Composite Structures 127 (2015) 395–398

Contents lists available at ScienceDirect

Composite Structures

journal homepage: www.elsevier.com/locate/compstruct

The effect of crosslinking to elasticity of cornea with various composite solutions

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ARTICLE INFO

Article history:
Available online 20 March 2015

Keywords:
Cornea
Elasticity
Keratoconus
Riboflavin
Dextran

ABSTRACT

The elasticity of cornea was investigated with and without dextran content in the 0.1% Riboflavin solutions including salt and glucose, respectively. These five different solutions were studied for keratoconus treatment alternatively. The elasticity of the treated and untreated cornea was characterized by using compressive technique. The cornea can be modeled by the mass–spring–damping system. It is understood that the compressive elastic modulus of the cornea changes before and after treatment by the alternative solutions. The mechanical behavior of cornea likes composite anisotropic material due to the structure of cornea which consists of collagen fibrils. The sheep cornea was used as a pilot study before the application of treatment to human cornea. In the cornea, the nonlinear pressure deformation was observed as the behavior of elastomer materials. We found a significant increase in biomechanical rigidity after the cross-linking processes. The observed elasticity properties are consistent with the suggested values in the literature. This study is one of the alternative solutions studies for the treatment of corneal deformation illness after our spectroscopic and mechanical investigations.

1. Introduction

Keratoconus is one of the diseases that occur in the form of more conical shape and progressive thinning of the cornea. It can lead to serious consequences that may require corneal transplantation. Patients whose corneas have proper thickness can be treated by increasing cross-ties and inner layer of the cornea by using a low drop of riboflavin (vitamin B2) and ultraviolet A [1,2]. After crosslinking of the cornea, it could be provided to become more resistant and rigid.

Collagen crosslinking (CXL) is a unique treatment method which obtained painless, quick recovery and successful results to prevent progressive of the corneal ectatic diseases. But this treatment cannot be applied to patients with corneal thickness below 400 μm [3,4] due to the risk of the damage the lower layers of the eye by UV and sometimes can be caused to edema on the eye [5]. In the keratoconus treatment, an absorption peak for UV at a wavelength of 365 nm of the photosensitizer riboflavin is used [6]. Singled form of the riboflavin is excited by UV and then while transferring to triplet form it emits green lights. As a result of this reaction singlet oxygen is formed to tide amino groups of the collagen nano fibrilles by covalent bond. Hence, the cross-linking of nano fibrilles enhances the resistant and rigidity of the cornea.

The effect of true intraocular pressure and modulus on the elasticity of the human cornea was studied [7]. The mathematical model was developed to improve results from applanation tonometry and to estimate the mechanical property of the cornea. The comparison of biomedical properties between human and porcine cornea were obtained [8]. The tensile strength and stress–strain relation were very similar but significant differences between the two tissues were observed in the stress–relaxation relationship. Stress–strain measurements of human and porcine corneas after riboflavin–ultraviolet a induced crosslinking evaluated [9]. Riboflavin–UV induced collagen crosslinking led to an increase in mechanical rigidity in porcine corneas and an even greater increase in human corneas. Dendrimer crosslinked collagen was used to generate highly crosslinked collagen with mechanical properties that would make it appropriate for use as a corneal tissue engineering scaffold [10]. Dendrimer crosslinked collagen gels showed promising properties that suggest that these might be suitable scaffolds for corneal tissue engineering and potentially other tissue engineering applications. Young's modulus in normal corneas and the effect on applanation tonometry were performed

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http://dx.doi.org/10.1016/j.compstruct.2015.03.047
0263-8223/

[11]. The statistically normal range of corneal Young's modulus in vivo in a young healthy normal population was determined and established if this variation is likely to have a clinically significant influence on intraocular pressure (IOP) measurement using the Goldman applanation tonometer.

In the present study, compressive technique was used to characterize keratoconus treatment. It is mainly focused on investigating the elasticity of the treated and untreated cornea with and without dextran content in the 0.1% Riboflavin solutions including salt and glucose, respectively. This technique was employed to measure force versus compression, stress and strain for the treated and untreated of the cornea. The behavior of compressive elastic modulus was explained by the mass–spring–damping system. Investigations indicated that the modulus can be improved after the cross-linking processes. Diffusion coefficients are compared with the modulus. Results of the fluorescence and compression measurements showed that the treated cornea preserves the ability of elasticity. Overall, the elasticity of the cornea is improved after crosslinking structure as a result of the application of CXL, as compared to an untreated cornea.

2. Experimental

2.1. Preparation of solutions

Dextran–Riboflavin solution has been used routinely in the standard CXL procedure. This solution was prepared by 0.001 gr riboflavin in 0.2 ml distilled water (dH2O) and 0.2 gr dextran, 0.072 gr sodium chloride (NaCl) or glucose in the 0.8 ml of distilled water, separately. They were mixed in the same beaker to prepare 0.1% Riboflavin (RF) was diluted for varying concentrations such as 0.01%, 0.02%, 0.04%, 0.06%, 0.08%, 0.1%, 0.2%, 0.4%, 0.6%, 0.8%, and 1%.

2.2. Measurements of spectroscopy

Dextran–riboflavin solutions have been analyzed in UV and visible wavelengths between 300 and 700 nm to decide absorption spectrums emission wavelength of the solutions [12]. After deciding excitation and emission wavelength of the solutions, the corneas were saturated with the riboflavin solutions during 30 min separately. After this state, the saturated cornea was placed into a quartz cuvette gently, and then excited with the appropriate wavelength which depends on the solution dextran–riboflavin is used. The changes of intensity of the emission of the cornea have been measured at time-drive mode of fluorescence spectrometer. This data gives information about amount of riboflavin was used during the CXL reaction with respect to the reaction time. The diffusion coefficient was calculated by using the correlation of emission intensity changes with time.

2.3. Measurements of compression

After CXL reaction, the cornea is removed from the quartz cuvette. Before the compression measurements, the dimensions of the cornea were decided. The compression experiments of the cornea were performed at room temperature around 25 °C. INSTRON 3345 model compressive testing machine, settled a crosshead speed of 0.5 cm/min and a load capacity of 10 N was used to perform uniaxial compression experiments on the cornea of each solutions. Any loss of solutions and changing in temperature during the measurements was not observed because of the compression period being less than 1 min. There is no deswelling during the compressive deformation stage: which means that our experiment corresponds to the case where we can assume the uniform compressive elastic modulus, S which of each copolymer was determined from the slope of the linear portions of compression stress–strain curves.

3. Results and discussion

Both dextran–riboflavin in saline and glucose solutions have been analyzed between 300 and 700 nm wavelength. Absorption spectrum gives pick at 370 and 450 nm for all solutions. Fluorescence spectrums of all solutions have been taken to decide emission wavelength of the solutions which were analyzed in UV and visible wavelengths between 300 and 700 nm [13]. After deciding the emission and excitation wavelength for each solution, we investigated the critical solution content for each solutions from the maximum absorption coefficient graph with respect to the ratio of riboflavin. Our critical riboflavin ratio for each case is 0.1% which agrees with the literature [12]. For CXL treatment, time drive measurement at 365 nm was performed for each solution with sheep. This is the pilot application for human cornea. After 1800s, the cornea was treated as shown in Fig. 1(a) and (b), respectively. This behavior was modeled by moving boundary diffusion [14] which was defined by Eq. (1)

$$\frac{I}{I_\infty} = 2\left[\frac{D}{\pi a^2}\right]^{1/2} t^{1/2} \tag{1}$$

where I is the intensity of light, D is the diffusion coefficient and a is the thickness of the sheep cornea. Diffusion coefficients of sheep cornea were calculated with critical solutions 0.1% for each solution as given in Table 1 and Fig. 2.

It was seen that the diffusion coefficients D from the cornea with various solutions (S0: cornea + RF + dH2O + dextran + salt, S1: cornea + RF + dH2O + dextran, S2: cornea + RF + dH2O + salt, S3: cornea + RF + dH2O + dextran + glucose, S4: cornea + RF + dH2O + glucose) increased in the following order: $D_{S4} > D_{S3} > D_{S2} > D_{S1} > D_{S0}$. The diffusion coefficient of the solution was used

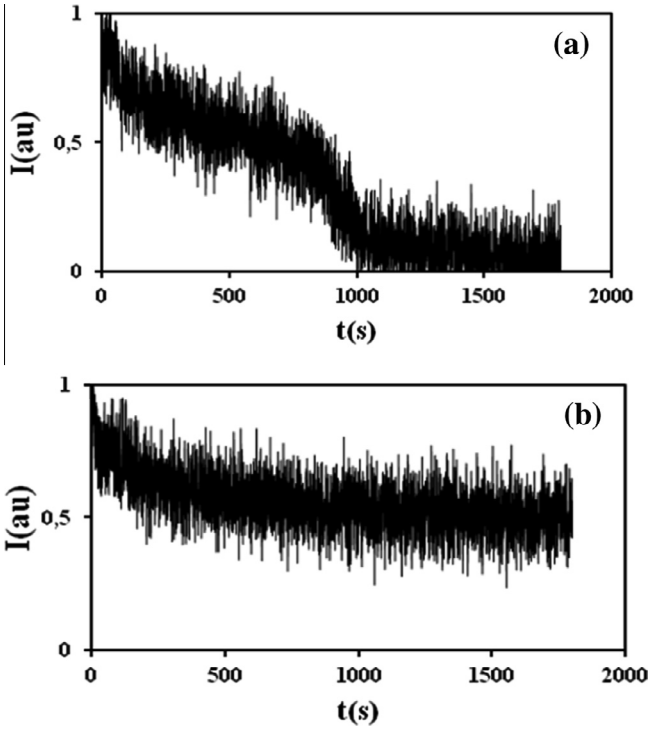

Fig. 1. Emission fluorescence intensity of riboflavin in the 0.1 M% dextran in (a) saline and (b) glucose solution with sheep cornea (S0 and S3) at 542 nm, respectively.