LABORATORY SCIENCE

Relationship between initial corneal hydration and stiffening effects of corneal crosslinking treatment

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Purpose: To characterize the mechanical property improvement of riboflavin and ultraviolet light corneal crosslinking (CXL) procedure in artificially swollen human and porcine corneas.

Setting: Computational Biomechanics Research Laboratory, Mechanical and Industrial Engineering Department, University of Illinois at Chicago, Illinois, USA.

Design: Experimental study.

Methods: Porcine and human donor corneas were crosslinked at different hydration levels using riboflavin–dextran solutions of different osmolality. Four porcine groups (H_w [hydration in mg H_2O/mg dry tissue] = 3.3 ± 0.2 [SD]; 4.0 ± 0.1; 5.1 ± 0.1; 5.6 ± 0.1) and 3 human groups (H_w = 3.2 ± 0.1; 3.9 ± 0.2; 5.3 ± 0.3) were considered. The mechanical properties were measured by uniaxial tensile experiments during which the hydration of samples was the same as the hydration at which they were crosslinked. Tensile properties of 2 porcine groups (H_w = 5.1 ± 0.1; 5.6 ± 0.1) were also measured when their average hydration was lowered to 4.0 mg H_2O/mg dry tissue.

Results: The CXL procedure significantly increased tensile properties of both human and porcine samples in each hydration group ($P < .05$). The improvement in tensile properties was hydration-dependent, that is, samples crosslinked at higher hydration levels showed lower maximum tensile stress. The behavior of samples crosslinked at different initial hydration but tested mechanically at similar hydration showed insignificant difference ($P = .7$).

Conclusion: Increasing the hydration of porcine and human corneal samples before the CXL treatment had insignificant influence on tensile property improvement, as measured by testing specimens at similar hydration.

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The cornea is a load-bearing collagenous tissue that forms with sclera the outermost layer of the eye. The cornea with the help of the lens refracts light rays such that they focus on the retina. Keratoconus is an eye disease that results in blurry and distorted vision. In keratoconus, the cornea thins out and becomes conical in shape. The progressive outward bulging of the cornea results in visual disorders such as myopia, irregular astigmatism, and double vision. Although the exact underlying cause of keratoconus has not yet been determined, it is associated with genetic disorders, such as familial inheritance and Down syndrome, and environmental factors, such as excessive eye rubbing and hard contact lens wear.[1–3]

Riboflavin–ultraviolet light (UV) corneal crosslinking (CXL) is a clinical treatment procedure that improves the mechanical strength of the keratoconus cornea. Over the past decade, the effectiveness of CXL treatment in stiffening the cornea and halting keratoconus has been established by different in vitro and in vivo studies.[4–10] The common CXL procedure often known as the Dresden protocol has 2 steps. The first step involves removing the corneal epithelium and soaking the stromal layer in a photosensitizer solution made up of riboflavin 0.1% and dextran 20.0%. Dextran prevents excessive swelling of the cornea during the application of the riboflavin solution. In the second step, the tissue is exposed to UV rays while drops of the photosensitizer solution are applied on the corneal surface.[6,7]

This treatment procedure is not recommended for patients whose corneal thickness is less than 400 μm, because UV irradiation will damage the endothelial cells.[8] In a recent study, hypoosmolar riboflavin solution that does not contain dextran was used to increase the thickness of thin corneas before CXL. This modified CXL treatment

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successfully and without any complication arrested the progression of the disease in patients with thin corneas.[11] Nevertheless, the exact biomechanical stiffening effects of the modified CXL treatment have not yet been investigated.

The primary objective of the present study was to determine how hypoosmolar riboflavin solution would influence the overall stiffening effect of the CXL treatment. For this purpose, we used riboflavin solutions of different osmolarity and crosslinked corneal specimens at different levels of hydration. We then conducted strip extensometry tests to measure tensile properties of the crosslinked corneas and compared the results with the mechanical properties of untreated samples. We used both human and porcine corneas to characterize possible differences in the mechanical behavior of the cornea from different species.

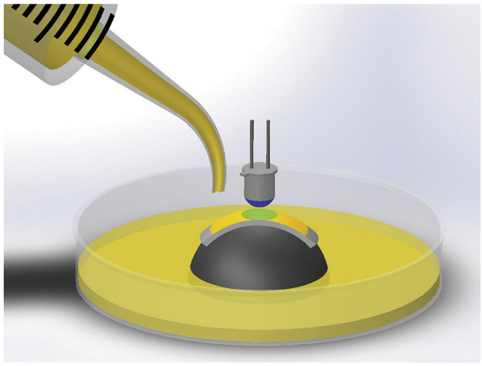

Figure 1. Schematic plot for the CXL treatment. The samples were placed on a hemisphere stand and were exposed to UV rays while a few drops of the riboflavin solution were added (CXL = corneal crosslinking; UV = ultraviolet).

MATERIALS AND METHODS

Both porcine corneas and human donor corneas were used in this study. Porcine corneas were dissected from fresh porcine cadaver eyes and tested within 1 day. Human donor corneas had been stored in a chondroitin sulfate–based corneal storage medium (Optisol) solution for approximately 1 month postmortem before they were brought to the laboratory. To crosslink specimens at different hydration levels, sample thickness was used as a surrogate for their hydration. The thickness–hydration relationships $H_w = 7.0\,t - 0.64$ and $H_w = 3.0 \ln(t/0.2)$ were used to estimate the hydration of porcine and human samples, respectively.[12,13] Here, H_w is the hydration in mg H_2O/mg dry tissue and t is the thickness in mm. The specimens were air-dried or artificially swelled by immersion in saline solution to adjust their initial hydration before being crosslinked.

Five samples in each thickness group were crosslinked and 5 specimens were used as control. The photosensitizer solution was prepared using riboflavin and Dextran T-500 (Sigma-Aldrich Co. LLC). The blunt edge of a scalpel was used to remove the epithelial layer from corneal specimens. All samples were soaked for 30 minutes in the photosensitizer solution for complete penetration of riboflavin into the corneal stroma. Then, 5.0 mm wide strips were punched in the nasal–temporal direction using a custom-built double-bladed punch. Strips dissected from the nasal–temporal direction were only used to prevent variations in experimental measurements due to the corneal anisotropic response.[14,15] The strips were exposed to UV rays of intensity 3 mW/cm^2 at approximately 1 to 2 cm distance for 30 minutes while a few drops of the photosensitizer solution were added every 5 minutes (Figure 1). The irradiance was measured using a radiometer (Solarmeter, Solar Light Co., Inc.). The CXL procedure was performed inside a dark room to minimize the exposure of the specimens to white light. The samples in the control group were also soaked and treated with the solution but the UV light was turned off during the treatment period. These samples were referred to as controls or pseudo-crosslinked in this work.

The concentration of dextran influences the osmolality of the riboflavin solution and subsequently equilibrium thickness of the samples when they are immersed in this solution during the CXL treatment. Thus, a pilot swelling study was performed to determine the appropriate concentration of dextran T-500 in the photosensitizer riboflavin–dextran solution such that unwanted hydration changes caused by the deturgescence effects of dextran could be avoided. The objective of this preliminary study was to find the required dextran concentration for which the thickness (hydration) of samples remained almost constant when they were immersed in the photosensitizer solution. Riboflavin–dextran solutions of different osmolality were made by varying the concentration of dextran from 2.5% to 20.0%. The concentration of riboflavin-5-phosphate (Sigma-Aldrich Co. LLC) was kept at 0.1% in all solutions. The specimens were immersed in riboflavin–dextran solutions with a known dextran concentration and their thickness was measured at regular intervals using a digital pachymeter (Pachette 3, DGH Technology. Inc.). Using this study, dextran concentrations of 20%, 10%, 5%, and 2.5% were used for porcine samples in order to divide them into 4 groups. Because of the limited number of available human donor tissues, this pilot swelling study was not performed on these samples and dextran concentrations of 20%, 15%, and 10% were used to divide the samples into 3 groups. Table 1 shows the mean thickness, hydration, and dextran concentration of porcine and human specimens.

Before starting the mechanical tests, the thickness of treated and control samples was measured using a digital pachymeter and the width of strips was measured using a digital caliper (Mitutoyo Corp.). A dynamic mechanical analyzer (RSA-G2, TA Instruments) was used to perform uniaxial tensile tests and measure the biomechanical properties of the strips. Sandpaper was used to ensure that there was no slippage. The strips were mounted at a loading gap of about 7.0 mm. After mounting the strips, a force of 20 mN was applied to remove any slack from the specimens.[14,16] The length of samples at this stage was noted as their initial length L_0 for strain calculations. The strips were then stretched to a strain level of 10% using a displacement rate of 2.0 mm/min. The tensile stress was obtained from dividing the experimentally measured force by the cross-sectional area of the strips.

The experimental stress–strain curves were curve-fitted using an exponential expression $\sigma = A(e^{B\varepsilon} - 1) + \sigma_0$, where ε is the strain, σ is the stress, σ_0 is the tare stress, and A and B are fit constants. The goodness of these numerical fits was assessed by calculating the coefficient of determinations R^2. Furthermore, the tangent modulus and the tensile stress at 6%, 8%, and 10% strain were calculated and reported as means $\pm$ SD. The maximum tensile, σ_{max}, is defined as the stress at 10% strain, $\varepsilon_{max} = 10\%$. One-way analysis of variance with a P value of 0.05 was performed to determine the significant difference between the mechanical behavior of various groups.

RESULTS

Figures 2 and 3 show the stress–strain behavior of crosslinked and control porcine and human samples. It was observed that with increasing the hydration (thickness), the mechanical response of both human and porcine specimens became softer. Table 1 shows the numerical values of the maximum tensile stress for control porcine and human samples. The difference between various control porcine hydration groups was significant ($P < .05$). Except for