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Macromolecular diffusion through collagen membranes

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Summary

Collagen is a major constituent of connective tissue and is unique in possessing different levels of structural order: primary, secondary, tertiary, and quaternary. The membrane fabrication method altered the collagen structure and porosity. The effect of collagen structure, crosslinking density, hydrophilicity of crosslinker, and membrane porosity on macromolecular diffusion were studied. Permeability, partition, and diffusion coefficients were determined for proteins varying in molecular weight (14,000–66,000). 'Pore' type permeation was the predominant mechanism. The quaternary structure, crosslinking density, and membrane porosity of the membranes were found to regulate the diffusion of macromolecules. The highest diffusion coefficients were found for the least crosslinked membranes, random collagen fibril structure, and membranes fabricated with the hydrophilic crosslinking agent.

Introduction

With the advances made in molecular genetics, peptides and proteins are rapidly emerging as a major class of possible therapeutic agents. Due to the instabilities of macromolecules via an oral route they may be more suited for controlled delivery systems than the more stable low molecular weight drugs. Previous work by Langer et al. (1980), Langer (1982), Korsmeyer and Peppas (1981), and Reinhart and Peppas (1984) using a variety of polymers including polyvinylalcohol and ethylene-vinyl acetate have been shown to deliver macromolecules. Ethylene-vinyl acetate studied extensively by Langer and co-workers is not biodegradable, thus requiring surgical removal.

Sato and Kim (1984) investigated macromolecular diffusion through cellulose, hydrogels, and biodegradable polymers. The mechanisms of diffusion was determined using solutes varying in molecular size. Depending on the polymer fabrication method, macromolecules were found to diffuse via bulk water channels and through the polymer matrix.

Collagen is a potentially useful biomaterial since it is a major constituent of connective tissue. Reconstituted collagen has been investigated for different applications including dialysis membranes, wound dressings and vitreous implants. The use of collagen as a biomaterial has the advantage that the biological characteristics of the molecule including its structure and modes of polymerization have been extensively studied (Stenzel, 1969).

Collagen is a major structural component of many tissues such as skin, bone, cartilage, tendons, and basement membrane. The basic collagen molecule (tropocollagen) has 3 polypeptide chains intertwined to form a triple helix. Each of the 3

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chains are designated as alpha (α) chains and consist of 1000 amino acid residues with an approximate molecular weight of 100,000. Each individual chain forms a left-handed helix (secondary structure). The 3 individual chains then assume the right-handed 'superhelical' structure (tertiary structure) having an average molecular weight of 300,000, a length of 300 nm and a diameter of 1.5 nm (Stryer, 1981). The orderly arrangement of the triple helices or tropocollagen molecules results in the formation of fibrils which are formed with a distinct periodicity. Tropocollagen molecules forming the fibrils are staggered by 67 nm between adjacent rows. In addition, a gap of 40 nm exists between succeeding molecules and this staggered arrangement can be denoted as the quaternary structure, as noted in electron microscopy (Bovey, 1979). Previous work by Wang et al. (1978) showed that the collagen quaternary structure is essential for collagen-induced platelet aggregation. Therefore, it was of interest to examine the effect of the quaternary structure on diffusion of macromolecules through collagen membranes.

The objective of this work is to examine the effect of a collagen matrix on the diffusional properties and biodegradation rate so that a macromolecular drug delivery system can be designed. This paper will discuss the effects of fabrication methods, crosslinking density, nature of crosslinker, and quaternary structure on the transport of macromolecules in diffusion experiments. The techniques to fabricate collagen membranes with native vs non-native quaternary structure and porous fibril vs dense aggregate collagen membranes were developed. Crosslinking density was varied by either the amount of crosslinker added or length of time the membranes remain in the crosslinking solution. Solutes varying in molecular size (lysozyme, carbonic anhydrase, ovalbumin, and bovine serum albumin) were used to examine the diffusion process. The mechanism of diffusion was determined according to the dependence of diffusivity on solute size and membrane hydration.

Materials and Methods

Preparation of collagen membranes

Enzyme-solubilized collagen (Koken Co., Tokyo, Japan) was used in preparation of dense and porous fibril collagen membranes. Dense collagen membranes of random aggregates were fabricated by air-drying at room temperature. Lyophilized collagen was dissolved in 0.01 M acetic acid to make a 0.5% solution by weight. Glutaraldehyde (25% aqueous solution, Fisher Scientific) diluted to a 1% solution in water, was mixed with 0.5% collagen solution for 24 h at 0 °C. For every mole of ϵ-amino groups on collagen 0.05, 0.10, 0.25 and 0.60 mol of glutaraldehyde was added. The collagen–glutaraldehyde solution was poured into polystyrene petri dishes and evaporated at room temperature. Porous fibril collagen membranes were fabricated by coagulation in sodium chloride or phosphate-buffered saline (PBS, pH 7.4). Lyophilized collagen was dissolved in 0.01 M acetic acid to make a 5% gel by weight. Collagen gel was cast onto a glass surface using a Gardner casting knife (Gardner Instruments, MD) and immediately coagulated in 5% sodium chloride or PBS (pH 7.4). Membranes were crosslinked in 50% MeOH (pH 12) containing either 0.01% glutaraldehyde or 5% polyglycerol polyglycidyl ether (PPE) (Denacol EX-512, mol. wt. 539 g/mol, Nagase America Corp., New York). Prior to use, membranes were soaked in PBS to remove unreacted crosslinker. The soaking solutions were monitored by UV until no residual crosslinker was detected. A schematic representation of the collagen membranes which should be obtained from the different fabrication methods are illustrated in Fig. 1. Confirmation of the different structures by scanning electron microscopy and transmission electron microscopy is described in the Results and Discussion.

Characterization

Infrared spectroscopy. Collagen purity was confirmed from an infrared spectrum using a Digilab FTS 20/80 Fourier Transform Infrared Spectrophotometer over the range of 4000–600 cm^{-1}. The Amide III band at 1235 cm^{-1} is sensitive to the tertiary structure of native collagen, whereas the absorbance at 1450 cm^{-1} is insensitive. The ratio of absorbances of these two bands is a measure of the tertiary structure (Gordon et al., 1974). The film was obtained by casting a 1% collagen solu-

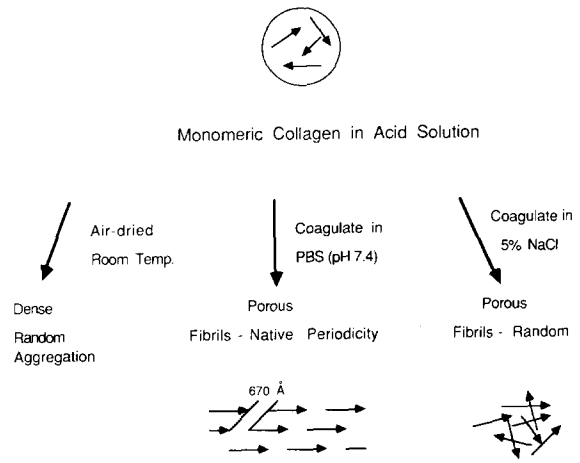


Fig. 1. Schematic representation of fabrication techniques used to fabricate collagen membranes with varying porosity and microstructure.

tion of 0.05 M acetic acid onto a 25 × 2 mm ZnSe crystal and drying overnight at room temperature.

Scanning electron microscopy (SEM). Dense collagen samples were vacuum-dried (30 μm Hg) overnight at room temperature. Fibril collagen samples were critically point-dried (Bomar Critical Point Dryer SPC-1500), mounted on stubs, and sputter coated with Au/Pd using Technics Sputter Coater (Hammer 3). The upper surface, lower surface and cross-section were examined with a scanning electron microscope (JEOL JSM-35).

Transmission electron microscopy (TEM). Collagen was dissolved in 0.01 M acetic acid so that the final concentration was 1 mg/ml. The collagen solution was added to 5% NaCl or PBS to precipitate random and native-type fibrils, respectively. The suspension was dropped on carbon coated grids and air-dried. The grids were stained with 0.4% phosphotungstic acid (pH 3.5) for 15 min and 1% uranyl acetate for 10 min (Ruggiero et al., 1985). Grids were rinsed with distilled water, air-dried and examined in a transmission electron microscope (JEOL JEM-100CXII).

Tensile test. Tensile tests were performed in the wet state as per the procedure outlined in ASTM D 1708-79 (1979) using an Instron (Universal Testing Machine Model 1130) at a crosshead speed of 2 inch/min. Dogbone samples 0.720 × 0.176 inch were used.

Percentage of crosslinking. A procedure described by Wang et al. (1978) was used to determine the number of ϵ-amino groups blocked due to crosslinking. 1 ml of 4% $NaHCO_3$ was added to 5–10 mg of collagen sample. 1 ml of freshly prepared 0.5% 2,4,6-trinitrobenzenesulfonic acid (TNBS, Sigma Chemical Co.) then was added. The mixture was shaken at 40 °C for 2 h, followed by the addition of 3 ml of 6 N HCl and finally heated at 60 °C for 1 h. The solution was diluted with 5 ml of distilled water and washed twice with 10 ml of ethyl ether to remove excess TNBS and trinitrophenyl *N*-terminal amino acid. The aqueous layer was heated in hot water for 10 min to remove ether and diluted as necessary. The absorbance was measured at 345 nm. A blank was run according to the same procedure except HCl is added before the addition of TNBS.

Determination of partition coefficient and hydration

Partition coefficients (K_d) are defined as the ratio of the solute concentrations in the membrane to those in the bulk aqueous phase. A solution depletion method was used to determine partition coefficients. Protein solution (1 mg/ml) was equilibrated in a scintillation vial with a known volume of collagen membrane. Partition coefficients were calculated using the following equation:

$$K_d = \frac{V_s(C_0 - C_s)}{V_m C_s} \quad (1)$$

where V_s is volume of protein solution, V_m is volume of collagen membrane, C_0 is the initial protein concentration in the solution and C_s is the protein concentration in the solution at equilibrium. The average of 5 measurements were used in the determination of K_d. The protein concentrations in the solution were measured until a constant value was obtained by UV spectroscopy. The volume of collagen membrane (V_m) was determined according to its buoyant force and determined using the following equation:

$$V_m = \frac{W_a - W_s}{d_s} \quad (2)$$

where W_a and W_s are the weights of collagen in air and PBS respectively, and d_s is the density of

PBS. W_s was measured by supporting the membrane in PBS with a thin wire which was directly attached to a balance.

Hydration of collagen membranes (H) was calculated using the following equation:

$$H = \left(\frac{d_m}{d_w}\right) W_f \tag{3}$$

where d_m is the density of the wet collagen membrane, d_w is the density of water, and W_f is the water content of the collagen membrane. W_f is determined from the dry and wet weight of collagen membranes according to the following equation:

$$W_f = \frac{\text{wet weight} - \text{dry weight}}{\text{wet weight}} \tag{4}$$

Diffusion experiment

Diffusion studies were carried out at room temperature (23° C) in a two-compartment glass diffusion cell, each compartment having a volume of 170 ml. The collagen membrane was clamped between the two compartments. Initially, one compartment (receiver) was filled with phosphate-buffered saline (pH 7.4) containing 0.1% sodium azide and the other compartment (donor) was filled with phosphate-buffered saline (pH 7.4), 0.1% sodium azide, and the protein being examined. The initial protein concentration was 1 mg/ml for lysozyme, ovalbumin, and bovine serum albumin and 0.1 mg/ml for carbonic anhydrase. The diffusion of the protein through the membrane was studied by determining its concentration in the receiver compartment as a function of time by a standard protein assay (Bio-Rad) for carbonic anhydrase and UV spectroscopy (Perkin-Elmer Lamda 7 UV/VIS spectrophotometer, Perkin-Elmer) for the remaining proteins.

Permeation coefficients were calculated from the following equation (Zentner et al., 1979) which was derived from Fick's law:

$$\ln\left(1 - \frac{2C_t}{C_0}\right) = \frac{-2AU}{VL} t \tag{5}$$

where C_t is the concentration in the receiver compartment at time t, C_0 is the initial concentration in the donor compartment, A is the effective membrane area, U is the permeability coefficient, V is the compartment volume, L is the membrane thickness. A plot of:

$$\frac{-VL}{2A} \ln\left(1 - \frac{2C_t}{C_0}\right) \text{ versus } t \tag{6}$$

is a straight line with a slope equal to U. The average of 3 experiments were used in the determination of the permeability coefficients. The apparent diffusion coefficient (D) was obtained from the following equation:

$$D = \frac{U}{K_d} \tag{7}$$

Calculation of solute molecular radius and self diffusion coefficient

The solute molecular radii were calculated from the following equation assuming that the solutes were spherical.

$$r^2 = \left(\frac{3V}{4\pi N_0}\right)^{2/3} \tag{8}$$

where r is the radius of the solute, V is the molal volume of solute and N_0 is Avogadro's number. The molecular volume was derived from the partial specific volume. Protein self diffusion coefficients (D_0) and partial specific volumes were obtained from the Handbook of Biochemistry (Sober, 1968). The calculated radii of the proteins are listed in Table 1.

TABLE 1

Molecular weight and radius of proteins

Protein	Molecular weight (Da)	Radius (nm)
lysozyme	14500	1.58
carbonic anhydrase	29000	2.04
ovalbumin	45000	2.38
bovine serum albumin	66000	2.69

Results and Discussion

Characterization of collagen and collagen membranes

Infrared spectroscopy was used to characterize the structure of collagen obtained from Koken Co. The characteristic bands in the collagen spectrum sensitive to secondary and tertiary structural changes include the N–H stretching (3330 cm^{-1}), Amide I: C=O stretching (1650 cm^{-1}), Amide II: N–H bending and C–N stretching (1550 cm^{-1}), and Amide III: C–N stretch, N–H bending vibrations (1250–1150 cm^{-1}). The 3330 cm^{-1} N–H stretching band shifts to 3300 cm^{-1} in gelatin. The presence of these bands in the spectrum illustrated in Fig. 2 indicates our material is collagen. The absorbance ratio of the 1450 cm^{-1} band (C–H deformations) to the 1235 cm^{-1} band (Amide III) has been used by other investigators to qualitatively determine the tertiary structure. The exact ratio associated with the tertiary structure of collagen is not known. However, the 1235 cm^{-1} band in the Amide III region is not present in the spectrum of denatured collagen following a 60 °C heat treatment. Yannas et al. (1980) has reported absorbance ratios ranging from 1.43 to 1.25, depending on the source of collagen. The absorbance ratio for the collagen used in this study was 1.1, indicating that the collagen was pure.

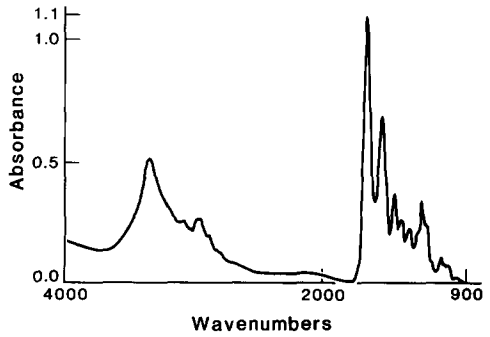
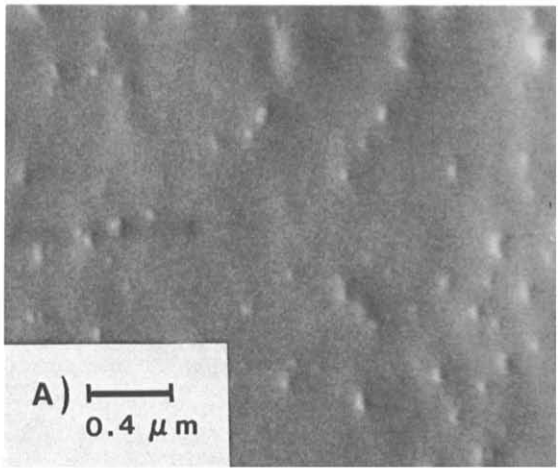
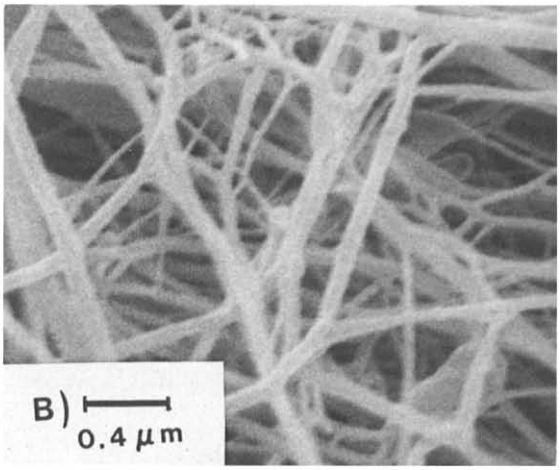


Fig. 2. Infrared spectrum of collagen. Collagen films cast at 23 °C from 0.05 M acetic acid onto a 25×2 mm ZnSe crystal.





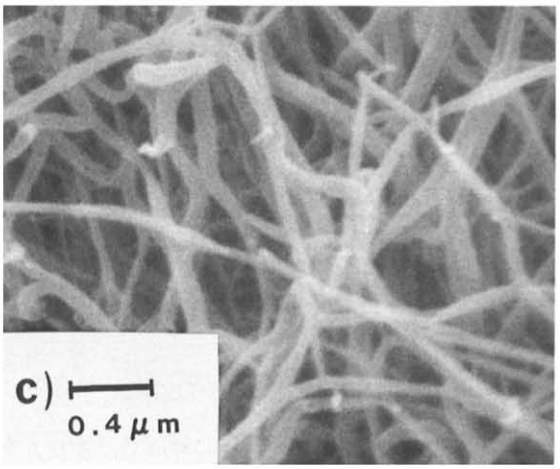
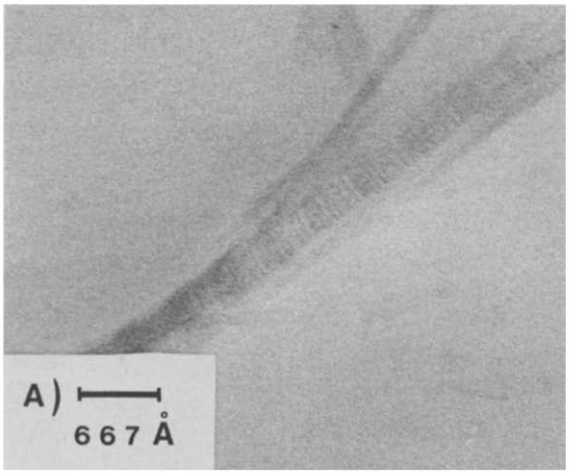


Fig. 3. SEM micrographs of collagen membranes (25,000×) A: air-dried dense membrane. B: porous fibril membrane-native periodicity. C: porous fibril membrane-random structure.

A schematic representation of the collagen membrane obtained from the different fabrication techniques are illustrated in Fig. 1. Fig. 3 includes SEM photographs of the dense aggregate and porous fibril collagen membranes. Only the upper surface pictures are shown in Fig. 3, since no differences were detectable between the upper surface, lower surface, and cross-section of the membranes. The surface of the air-dried dense collagen membrane was smooth with no visible pores. On the other hand, the porous fibril collagen membranes contains pores on the surface



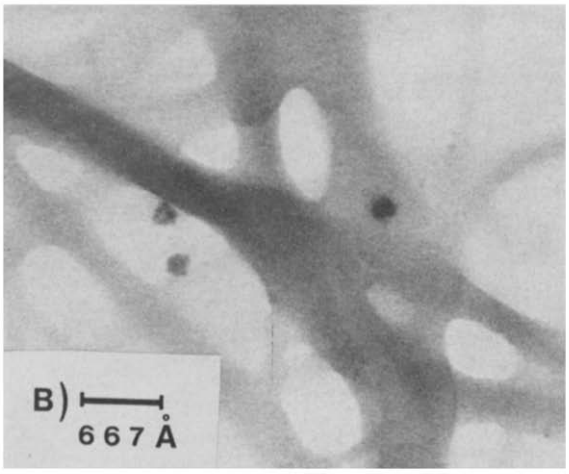


Fig. 4. TEM micrographs of collagen fibrils (150,000×). A: native periodicity. B: no periodicity.

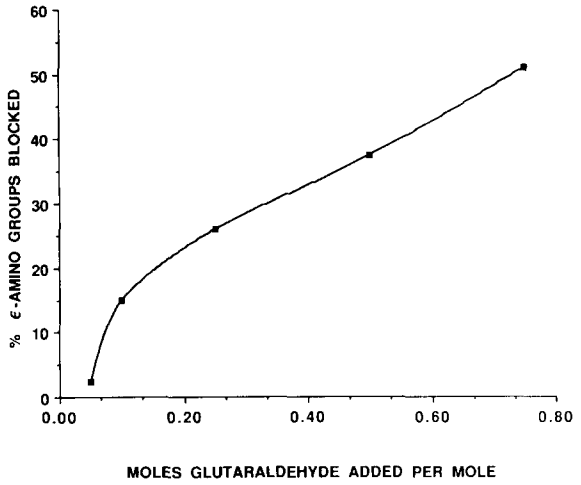


Fig. 5. The % ε-amino groups blocked as a function of moles of glutaraldehyde added for air-dried dense collagen membrane.

and throughout the membrane. TEM photographs of the fibrils are shown in Fig. 4. Coagulation of the collagen gel in PBS precipitates fibrils with native periodicity (64 nm) while 5% NaCl precipitates fibrils with no periodicity. The resulting periodicity or banding pattern of collagen fibers is due to the staggered arrangement of the tropocollagen molecules, which form the fibrils. The native periodicity is a result of the tropocollagen molecules being staggered by 64 nm between adjacent rows. The fibrils lacking periodicity is due to random alignment of the tropocollagen molecules when forming fibrils.

Crosslinking of the collagen membranes was necessary to prevent dissolution. The crosslinkers, glutaraldehyde and PPE, react with ε-amino groups on collagen. The crosslinking density was controlled by the amount of crosslinker added to the solution for the dense collagen membranes, or length of time the collagen membranes remain in the crosslinking solution for the porous fibril collagen membranes. Figs. 5 and 6 illustrate the percent of ε-amino groups on collagen which were blocked as a function of time or amount of crosslinker for the different membranes and cross-linkers.

Tensile tests, of the random fibril membrane in the wet state, crosslinked with the hydrophobic

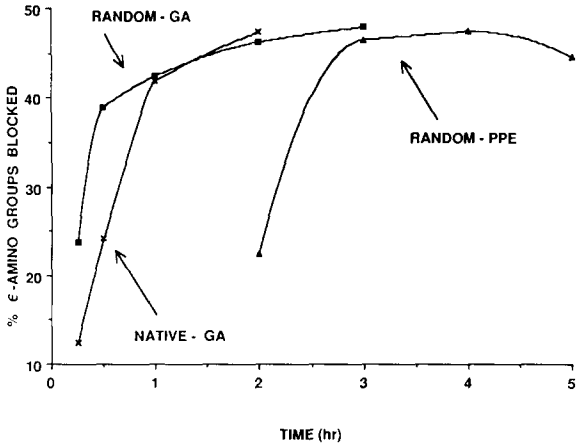


Fig. 6. The % ϵ-amino groups blocked as a function of time. The results are shown for porous random fibril collagen membrane crosslinked with glutaraldehyde, GA (■), random fibril collagen membrane crosslinked with PPE (△), and native fibril collagen membrane crosslinked with GA (×).

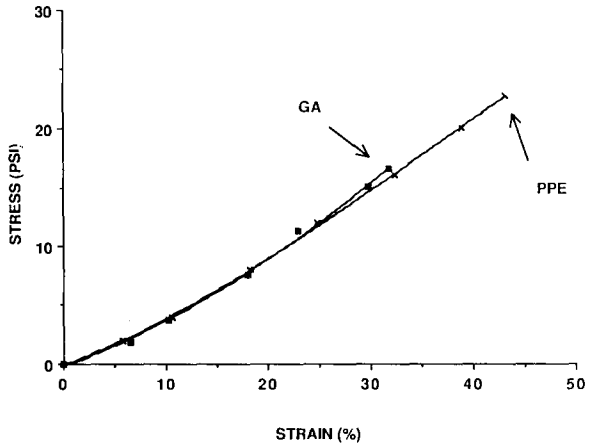


Fig. 7. Stress–strain curve for porous random collagen fibril membrane crosslinked with glutaraldehyde, GA (■) and polyglycerol polyglycidyl ether, PPE (×). Both membranes had 45% of ϵ-amino groups blocked. Tensile tests in the wet state were performed according to the procedure outlined in ASTM Standards (1979).

crosslinker, glutaraldehyde, and the hydrophilic crosslinker, PPE, with 45% of ϵ-amino groups blocked, are shown in Fig. 7. Increasing the crosslinking density prevented dissolution, but the membranes became more brittle. The resulting brittleness was more pronounced for the hydrophobic crosslinker, glutaraldehyde, as opposed to the hydrophilic crosslinker, PPE. Tensile tests of the random fibril membrane confirm this observation. The strain at fracture for the fibril membrane crosslinked with PPE was approximately 40% higher as compared to the fibril membrane crosslinked with glutaraldehyde. The stress for both membranes was identical, as illustrated in Fig. 7. The differences seen in strain could be important in applications where a more elastomeric membrane is desirable.

Dependence of diffusion coefficient on size and hydration

The membrane diffusion coefficients of several globular proteins (lysozyme, carbonic anhydrase, ovalbumin, bovine serum albumin) were determined for dense and porous fibril collagen membranes and different crosslinkers. Tables 2–5 summarize the results with the values of permeability coefficients (U), partition coefficients (K_d), and diffusion coefficients (D). The stirring in the two-compartment diffusion cell did not induce protein denaturation as confirmed by circular dichroism. The diffusion experiments revealed that the permeability coefficients were dependent on the size of the diffusing species and hydration of collagen membranes. Fig. 8 demonstrates the size

TABLE 2

Water content (W_f), % ϵ-amino groups blocked, permeability (U), partition (K_d), and diffusion coefficients (D) for lysozyme across dense collagen membranes crosslinked with glutaraldehyde

W_f	% ϵ-amino groups blocked	U (cm^2/s) [a]	K_d [b]	D (cm^2/s)
85%	2%	$1.40\ (\pm 0.45) \times 10^{-7}$	1.0	1.40×10^{-7}
78%	15%	$3.02\ (\pm 0.52) \times 10^{-8}$	1.9	1.59×10^{-8}
73%	26%	$5.72\ (\pm 1.05) \times 10^{-9}$	2.5	2.29×10^{-9}
71%	45%	$2.78\ (\pm 0.28) \times 10^{-9}$	5.0	5.56×10^{-10}

[a] $n = 3$,
[b] $n = 5$.

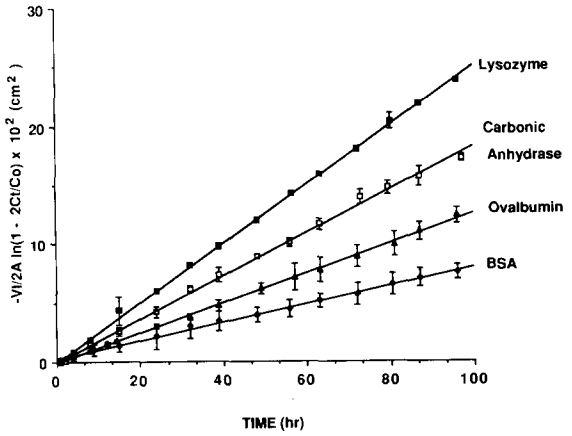


Fig. 8. Variability of permeability coefficients with protein size for native fibril collagen membrane crosslinked with glutaraldehyde. The slopes are the permeability coefficients for lysozyme (■), carbonic anhydrase (□), ovalbumin (▲) and bovine serum albumin (◇) through collagen membranes.

dependence of solute flux for native fibril collagen membrane. The permeability coefficients were obtained from the slope of the plot in Fig. 8. The permeability coefficients decrease with increasing protein size. The results were analyzed using the free volume relationship described by Yasuda et al. (1968, 1969) and Yasuda and Lamaze (1971) for solute diffusion in swollen polymeric networks:

$$\ln\left(\frac{D}{D_0}\right) \propto \frac{-Br^2}{V_{fw}}\left(\frac{1-H}{H}\right) \tag{9}$$

where D is the diffusion coefficient of the solutes in the membrane, D_0 is the solute self-diffusion coefficient, Br^2 is proportional to the solute cross-sectional area (πr^2), V_{fw} is the free volume of water in the collagen membrane, and H is the degree of hydration. This equation describes the relationship between the solute diffusion coefficient and size of solute and degree of hydration. Free volume theory was verified by first, varying the solute size diffusing across a membrane of constant hydration and second, studying the diffusion of one protein for membranes varying in hydration.

Tables 3–5 summarize the permeation results for various solute sizes while the hydration of the membrane (95%) and % ϵ-amino groups blocked (45%) were kept constant. The results for native fibril membrane crosslinked with glutaraldehyde, random fibril membrane crosslinked with glutaraldehyde and PPE are shown. Comparison of partition coefficients for random fibril collagen membrane crosslinked with glutaraldehyde (Table 3) to those crosslinked with PPE (Table 4) reveals differences. The partition coefficients for collagen membrane crosslinked with the hydrophilic crosslinker, PPE, was approximately 1.0 for all proteins whereas when glutaraldehyde was the crosslinker the partition coefficient increased with increasing protein size. The collagen chains, secondary or tertiary structure, in the collagen membrane cross-

TABLE 3

Permeability (U), partition (K_d) and diffusion coefficients (D) for various proteins across porous random fibril collagen membrane crosslinked with glutaraldehyde

Protein	U [a] (cm^2/s) $\times 10^7$	K_d [b]	D (cm^2/s) $\times 10^7$
lysozyme	8.68 ± 0.64	1.06 ± 0.12	8.19
carbonic anhydrase	5.57 ± 0.36	1.32 ± 0.19	4.22
ovalbumin	4.37 ± 0.23	1.64 ± 0.24	2.67
bovine serum albumin	3.16 ± 0.24	2.13 ± 0.15	1.48

[a] $n = 3$.
[b] $n = 5$.

TABLE 4

Permeability (U), partition (K_d) and diffusion coefficients (D) for various proteins across porous random fibril collagen membrane crosslinked with PPE

Protein	U [a] (cm^2/s) $\times 10^7$	K_d [b]	D (cm^2/s) $\times 10^7$
lysozyme	8.80 ± 0.51	1.01 ± 0.11	8.71
carbonic anhydrase	6.68 ± 0.31	1.03 ± 0.07	6.49
ovalbumin	5.22 ± 0.24	1.06 ± 0.04	4.92
bovine serum albumin	3.85 ± 0.23	1.15 ± 0.07	3.35

[a] $n = 3$.
[b] $n = 5$.

TABLE 5

Permeability (U), partition (K_d) and diffusion coefficients (D) for various proteins across porous native fibril collagen membrane crosslinked with glutaraldehyde

Protein	U [a] (cm^2/s) $\times 10^7$	K_d [b]	D (cm^2/s) $\times 10^7$
lysozyme	7.01 ± 0.12	1.33 ± 0.14	5.27
carbonic anhydrase	5.13 ± 0.04	1.52 ± 0.17	3.38
ovalbumin	3.51 ± 0.32	1.90 ± 0.11	1.84
bovine serum albumin	2.16 ± 0.17	2.45 ± 0.24	0.88

[a] $n = 3$,
[b] $n = 5$.

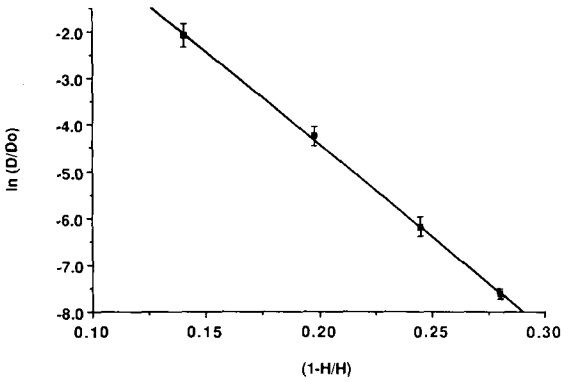


Fig. 10. Linear relationship between diffusion coefficient and degree of hydration. Results are shown for diffusion of lysozyme across air-dried dense collagen membrane crosslinked with varying amounts of glutaraldehyde.

linked with hydrophilic PPE could have greater flexibility and therefore reduce the interaction of the protein with collagen chains in contrast to a membrane fabricated with the hydrophobic crosslinker glutaraldehyde. Permeability coefficients were lower for random fibril collagen membranes crosslinked with glutaraldehyde compared to PPE. This can also be attributed to greater flexibility of chains when the hydrophilic crosslinker, PPE, is used. The differences in permeability coefficients were more pronounced for the larger proteins. Differences were detected in permeability coefficients for random fibril collagen membrane (Table 3) versus native fibril collagen membrane (Table 5). Permeability coefficients were lower for the native collagen membrane. This could be attributed to the packing of the tropocollagen molecules in the formation of fibrils. The random fibrils are less ordered and possibly have larger pores, less tortuosity, when compared to the packed native fibrils. A plot of $\ln(D/D_0)$ versus r^2 is shown in Fig. 9. The linearity supports the hypothesis that a correlation exists between diffusivity and solute size as described by Wisniewski and Kim (1980) and Zentner et al. (1979). Based on the linear dependence from the plot of $\ln(D/D_0)$ versus r^2, it was concluded that permeation occurs through the 'bulk-like' water regions or by a 'pore' type mechanism. Therefore, the macromolecules examined in this work permeate via a pore type mechanism due to the high water content of the fibril membranes.

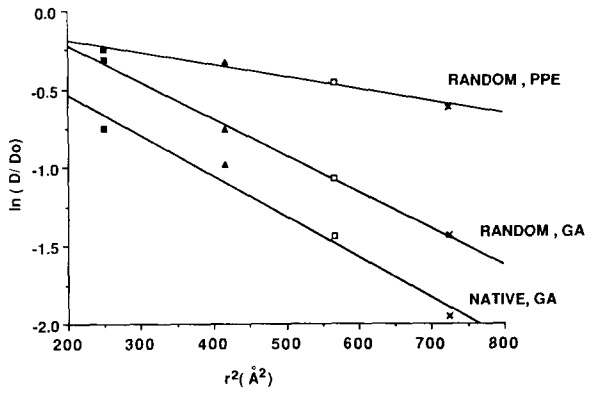


Fig. 9. Linear relationship between diffusion coefficient and radius of protein. Results are shown for diffusion of lysozyme (■), carbonic anhydrase (▲), ovalbumin (□), and bovine serum albumin (×) through collagen membranes with varying structures as noted.

The results for the dense collagen membrane varying in hydration are in Table 2. The diffusion of lysozyme across the dense membrane crosslinked with glutaraldehyde was determined. Carbonic anhydrase and larger proteins did not diffuse across the dense membrane with 45% ϵ-amino groups blocked. As the crosslinking density increased, the hydration decreased and the permeability and diffusion coefficients decreased by 2 and 3 orders of magnitude, respectively. The partition coefficient was found to increase slightly as the hydration decreased. The increase in cross-lin-

king density would increase the entanglements of the collagen chains, possibly the tertiary structure, thereby increasing the tortuosity and decreasing the effective areas for diffusion. The increase in partition coefficients with decreasing hydration can be attributed to a reduction of 'bulk-like' water regions and therefore a greater interaction of lysozyme with collagen. A linear relationship was found to exist between $\ln(D/D_0)$ and $(1 - H)/H$ as shown in Fig. 10. According to free volume theory this relationship is indicative of a 'pore' type permeation mechanism.

It would be of interest to compare the permeability coefficients obtained from this work with membranes examined by other authors for transport of proteins. This is difficult to do since a majority of the research, i.e. Langer et al. (1980, 1982) examines the release from matrix-loaded devices, as compared to permeation experiments through the membranes as is described in this work. Permeability coefficients are similar to values reported by Sato and Kim (1984) and Reinhart and Peppas (1984). Therefore the use of collagen for a biodegradable macromolecular drug delivery system is feasible.

Conclusion

Macromolecular diffusion is possible with collagen membranes. Techniques used to fabricate collagen membranes with quaternary vs random structure were developed. The diffusion of macromolecules can be regulated by fabrication technique, crosslinking density, nature of crosslinker, and quaternary structure. 'Pore' type permeation was the predominant mechanism of diffusion. The diffusion coefficients for macromolecules through collagen membranes were higher for membranes with porous fibril structure, low crosslinking densities, membranes crosslinked with hydrophilic crosslinker, PPE, and non-native quaternary structure. Collagen is a viable material in designing a macromolecular drug delivery system.

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