Water Transport Through Reconstituted Collagen Hollow-Fiber Membranes

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Synopsis

The hydraulic permeability κ of reconstituted collagen hollow-fiber membranes has been determined from measurements of the volumetric flow rate of aqueous solutions through the membrane for various pressure differences across the membrane. Increasing the ultraviolet crosslinking time during manufacture of the membrane markedly decreases κ, whereas decreasing the pH of the aqueous solution increases κ. Applying a tensile strain of only 10% along the fiber axis decreases κ approximately fivefold. These results are in good agreement with those expected from a recent model of the membrane structure in which collagen fibrils, oriented preferentially along the hollow-fiber axis, bend between crosslinks to accept water in a pore structure. If the average fibril volume fraction and diameters are known, estimates can be made of κ. These estimates, based on direct small-angle x-ray scattering measurements of fibril diameters, are in good agreement with the measured κ values.

INTRODUCTION

Reconstituted collagen membranes are promising candidates for artificial kidney dialysis and other biomedical applications.[1] They are attractive not only because similar membranes are already produced at low cost for edible sausage casing, but also because the properties of these membranes can be altered substantially by relatively minor changes in processing conditions. The present investigation is part of a long-term study of the relationships between the structure of the membrane as influenced by processing conditions and membrane properties. Of all these properties perhaps the most sensitive to structural changes is the hydraulic permeability, which is the subject of this paper.

MEMBRANE PROCESSING AND STRUCTURE

The tropocollagen molecule, which is the basic building block of the membrane, is approximately 2800 Å long and consists of three helical polypeptide chains wound together into a triple helix over most of its length.[2] A solution of tropocollagen is prepared in a process developed by the Japan Leather Co. in which steer hide is treated with proctase, a proteolytic enzyme that removes the small nonhelical appendages at the ends of the molecules known as telopeptides.[3] The hollow-fiber membranes, about 400 μm in diameter and 15–20 μm in wall thickness (dry dimensions), are produced by extruding an acid solution (~pH 3) of these molecules through an annular nozzle into a saturated NaCl coagulating bath. The fiber is subsequently crosslinked by ultraviolet irradiation for a given time in the wet state, washed, and neutralized.[4]

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Previous structural and mechanical property studies[5–7] have resulted in the structural model of the membrane shown in Figure 1. In the dry state the membrane consists of large fibrils (~1500 Å diam) of tropocollagen molecules oriented along the axis of the hollow fiber (the extrusion direction). When the membrane is placed in water, the large fibrils split up laterally into many finer fibrils (~30 Å diam at pH 7) with water in pores between fibrils. The water-filled pores effectively crimp the fibrils on a microscale, giving rise to a toe in the tensile stress–strain curve[6] of the hollow fiber, which is accentuated by any environment (e.g., low pH) that results in an increased swelling and increased crimp.[7]

For the purpose of modeling the transport properties of the membrane, the two most useful structural parameters are the average fibril diameter and the volume fraction of fibrils in the swollen membrane. The fibril volume fraction v_f is taken to be the volume fraction of dry collagen in the membrane which is determined from optical microscope measurements of the swelling of the dry membrane when placed in the environment of interest. The assumption that the fibril volume fraction is the volume fraction of dry collagen is controversial since it implies that no water is absorbed in the fibrils. Nevertheless, it is felt to be more appropriate for the small fibrils considered here, which consist of only a few tropocollagen molecules, than the procedure used in reference 5 where a fraction of water is assumed to be bound in the collagen phase. The error induced by ignoring the water bound in the fibril is also partly canceled by the fact that the dry dimensions of the hollow fiber include a certain volume of voids, evidence for which can be obtained from the SAXS pattern of the dry fiber.[5]

The fibril volume fraction under different conditions of pH and crosslinking is tabulated in Table I. Decreasing the pH (positively charging the tropocollagen molecules) greatly increases the water uptake of the membrane and decreases v_f, whereas increasing the crosslink density by increasing the UV irradiation dose greatly increases v_f at neutral pH.

Fibril diameters can be determined from the SAXS pattern from an unoriented

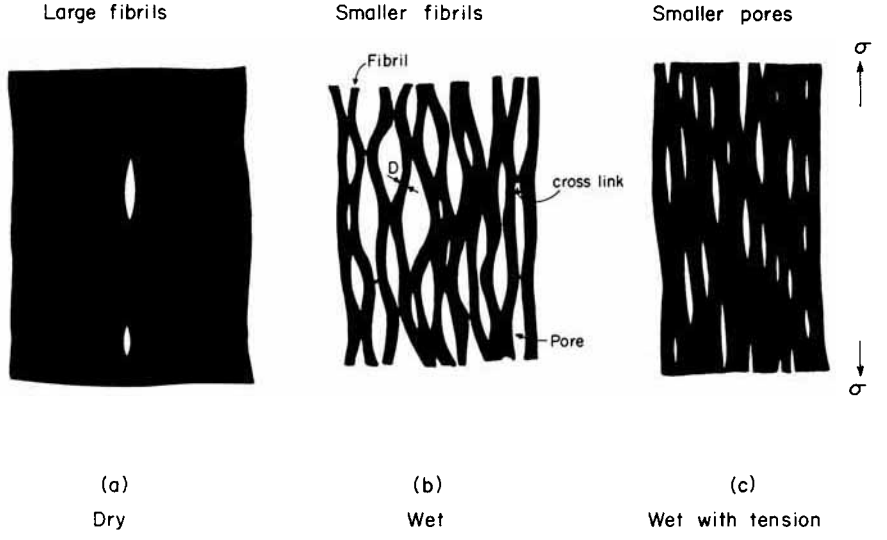

Fig. 1. Schematic model of the fibril structure of the collagen hollow fiber under dry, wet, and wet with tension conditions.

TABLE I
Structural and Transport Parameters of Collagen Membranes

Hollow fiber	UV Irradiation time, min	pH	SAXS D, Å	κ, Å^2	v_f
A	5	7	28	536	0.072
B	10	7	23	226	0.120
C	30	7	31	76	0.270
D	22.5	7	29	104	0.215
D	22.5	3.5		160	0.117
D	22.5	3.05		180	0.101
D	22.5	2.2	20	208	0.0875

array of fibers following the procedure outlined in reference 5. A typical pattern from such an array is presented in Figure 2, which shows intense continuous scattering near the origin. For our two-phase collagen–H_2O system, Porod's law[8,9] holds:

$$\lim_{s \to \infty} I(s)s^4 = \text{constant} \tag{1}$$

where $I(s)$ is the intensity at scattering vector s ($|s| = 2 \sin \theta/\lambda$, where λ is the x-ray wavelength and θ is the scattering angle). Combining this limiting value, which is proportional to the surface area between the two phases, with the invariant $\int_0^\infty I(s)s^2\,ds$, which is proportional to the volume of material irradiated,

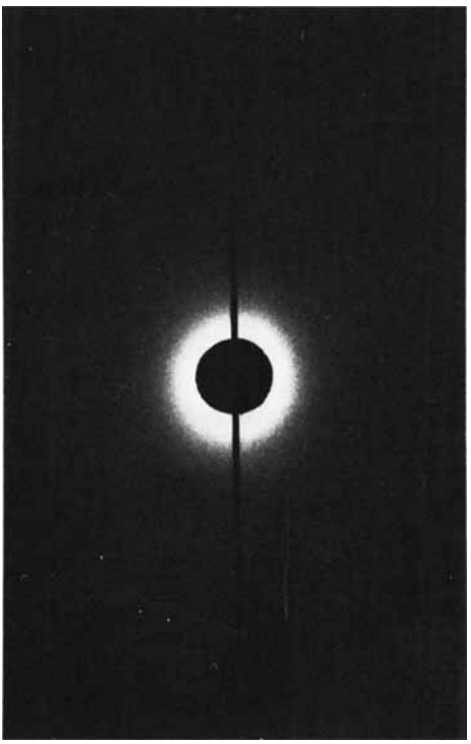

Fig. 2. Small-angle x-ray scattering pattern from an unoriented array of collagen hollow fibers.

one can define the correlation distance l_p as

$$l_p \equiv \frac{2}{\pi^2} \frac{\int_0^\infty I(s)s^2\,ds}{\lim_{s\to\infty} [I(s)s^4]} \tag{2}$$

The correlation distance in turn is related to the range of inhomogeneity in the two phases, l_f and l_w, viz.,

$$l_p = l_f(1 - v_f) = l_w v_f \tag{3}$$

The l_f and l_w are the average lengths of segments of straight lines drawn at random in the fibril and water phases, respectively.[8] Conveniently for an array of long, straight cylindrical fibrils, l_f is exactly the fibril diameter D. Carrying out this analysis for fibers UV-crosslinked for various times results in the D values at pH 7 shown in Table I. As expected, the fibril diameter increases with increasing crosslinking time. Also shown for one crosslinking time is the decrease in fibril diameter caused by decreasing the pH to 2. This decrease can be rationalized based on the repulsion between charged tropocollagen molecules at low pH.

HYDRAULIC PERMEABILITY

The hydraulic permeability κ of a hollow fiber is given by[10]

$$\kappa = \mu Q \ln(r_0/r_i)/2\pi L\ \Delta P \tag{4}$$

where Q is the volume flow rate through, and ΔP is the pressure difference across, the wall of the hollow fiber; r_i, r_0, and L are its inner and outer radius and length, respectively; and μ is the viscosity of water. The volume flow rate Q is determined using the simple apparatus shown in Figure 3, which consists of a 0.1-ml water-filled pipet to one end of which a hypodermic needle is joined with epoxy. The hollow fiber is inserted onto the needle and securely fastened by a wet thread. The other end of the hollow fiber is sealed off in a similar manner. The hollow fiber is totally immersed in a bath of water, and a constant pressure of N_2 is maintained on a column of water in the pipet and monitored by means of a mercury manometer. By measuring the time necessary for the water column to fall a certain distance, Q can be determined.

The method used to seal the hollow fiber is found to be leak free up to a ΔP of 250 mm mercury. The absence of leaks was verified both by measuring Q for hollow-fiber segments of varying lengths and by tracking the motion of an air bubble in the hollow fiber.[11] (The height of the bubble should decay exponentially with time if there are no leaks below the bubble.)

The volume flow rate was found to increase nonlinearly with ΔP as shown in Figure 4. Optical microscopy of the fiber, however, reveals a highly nonlinear thinning of the fiber wall as a function of pressure.[12] If the raw flow rate data are corrected for the change in membrane geometry, i.e., the change in $\ln(r_0/r_i)$, with pressure, the flow rate at constant geometry varies nearly linearly with ΔP as shown by the dashed line in Figure 4. Since the correction at low ΔP is negligible, the κ values were determined from data at ΔP values of 40 mm Hg and below. The κ values for one membrane at several different pH values and for

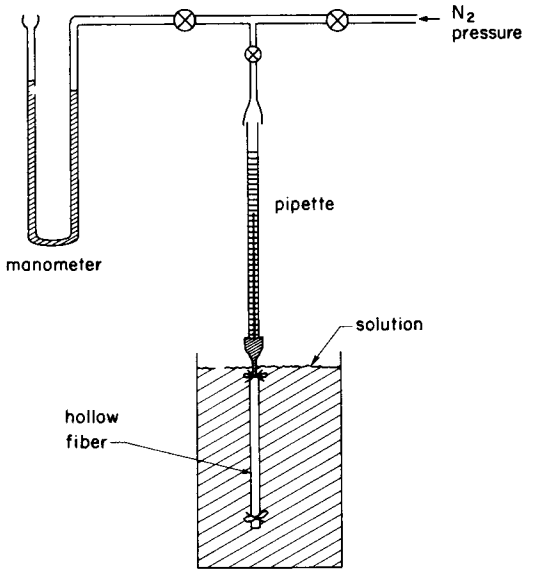

Fig. 3. Schematic of the apparatus used for measuring hydraulic permeability.

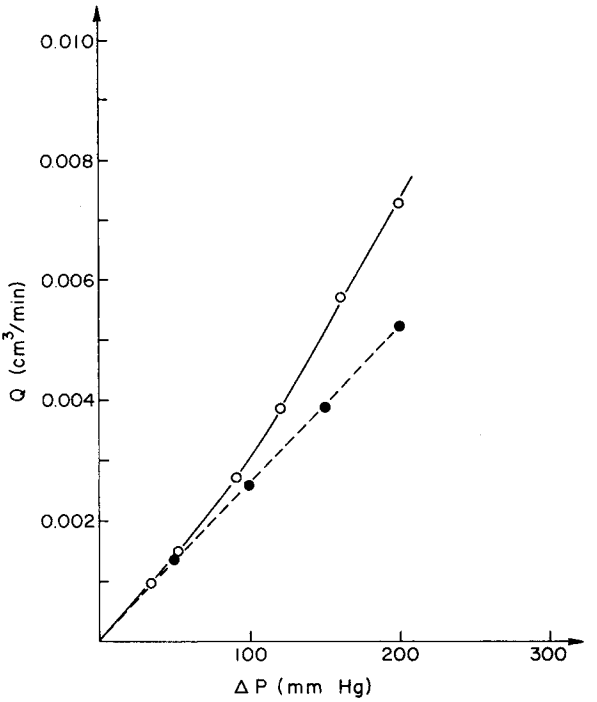

Fig. 4. Volume flow rate of water through membrane vs pressure difference across membrane. Open circles = raw data; filled circles = data corrected for changes in hollow fiber dimensions with ΔP.

several membranes in pH 7 crosslinked for different times are tabulated in Table I. The hydraulic permeability increases with increasing swelling of the membrane, but the extent of the increases is somewhat less than one would intuitively expect for such large increases in swelling. In fact, the increase in κ with pH is

almost entirely due to the increase in $\ln(r_0/r_i)$. The slope of Q versus ΔP is virtually unchanged over this pH regime.

Mechanical testing and SAXS results[5–7] have indicated that tensile strain of the hollow fiber along its axis decreases the size of the water pores between fibrils by straightening these fibrils and removing the microcrimp as indicated in Figure 1(c). If this picture is correct, a tensile strain of the hollow fiber should markedly decrease the hydraulic permeability of the membrane. The apparatus shown in Figure 3 was modified so that measurements could be made while the fibril was subjected to a longitudinal strain. The results shown in Figure 5 provide a dramatic confirmation of microcrimp model of tensile deformation. A tensile strain of only 10% decreases κ to a value only 20% of its original value. The tensile strains used are in the elastic (i.e., recoverable) range of the hollow fiber; and, in fact, if the tensile strain is released, κ recovers to its original value before straining.

DISCUSSION

It is of primary interest here to determine whether the large variation in κ from membrane to membrane can be accounted for by the changes in the structure of the membrane, i.e., changes in v_f and D. A model is thus needed to link the hydraulic permeability to the structure. Given the structure shown in Figure 1 and the rather low fibril volume fraction (the membranes are 80% or more water by volume), it seems reasonable to model the hydrodynamic flow in this case as the flow of water through a forest of straight, long, solid cylinders of diameter D. Unfortunately, analytic solutions to the problem of the flow perpendicular to a random array of oriented cylinders are not available. However, an ap-

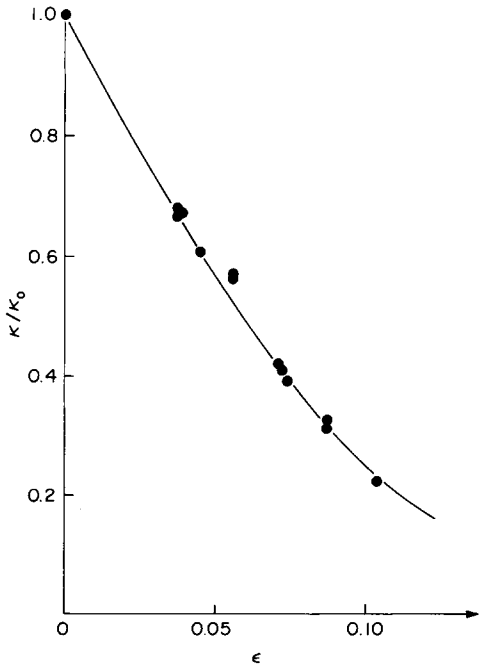

Fig. 5. Hydraulic permeability as a function of tensile strain applied parallel to the hollow-fiber axis.

proximate solution to this problem has been obtained by Happel.[13] He considers the problem of the flow of a cylindrical volume of fluid of diameter $D/\sqrt{v_f}$, for which all shear stresses are constrained to vanish at the outer boundary of the fluid cylinder, past a solid cylinder of diameter D at its center. The resulting expression for κ is

$$\kappa = \frac{D^2}{32v_f}\left\{\ln(1/v_f) - \frac{1 - v_f^2}{1 + v_f^2}\right\} \tag{5}$$

One way to test this model is to use the experimental values for κ and v_f and eq. (5) to estimate fibril diameters that can then be compared with the SAXS results. The D values derived from eq. (5) are plotted as a function of pH and UV irradiation time in Figures 6 and 7, respectively. Plots of κ versus pH and UV irradiation time are also given in these figures for comparison. The fibril diameters directly determined from SAXS are shown as the square data points. The agreement between the directly determined and derived fibril diameters, both in magnitude and functional dependence on pH and irradiation time, is unexpectedly good, especially considering the crude assumptions of the model and some of the uncertainties in the x-ray measurements. From this agreement, as well as the dramatic decrease in κ with tensile strain (Fig. 5), we may conclude that the structural model shown in Figure 1 is consistent with the measured hydraulic permeability data.

The sensitivity of κ to the structure of the membrane is both a nuisance and a potential advantage. On one hand, the sensitivity magnifies quality control problems. In particular, the membrane apparently undergoes additional crosslinking when aged under dry condition, which leads to a decrease in κ with

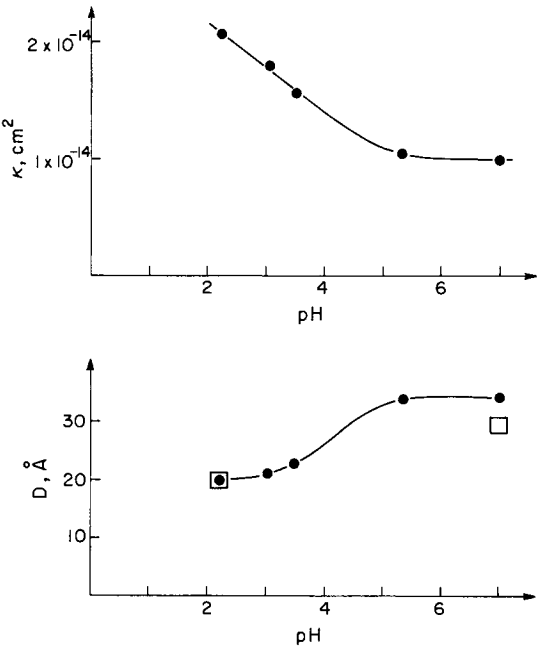

Fig. 6. Hydraulic permeability and fibril diameter as a function of pH for membrane UV-crosslinked for 22.5 min. Open squares represent D from SAXS, while filled circles represent D derived from eq. (5) using measured κ and v_f values.

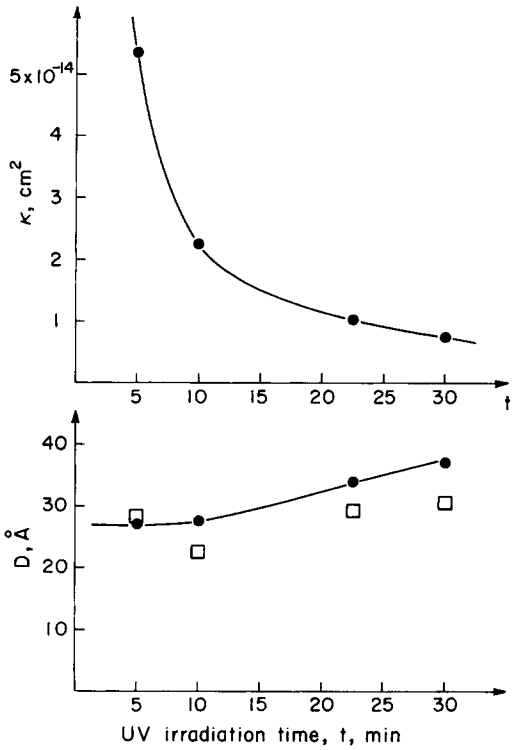

Fig. 7. Hydraulic permeability and fibril diameter as a function of UV irradiation (crosslinking) time (test environment = pH 7). Open squares represent D from SAXS, while filled circles represent D derived from eq. (5) using measured κ and v_f values.

aging. On the other hand, it is often desirable to be able to decrease κ relative to the solute permeability of the membrane,* which is much less sensitive to structure, varying essentially with $1 - v_f$ for small molecules such as urea. Clearly, the sensitivity of κ to structure, and in particular the ability to decrease κ as much as fivefold by applying a small tensile strain parallel to any array of fibers, should prove useful in adjusting κ to an optimum value.

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References

1. A. L. Rubin and K. H. Stenzel, in *Biomaterials*, L. Stark and G. C. Agarwal, Eds., Proceedings of the University of Illinois Workshop, Plenum Press, New York, 1969.

2. G. N. Ramachandran, in *A Treatise on Collagen*, G. N. Ramachandran, Ed., Academic Press, New York, 1967, p. 103.

3. A. L. Rubin, M. P. Drake, P. F. Davidson, D. Pfahl, P. T. Speakman, and F. O. Schmitt, *Biochemistry*, **4**, 181 (1965).

* If κ is too high relative to the solute permeability, patients may become dehydrated during dialysis.

4. K. H. Stenzel, A. L. Rubin, W. Yamayoshi, T. Miyata, T. Suzuki, T. Shode, and M. Nishizawa, *Trans. Am. Soc. Artif. Int. Organs,* **17,** 293 (1971).
5. R. K. Viswanadham and E. J. Kramer, *J. Mater. Sci.,* **10,** 1472 (1975).
6. R. K. Viswanadham and E. J. Kramer, *J. Mater. Sci.,* **11,** 1254 (1976).
7. R. K. Viswanadham, D. C. Agrawal, and E. J. Kramer, *J. Polym. Sci.,* **14,** 2195 (1976).
8. A. Guinier and G. Fournet, *Small-Angle Scattering of X-rays,* Wiley, New York, 1955.
9. L. E. Alexander, *X-Ray Diffraction Methods in Polymer Science,* Wiley, New York, 1969.
10. R. B. Bird, W. E. Stewart, and E. N. Lightfoot, *Transport Phenomena,* Wiley, New York, 1960, p. 151.
11. J. S. Parry, C. A. Steiner, and J. F. Stevenson, unpublished report.
12. C. W. Tyler and J. F. Stevenson, unpublished report.
13. J. Happel, *A.I.Ch.E.J.,* **5,** 174 (1959).

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