the fibril between adjacent crimp is l where $l \equiv l_0 \sec \theta_0$. The strain required to straighten out the crimp completely is $\epsilon_\infty = (\sec \theta_0 - 1)$. The crimped fibril after a certain tensile strain appears

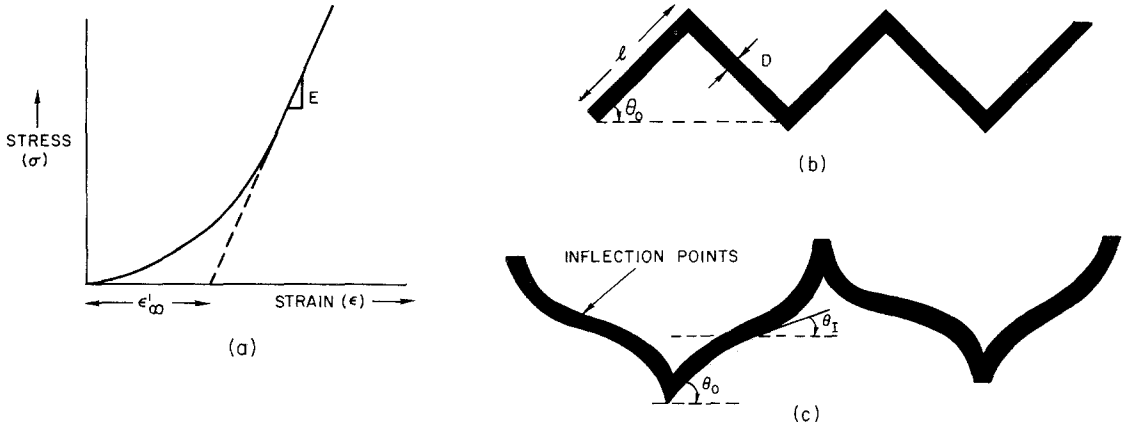

Figure 4(a) A typical stress–strain curve of the wet hollow fibre. The intercept on the strain axis of the extrapolated linear region is ϵ'_∞. The slope of the linear region is E. (b) Idealized "elastica" model of crimped fibrils of length l, diameter D and crimp angle θ_0. (c) The crimped fibrils at finite tensile strain. Equation 1 is valid for $\theta_0 \gg \theta_I \approx 0$.

as shown in Fig. 4c. Diamant *et al.* have discussed the deformation behaviour of such a model in detail and derived a mathematical expression for the stress–strain relation. This relation is given approximately by Equations 1 and 2, namely

$$\epsilon - \sigma/E = \epsilon_\infty - \lambda\sqrt{(E/\sigma)} \quad (1)$$

where

$$\lambda = D/l\left[\frac{1-\cos(\theta_0/2)}{\cos\theta_0}\right]. \quad (2)$$

Here ϵ is the strain, σ is the stress and D is the diameter of the fibrils. The stress–strain relation given by Equation 1 is valid only if the angle which the fibril makes with the tensile axis at the inflextion point θ_I is approximately zero and much less than the crimp angle θ_0, i.e. it is only strictly valid for strains approaching ϵ'_∞. This certainly is not the case at very low strains and the plot of $(\epsilon - \sigma/E)$ versus $\sqrt{(E/\sigma)}$ must deviate in this regime from the straight line relation predicted by Equation 1.

To see if Equation 1 can represent the stress–strain curves of the wet hollow fibre we have plotted our results as $(\epsilon - \sigma/E)$ versus $\sqrt{(E/\sigma)}$ in Fig. 5. A linear relationship obtains over an intermediate range of strains. The intercept of the extrapolated line on the $(\epsilon - \sigma/E)$ axis, which is ϵ_∞ in Diamant *et al.*'s paper can be used to calculate θ_0. Using the θ_0 value thus obtained and the slope λ of the straight line relation between $(\epsilon - \sigma/E)$ and $\sqrt{(E/\sigma)}$ we can calculate l/D. A direct extrapolation of the linear portion of the σ–ϵ curve to $\sigma = 0$ gives ϵ'_∞ which approximates (but is somewhat smaller than) ϵ_∞. Values for ϵ'_∞, ϵ_∞, θ_0 and l/D at different humidities are listed in Table I. It is quite clear that θ_0 increases with increase in relative humidity and is a maximum in the wet state.

TABLE I Crimp parameters determined from the stress–strain curves of the hollow fibre using Diamant *et al.*'s analysis

	Relative humidity			
	66%	90%	Wet	Wet
Temperature (°C)	23	23	23	12
ϵ'_∞	0.004	0.011	0.032	0.038
ϵ_∞	–	–	0.045	0.051
θ_0, degrees	5*	8.5*	17	18
l/D	–	–	4.6	4.4

* These two values were estimated assuming $\epsilon'_\infty = (\sec\theta_0 - 1)$ and are likely to be smaller than the actual values.

The structural model and the elastica analysis

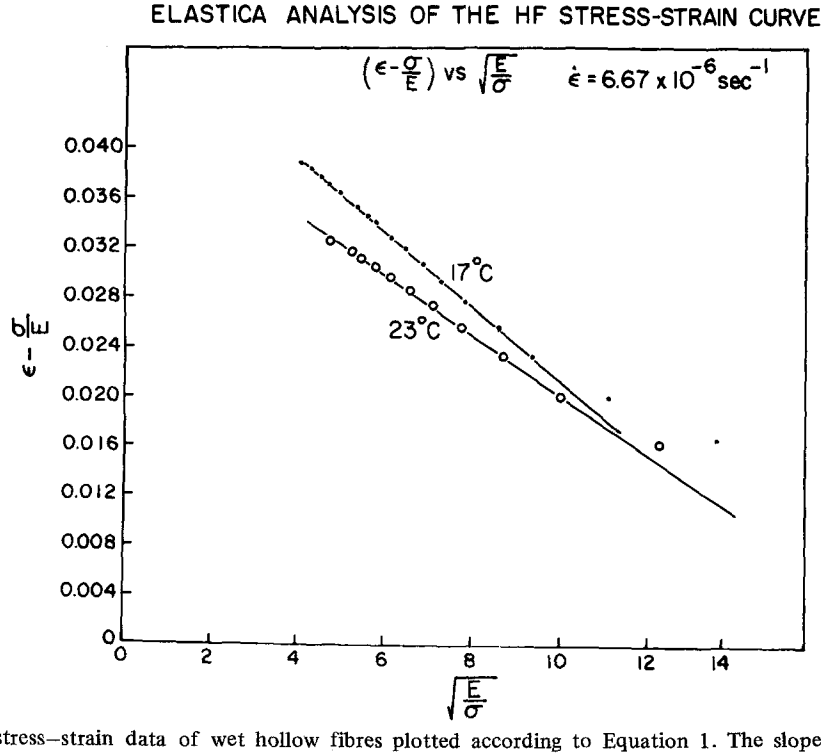

Figure 5 The stress–strain data of wet hollow fibres plotted according to Equation 1. The slope of the lines is λ (Equation 2).

of it can be checked for quantitative consistency since values of fibril diameter D and pore diameter are available from small-angle X-ray scattering (SAXS) studies. Starting from the SAXS value of $D = 60$ Å and the value of l/D from Diamant *et al.*'s elastica analysis (Table I) we find $l \simeq 280$ Å. Taking the zig-zag model literally, the maximum pore diameter is $2l \sin\theta_0$ which equals 160 Å which is to be compared with the experimental (SAXS) value of pore diameter, 160 Å [5]. The excellent agreement must be termed fortuitous, given the crudity of the model, but nevertheless it does reinforce the conclusion that the toe of the stress–strain arises from the straightening of a microcrimp associated with the water-filled pore structure.

At very low strains the data in Fig. 5 lie systematically above the straight line of Equation 1 as expected since θ_I is no longer close to zero. On the other hand, at very low strains the deflection of the zig-zag crimp can be treated as that of a bent cantilever (Appendix). One can derive a relationship between $\lim_{\epsilon\to 0}(\sigma/\epsilon)$, l/D, θ_0 and E, namely

$$(l/D)^2 = \frac{3E}{4}\frac{\cos\theta_0}{\sin^2\theta_0}\frac{1}{\lim_{\epsilon\to 0}(\sigma/\epsilon)}. \qquad (3)$$

Thus one can use the initial slope of the stress–strain curve to arrive at an independent estimate of $l/D \simeq 6$ for the wet fibril. Given the crudity of the linear zig-zag model this is in reasonable agreement with the value obtained via Diamant *et al.*'s analysis.

In spite of the fact that Diamant *et al.*'s model produces a reasonable fit to our data, the assumption of this analysis that the initial crimp is stress free is probably questionable. Indeed our picture (Fig. 1) of water molecules pushing in between fibrils to create pores implies repulsive forces between fibrils, acting transverse to the fibrils. The straightening of a fibril under transverse load by applying a tensile stress gives rise to a different, and more complicated set of equations than does the straightening of an initially unstressed linear zig-zag and one which, in general, must be solved numerically. We have analysed such a model in conjunction with a study of the elastic response of the hollow fibre in low pH media in which the fibrils are charged and repel one another strongly. The results of this numerical analysis show that Diamant *et al.*'s analysis, even though it conceptually cannot apply to this case, is still a good representation of the stress–strain curve and l/D values obtained from it are not far from those obtained using the more exact lateral force model. (The values of θ_0 from Diamant *et al.*'s analysis tend to be low since the real fibril forms a smooth curve and not a sharp crimp as the zig-zag implies.) The simple elastica analysis of the zig-zag crimp is thus suitable for our purposes in this paper but one should bear in mind that physically the model is probably quite unrealistic.

3.3. The fibril elastic modulus

The elastic modulus E of the fibrils making up the hollow fibre is defined as the slope of the linear portion of the stress–strain curve (see Fig. 4a) and is very sensitive to the relative humidity as

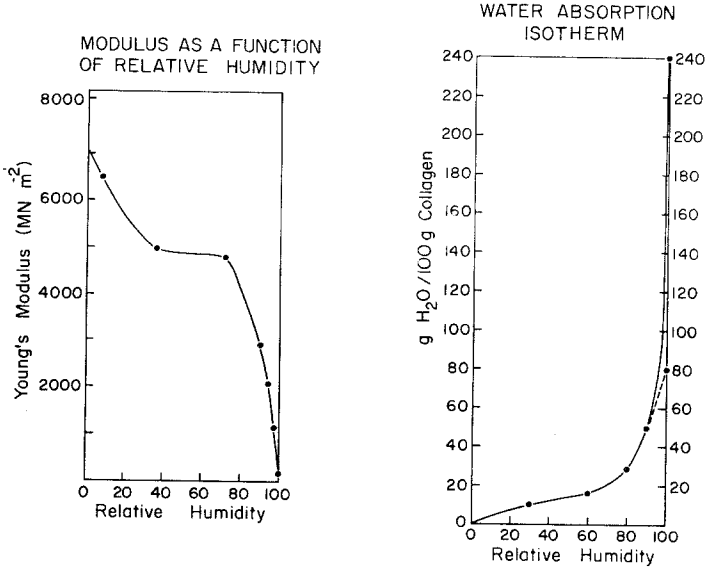

Figure 6 Changes in the elastic modulus of the hollow fibres as a function of relative humidity.

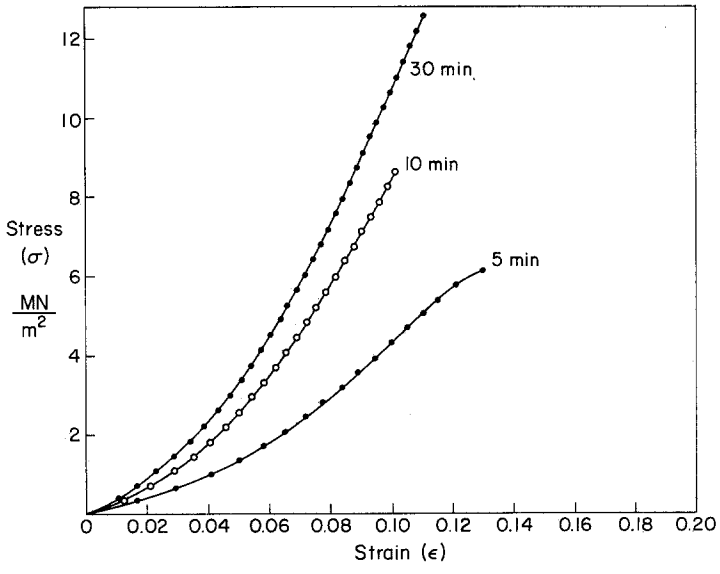

Figure 7 Tensile stress–strain curves of wet hollow fibres cross-linked by ultraviolet irradiation for 5, 10 and 30 min.

shown in Fig. 6. The absorption isotherm for water is also displayed in this figure. It is significant that the rapid increase in water absorption at high r.h. corresponds to the rapid decrease in modulus in this range. These changes in E are reversible in the sense that the r.h. can be cycled from high to low values and back again and the original E will be restored.

The effect of temperature on the modulus can be inferred from the stress–strain curves of Fig. 3. At the lower temperature the fibres have a higher modulus indicating that the elasticity is not completely entropy driven and that energy effects must be taken into account. All of our other evidence, however, points to the conclusion that the fibrils in the wet hollow fibre deform elastically in a predominantly rubbery manner. For one thing, although the magnitude of E in the dry fibre is typical of the modulus of a semicrystalline or glassy polymer, the E of the wet fibre is much too low to be produced by the stretching and bending of bonds. The magnitude of the wet modulus ($\sim 100\,\mathrm{MN\,m^{-2}}$) is more typical of a cross-linked elastomer than of a semicrystalline polymer. Experiments where the cross-link density is changed by varying the ultraviolet irradiation time reinforce this view. The wet stress–strain curves of three hollow fibres cross-linked by ultraviolet irradiation for 5, 10 and 30 min are shown in Fig. 7. There is a marked increase in E and σ_y with increased cross-link density and a modest decrease in ϵ_∞ and θ_0. The increase in E with cross-link density is typical of an elastomer. Finally, there are the wide-angle X-ray scattering results [5] which show that the relatively sharp reflections of the triple helix of the tropocollagen molecules in the dry fibre become very diffuse, not unlike the amorphous halo of cold-cast gelatin, although some trace of the 2.8 Å arc, which represents the fundamental repeat along the axis of the triple helix, remains. The best explanation of this pattern is that significant portions of the triple helix in our reconstituted collagen unwind in water to form a constrained random coil between intact portions of triple helix.*

Over two decades ago Bear [9] proposed that the amino acid sequence in collagen contained alternating regions rich in polar and non-polar residues, an hypothesis amply confirmed by recent cyanogen bromide sequence analysis [10]. A close examination of the sequences indicates, moreover, that a definite deficiency of imino residues (hydroxyproline and proline) exists in the regions rich in polar groups. Since imino residues are important in stabilizing the triple helix conformation as evidenced by the fact that the dilute solution helix–coil transition temperature (denaturation temperature) for collagen molecules from different species increases with increasing imino residue content [11, 12] the triple helix conformation in

* This is not true of native collagen where the reflections of the WAXS pattern actually becomes sharper in water; the structural charge in the reconstituted collagen in water is probably an important reason why it has much lower mechanical strength than native fibres.

regions rich in polar groups is probably not as stable as that in the non-polar regions. These polar regions also have a greater attraction for water molecules.

There is evidence that even in native collagen fibres the molecular conformation along the entire collagen molecule is not uniformly triple helical and that the deformation of the molecule under tensile load does not result from deformation of the triple helical regions [13, 14] but of regions deficient in imino residues [15]. In our wet, reconstituted collagen, these deformable, non-helical regions probably occupy a much larger fraction of the molecules and are more random-coil-like for two reasons. First, the tropocollagen molecules do not have the same lateral bonding to neighbouring molecules as revealed by the absence of the periodic hole arrangement, characteristic of the so-called quarter-stagger structure of native collagen fibrils [16]. It is known that the lateral aggregation of molecules into fibrils is an important stabilizing influence on the triple helix as the denaturation temperature of single tropocollagen molecules is raised some 40° C if these aggregate to form native fibrils. Second, the ultraviolet irradiation cross-linking treatment will also lower the stability of the triple helix by creating chain defects that cannot be incorporated into the triple helix except at a large expense in intramolecular bond energy. We have direct evidence of this effect for tropocollagen molecules in dilute solution where we have monitored the 221 nm circular dichroism absorption peak, which arises due to the helical character of the molecule and which is absent in gelatin. The height of this peak at room temperature decreases, and the helix–coil transition is depressed in temperature and broadened, with increasing ultraviolet irradiation time [17].

Nevertheless, the tropocollagen molecules in the wet hollow fibre cannot be completely random coil. Both the WAXS pattern and the fact that the shrinkage transition, which accompanies the complete unwinding of the triple helix, occurs at ~ 48° C in water indicate that considerable portions of the triple helix are intact. The reconstituted collagen fibril can be pictured as a composite consisting of an arrangement of constrained random-coil regions (elastomeric regions) of the molecule in series with stiff triple-helix regions. The elastic modulus of such a composite will be controlled by the more compliant elastomeric regions. Additions of water can cause a decrease in E for two reasons. In the first place water exerts a destabilizing influence on the triple helix as it lowers the helix–coil transition temperature and will create more constrained random coil regions, a process which is confirmed by the WAXS pattern changes observed [5]. In addition, since water acts as a very effective plasticizer for gelatin [18] it would be expected to lower the glass transition temperature T_g of the constrained random coil regions already created, thereby decreasing E if T_g is not too far below room temperature. The fact that the wet modulus decreases slightly with increasing temperature is consistent with a T_g close to but below room temperature for the constrained random coil regions in water.

The very low modulus of the wet hollow fibre may also have an important bearing on its strength. In particular since the critical stress intensity factor for crack propagation in the membrane can be approximated by*

$$K_{IC} = \sqrt{(G_{IC}E)},$$

where G_{IC} is the critical strain energy release rate (or the total work necessary to advance a crack over a unit area), the factor of ~ 100 decrease in E on wetting will produce a factor of ~ 10 decrease in K_{IC} if the surface work done is constant. This decrease is of the same order of magnitude as the actual decrease in strength observed on wetting. It would appear that attempts to improve the wet mechanical strength of the reconstituted collagen should concentrate on increasing the modulus of the fibrils by increasing the stability of the triple helix and decreasing the fraction of constrained, random coil sections of the tropocollagen molecule in the fibril. The fact that Nature can produce fibrils with these characteristics should give us some hope that significant improvements are possible by altering processing conditions.

Appendix

The deformation of the zig-zag crimp (Fig. 4b) at small strains can be found from the well known formula for the deflection of a cantilever beam. An applied tensile force F can be considered to act at an inflection point of the zig-zag (Fig. 4c) and can be resolved into components parallel to and perpendicular to the fibril. We can now treat

* Strictly speaking, this formulation is quantitatively valid only for linear elastic materials which the wet hollow fibre certainly is not. Nevertheless, for the qualitative purpose intended here it is adequate.

half the fibril as a cantilever beam whose base is fixed at an angle θ_0 to the axis of the fibril. The maximum displacement δ of the fibril at this point due to the perpendicular force $F \sin \theta_0$ is

$$\delta = \frac{F \sin \theta_0 l^3}{24EI} \qquad \text{(A1)}$$

where I, the moment of inertia of a cylindrical fibril, is $\pi D^4/64$. The longitudinal strain along the axis of the zig-zag due to the displacement δ is

$$\epsilon = \frac{2 \sin \theta_0 \delta}{l \cos \theta_0}; \qquad \text{(A2)}$$

but the applied force F equals $\sigma \pi D^2/4$, where σ is the stress based only on the area of fibril and, therefore, by combining Equations A1 and A2 we find

$$\epsilon = \frac{4}{3}\left(\frac{l}{D}\right)^2 \frac{\sin^2 \theta_0}{\cos \theta_0} \frac{\sigma}{E}.$$

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