Mass Transfer Characteristics of Glutaraldehyde-crosslinked and Epoxy-crosslinked Collagen Films

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Abstract

Collagen is a broadly used biomaterial and its mechanical strength can be enhanced by many crosslinking agents. Collagen matrices for drug delivery or tissue regeneration, however, are expected to have desired mass transfer characteristics. In this study, collagen films were crosslinked by two commonly used crossliking agents, namely glutaraldehyde (GTA) and epoxy. The mass transfer characteristics of the crosslinked collagen films were determined using theophylline (a hydrophilic drug) and benzocaine (a hydrophobic drug). The apparent diffusivities (D_{app}) of theophylline and benzocaine in untreated collagen films were about 1×10^{-6} cm^2/s and 3×10^{-7} cm^2/s, respectively. The crosslinking treatments caused a decline in D_{app} for both drugs. The D_{app} values of theophylline and benzocaine in GTA-crosslinked and epoxy-crosslinked films were about one third of the D_{app} values in the untreated films. In addition, swelling ratio of the GTA-crosslinked or epoxy-crosslinked films decreased by 50% as compared with untreated films. This study suggests that the decrease in the swelling ratio is due to the cross-linkages between collagen molecules, thereby increasing the mass transfer resistance and decreasing the D_{app} values. Further, as compared to the hydrophilic drugs such as theophylline, the permeation of hydrophobic drugs such benzocaine in collagen films is relatively poor.

***Keywords*:** Collagen films, Crosslinking, Drug permeation, Mass transfer

Introduction

In recent years, biomaterials have been applied to many aspects of medical technology. Among many kinds of biomaterials currently in use, collagen is a widely used one. Collagen is a major component of extracellular matrix [1]. The collagen molecules assemble spontaneously to form various fibers, which possess good mechanical strength [2-3]. Therefore, collagen can be manufactured as different forms such as: films, sponges, gels, etc. Good biocompability and low antigencity [4] are two major reasons that make collagen matrix a superior biomaterial [5]. Collagen matrices are widely used for wound dressing materials, artificial skin [6], artificial blood vessel [7-8], drug delivering apparatus [9], and guided tissue regeneration [10]. Recently, collagen matrices have received more attention due to its excellent application potential in tissue engineering [11].

Collagen matrices often need to be modified to fulfill the requirements for clinical applications. The modification could change the mechanical properties and mass transfer characteristics of collagen matrices. For example, collagen films can be crosslinked to gain sufficient mechanical strength. Additionally, drug releasing rate for a collagen-based biomaterial can be controlled by the extent of crosslinking. Therefore, the crosslinking of collagen films is a widely utilized technique.

In this study, collagen films were prepared using a vacuum drying method. Afterwards, the films were crosslinked by either glutaraldehyde (GTA) or epoxy. The effect of cross-linking on the mass transfer characteristics of collagen films were investigated using theophylline [12-14] and benzocaine. There two drugs having different properties were chosen because the former is hydrophilic and the latter is hydrophobic. Mass transfer characteristics of collagen films were determined in the side-by-side cells with the selected drug initially only present in the donor cell [15]. The concentrations of drug in the donor and receptor cells at pre-scheduled time points were determined spectrophotometrically. Our results indicated that the cross-linkages between collagen molecules increased the mass transfer resistance and decreased the diffusivities of theophylline and benzocaine in collagen films. Further, the hydrophilic theophylline had a higher permeation rate in collagen films than the hydrophobic benzocaine.

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Materials and Methods

Preparation of collagen films

Collagen (type I) was purified from porcine tendon as previously described [16]. Collagen was adjusted to 5 mg/ml in 0.5 M acetic acid solution. 20 ml solution was poured into a Petri dish (diameter: 5 cm) and vacuum dried.

Preparation of GTA-crosslinked collagen films

After vacuum drying, collagen films were immersed in 0.0075 wt% or 0.01 wt% GTA solution [17]. The GTA solution was made by adding aqueous 25 wt% GTA (Sigma, USA) into phosphate buffered saline (PBS) (pH=7.4). The crosslinking reaction was carried out at 37℃ for 24 hours. After reaction was completed, the films were washed in the solution which was used in the determination of mass transfer characteristics.

Preparation of epoxy-crosslinked collagen films

The procedure of crosslinking by epoxy was the same as crosslinking by GTA, except the concentrations of epoxy solution were 1 wt% or 4 wt% [18]. The epoxy solution was made by 100% liquid epoxy (EX-810) (Nagase, Japan) and sodium carbonate/bicarbonate buffer. The reaction durations for 1 wt% and 4 wt% epoxy solution were 72 hours and 24 hours, respectively.

Scanning electron microscope (SEM) analysis

Collagen Films were well swollen in water at 37℃ for 24 hours. Samples were fixed at least 2 hours in 2 wt% GTA solution at room temperature. After rinsing two times for 10 min in 0.1 M cacodylate buffer, samples were post-fixed for 30 min in 0.1 wt% osmium tetraoxide solution. Following dehydration in a series of graded ethanol (50%, 60%, 70%, 80%, 90%, 95%, 100%), samples were critical-point dried from 100% ethanol, coated with gold-palladium, then observed by using SEM.

Contact angle measurements

The hydrophilic/hydrophobic nature of the collagen films was determined from the measurement of water contact angle. Contact angles were determined by the sessile drop technique. A water droplet of 2 μl was placed on the dried film surface. The drop image was recorded using a videocamera mounted on a microscope within 15-30 seconds after the application of the droplet. Eight measurements were made and an average value was taken. This system was calibrated for pure water on the parafilm with the contact angle to be 109^{o}.

Determination of mass transfer characteristics

Mass transfer characteristics of collagen films were investigated in the side-by-side cells with the selected drug initially only present in the donor cell. The drug solution was either 0.45 wt% theophylline in PBS or 1.0 wt% benzocaine in 50 wt% ethanol solution. The concentrations of drug in the donor and receptor cells of equal volume (20 ml) at 0, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, 12, 24 hour time points were determined spectrophotometrically at 274 nm for theophylline and 284 nm for benzocaine. The permeability (P) of these two drugs was obtained as follows. Assuming that the accumulation of drug in the film is negligible, according to the theory of mass transfer, the relationship between concentration of drug and time can be written as:

$$\frac{dC_R}{dt} = A\frac{P}{LW_R}(C_D - C_R) \tag{1}$$

The concentration of drug in donor cell, C_D, can be calculated by the following equation:

$$C_D = \frac{C_0W_0 - C_RW_R}{W_D} = C_0 - C_R \tag{2}$$

where

A : the cross section of mass flux (cm^2)

C_R:the concentration of drug in receptor cell at each time point (wt%)

C_D:the concentration of drug in donor cell at each time point (wt%)

C_0 : the initial concentration of drug in donor cell (wt%)

L : film thickness (cm)

P : permeability of drug (cm^2/s)

t : time (s)

W_R: the total weight of solution in the receptor cell at each time point (g)

W_D:the total weight of solution in the donor cell at each time point (g)

W_0: the initial total weight of solution in the donor cell (g)

W_R, W_D, W_0 were the same in this research and thus they were all represented by the term "W". Combination Eq. 1 and Eq. 2, the following equation was obtained.

$$\frac{dC_R}{dt} = A\frac{PC_0}{LW}\left(1 - \frac{2C_D}{C_0}\right) \tag{3}$$

Integrated to:

$$\ln\left(1 - \frac{2C_R}{C_0}\right) = -\frac{2AP}{LW}t \tag{4}$$

From the plot of $\ln[1-2(C_R/C_0)]$ vs. t (time), P (permeability) can be calculated from the slope of regression line.

If the solution-diffusion model can be applied to the permeation of a drug through a film, the relationship between P, K (partition coefficient), and D (diffusivity) can be expressed as [19]:

$$P = D_{app} \times K_{app} \tag{5}$$

where

D_{app}: apparent diffusivity (cm^2/s)

K_{app}: apparent partition coefficient

To measure K_{app}, the films were immersed in drug solution (0.45 wt% theophylline in PBS or 1.0 wt% benzocaine in 50 wt% ethanol solution) at 37℃ for 48 hours to reach equilibrium. K_{app} can be calculated from the following equation:

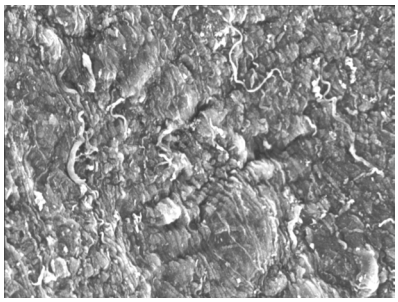
$$K_{app} = \frac{C_f}{C_s} \tag{6}$$

where

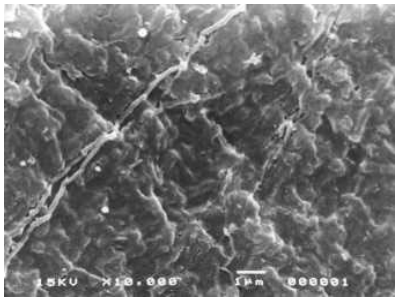
C_f: drug concentration in the film (wt%)

C_s: drug concentration in the solution (wt%)

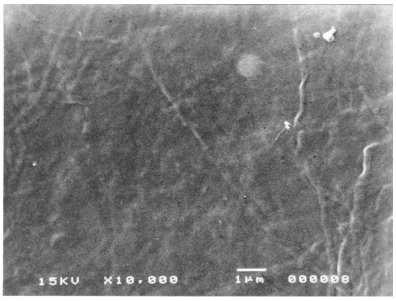
In order to determine C_f, the films pre-equilibrated with drug



(a)



(b)



(c)

Figure 1. The SEM micrographs of surface of different collagen films. The treatment of each graph was：(a) untreated control; (b) 0.01 wt% GTA; (c) 4 wt% epoxy.

solution were put into a drug-free solution of large volume until reaching steady state, i.e., nearly all drug being present in the solution. Assuming the final amount of drug in the films can be neglected. Thus C_f can be calculated by:

$$C_f = \frac{C_{sol} \times W_{sol}}{W_f} \tag{7}$$

where

C_{sol}: the concentration of drug in solution (wt%)

W_{sol}: the weight of solution (g)

W_f: the weight of film pre-equilibrated with drug solution (g)

Results

Surface morphology of collagen films

The complex fibril surface structure of untreated control collagen films was shown in Fig. 1(a). Fig. 1(b) and Fig. 1(c) showed the surfaces of collagen films crosslinked by GTA and epoxy, respectively. The crosslinking treatments made the surface of collagen films smoother than control, suggesting that the cross-linkages between collagen molecules resulted in such effect.

Table 1. Contact angle of different collagen films.

Treatment	Control	GTA (wt%)		Epoxy (wt%)	
		0.0075	0.01	1	4
Contact Angle	105	68	56	82	96

Table 2. Mass transfer characteristic of theophylline in collagen films.

Treatment	Control	GTA (wt%)		Epoxy (wt%)	
		0.0075	0.01	1	4
Thickness (cm)	0.010	0.007	0.011	0.013	0.008
Flux ($\times 10^{-5}$ g/cm^{2}s)	4.08	3.40	2.14	3.03	3.62
Partition Coefficient, K	0.94	1.60	1.66	1.64	1.78
Swelling ratio	3.23	2.62	2.26	3.47	3.17
Permeability, P($\times 10^{-7}$cm^2/s)	9.27	5.22	5.01	8.57	6.62
Apparent Diffusivity, D_{app}($\times 10^{-7}$cm^2/s)	9.88	3.33	3.03	5.24	3.77

Water contact angle

The hydrophilic/hydrophobic nature of the collagen films was determined from the measurement of water contact angle. Table 1 showed the water contact angles of untreated and crosslinked collagen films. The GTA-crosslinked films were the most hydrophilic among all the films and had contact angles between 56°-68°. The epoxy-crosslinked films were more hydrophobic than GTA-crosslinked films, and their contact angles were 82°-96°. The water contact angle of untreated collagen films was about 105°, indicating that these films were relatively hydrophobic.

Diffusivities of theophylline in collagen films

The mass transfer characteristics of theophylline in untreated, GTA-crosslinked, and epoxy-crosslinked films were shown in Table 2. The crosslinking treatments resulted in the decline in the apparent diffusivity (D_{app}) of theophylline. The D_{app} values for theophylline in GTA-crosslinked and epoxy-crosslinked films were $3.03 - 5.24 \times 10^{-7}\, cm^2/s$, about one third of that in the untreated films which was $9.88 \times 10^{-7}\, cm^2/s$. The swelling ratios of GTA-crosslinked collagen films (2.26-2.62) were slightly lower than that of the epoxy-crosslinked (3.17-3.47) and untreated films (3.23).

Diffusivities of benzocaine in collagen films

Table 3 shows the mass transfer characteristics of benzocaine in untreated, GTA-crosslinked, and epoxy-crosslinked films. Again, the crosslinking treatments resulted in the decline in the apparent diffusivity (D_{app}) of benzocaine. The D_{app} values for benzocaine in GTA-crosslinked and epoxy-crosslinked films were $0.97 - 1.26 \times 10^{-7}\, cm^2/s$, about one third of that in the untreated films which was $3.22 \times 10^{-7}\, cm^2/s$. The swelling ratios of GTA-crosslinked and epoxy-crosslinked collagen films (2.08-2.46) were also reduced to about half of that of the untreated films (4.06).

Table 3. Mass transfer characteristic of benzocaine in collagen films.

Treatment	Control	GTA (wt%)		Epoxy (wt%)	
		0.0075	0.01	1	4
Thickness (cm)	0.009	0.006	0.007	0.008	0.007
Flux ($\times 10^{-5}$ g/cm^{2}s)	4.09	3.80	3.49	2.83	3.49
Partition Coefficient, K	1.30	2.02	2.09	2.47	2.10
Swelling ratio	4.06	2.08	2.46	2.14	2.43
Permeability, P($\times 10^{-7}$cm^2/s)	3.84	2.54	2.74	2.31	2.20
Apparent Diffusivity, D_{app}($\times 10^{-7}$cm^2/s)	3.22	1.26	1.22	0.97	1.05

Table 4. Intrinsic diffusivity of benzocaine and theophylline.

Treatment	Control	GTA (wt%)		Epoxy (wt%)	
		0.0075	0.01	1	4
ε/τ ($\times 10^{-2}$)	5.49	3.63	3.91	3.30	3.14
Intrinsic Diffusivity, D_{int} of theophylline ($\times 10^{-6}$ cm^2/s)	16.9	14.39	12.80	25.97	21.08
Intrinsic Diffusivity, D_{int} of benzocaine ($\times 10^{-6}$ cm^2/s)	7	7	7	7	7

Discussion

In this research, all the drug solutions used were generally recognized as hydrophilic ones (either PBS or 50 wt% ethanol in H_2O). Thus, theophylline was supposed to transfer more efficiently than benzocaine. As the results in Tables 2 and 3 showed, the D_{app} values of theophylline were larger than that of benzocaine in all three kinds of films including untreated, GTA-crosslinked, and epoxy-crosslinked collagen films, thereby consistent with our prediction.

A comparison between Table 2 and Table 3 also revealed that the D_{app} of theophylline in GTA-crosslinked films were smaller than that in epoxy-crosslinked films, but benzocaine had larger D_{app} in GTA-crosslinked films than in epoxy-crosslinked films. The phenomena could be explained by the dissimilar polarities of these two types of films. The truth that polar molecules will attract each other implies that the molecules possessing polarities similar to that of the films will be more inclined to be attracted to the films, thus retarding the permeation of drug molecules through the film. According to Table 1, the GTA-crosslinked films were more hydrophilic than epoxy-crosslinked films thus theophylline, a hydrophilic molecule, had smaller D_{app} in GTA-crosslinked films. On the contrary, benzocaine, due to its hydrophobic nature, had smaller D_{app} in epoxy-crosslinked films.

The above mentioned equations are appropriate if applied to a homogeneous dense film. However, considering the high swelling ratios of films used in this research, the collagen films probably resemble microporous membranes. Thus Eq. 5 should be corrected as [20]:

$$P = D_{app} \times K_{app} = \frac{D_{int} \times K_{int} \times \varepsilon}{\tau} \quad (8)$$

where

D_{int}: intrinsic diffusivity (cm^2/s)

K_{int}: intrinsic partition coefficient (usually taken as 1.0)

ε: porosity

τ :tortuosity factor

The intrinsic diffusivity (D_{int}) of benzocaine can be obtained in literature, i.e., $7\times10^{-6}\,cm^2/s$ [21]. By using this D_{int} value of benzocaine, the ε/τ value of collagen film can be calculated by Eq. 8. Using the obtained ε/τ value, the D_{int} value of theophylline can then be calculated also by Eq. 8. The results were shown in Table.4. Theoretically, the D_{int} value of theophylline was supposed to reach constant, and our data were roughly consistent with the prediction. Comparing the D_{app} values in Tables 2, 3 with the D_{int} values in Table 4, the D_{app} values were much lower than the D_{int} values, indicating that collagen films caused significant resistance to the permeation of drugs. The D_{app} value of benzocaine ($3.22\times10^{-7}\,cm^2/s$) was about 5% of its D_{int} values ($7\times10^{-6}\,cm^2/s$) [20]. The composition of drug solution could affect the value of ε/τ. Since the two model drugs were prepared in different solutions, either 0.45 wt% theophylline in PBS or 1.0 wt% benzocaine in 50 wt% ethanol solution, thus even the same kind of films were supposed to have different values of ε/τ when the films were tested by different drugs. However, because the D_{int} value of theophylline was not available, the values of ε/τ for the films immersed in theophylline solution could not be calculated by Eq. 8. Therefore, we had to use the same ε/τ value even in two different drug solutions to find D_{int} value of theophylline, and this might cause incorrectness in the obtained D_{int} value.

Conclusions

Collagen films caused a great resistance to the mass transfer of drugs such as theophylline and benzocaine. The crosslinking treatments leaded to the decline in the apparent diffusivities of the two drugs. In addition, swelling ratio of the crosslinked films decreased by 50% as compared with untreated films. Further, as compared to the hydrophilic drugs such as theophylline, the permeation of hydrophobic drugs such benzocaine in collagen films is relatively poor. This research provides important information that is essential to the design and application of collagen-based biomaterials.

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